CHAPTER 2

REVIEW OF LITERATURE

Algae are highly diverse group of photosynthetic organisms, with respect to habitat, size, organization, physiology, biochemistry and reproduction (Lukow et al. 2000). They are autotrophic and range from small, unicellular organisms to multi-cellular organisms, with well-varied morphology and cell structure such as spherical, rod, club or spindle and multicellular organisms appear as filaments. Algae are microscopic, prokaryotic or eukaryotic, and uni- or pluri-cellular organisms (Chisti 2007). They are a broad term without taxonomic value, phylogenetically covering prokaryotic or eukaryotic organisms.

2.1. GENERAL CHARACTERISTICS OF ALGAE

The term algae refer to microscopically small, unicellular organisms, some of which form colonies are usually distributed throughout the water and may give considerable turbidity if they attain high densities. Algae have a wide range of sizes and shapes. Unicellular species occur in different shapes that may be spherical, rod- shaped, club shaped or spindle-shaped. Multicellular species appear in every possible form and shape like membranous colonies, filaments grouped singly or in clusters with individual strands that may be branched or unbranched. Algae principally require sunlight, carbon-dioxide and water for its growth. Photosynthesis is an important bio-chemical process in which algae convert the energy of sunlight to chemical energy (Geitler 1932).
2.2. MORPHOLOGY OF ALGAE

Algae exhibit great variation in their thallus structure and organisation with respect to their size, shape, number and structure of cell, pigmentation, nutrition, reserve food, growth, movement and reproduction from species to species. Algae that are less than 2µm in diameter and can be viewed only with the aid of a microscope are known as microalgae and those which are large enough to be seen with the unaided eye are referred to as macro algae. Micro algae includes both unicellular and multicellular forms.

2.2.1. Unicellular Algae

The microalgal species which occur as solitary cells are referred to as unicellular algae. Species like *Chlorella* occur as non-motile solitary cells which are described as “The Coccoid Habit” by Fritsch (1935) and as “protococcoidal type” by Round (1973) whereas species like *Chlamydomonas* are motile. Motile cells may possess one or more flagella or they move by gliding. Among the unicellular structures, number of different forms exist including the cells having flexible shapes, cells with flagella of equal or unequal length, those contained within a gelatinous sheath, with intricate cell walls. Round (1973) divided the unicellular thalli of algae into three categories, viz. Rhizopodial type, Protococcoidal type and Flagellated unicells.

2.2.2. Multicellular Algae

Multicellular thalli are of five major types and are described as colonial, filamentous, siphonaceous, foliose and heterotrichous forms. A colony type of multicellular algae found with definite number of cells and possessing a
constant shape and size is said to be coenobium which could be motile like *Volvox* or nonmotile like *Pediastrum*. Huge masses of cells that remain embedded irregularly in mucilage constitute Palmelloid colony as in the case of *Microcystis*. A number of cells joined end to end by the production of localised mucilage forms a dendroid colony as in *Dinobryon*. An aggregation of cells in an end-to-end fashion contained in a common gelatinous matrix but are not directly connected to each other constitute a Pseudo filament as that of *Chroodactylon*. A series of cells that are arranged in an end-to-end manner, where adjacent cells share a common cross wall forms a trichome. A trichome surrounded by a sheath is called a filament. When the cells are arranged in a single series, the filaments are said to be uniseriate as in *Spirogyra* and when there are more than one series of cells it is said to be multiseriate like in *Bangia*. Filaments may be unbranched as that of *Zygnema* or branched like in *Chladophora*. False branches formed by the fragmentation and continued growth of one or both fragments are seen in some cyanobacteria such as *Scytonema*.

2.3. **CLASSIFICATION OF ALGAE ON THE BASIS OF THEIR HABITAT**

2.3.1. **Aquatic algae:** These algae are found growing in aquatic environments including freshwater, brackish water and sea water.

- **Freshwater algae:** Species of *Oedogonium, Chara, and Zygnema* etc. grow in the still freshwater bodies whereas *Cladophora* grow in the running freshwater bodies.
- **Brackish water algae:** *Anabaena, Oscillatoria* etc. are found in brackish waters.
- **Marine algae:** Algae like *Gracilaria, Fucus, Padina*, etc. grow in the sea water.
2.3.2. Phytoplanktonic Algae: Aquatic organisms that float on the surface of water are called Plankton. Those belonging to the plant kingdom are referred to as phytoplankton. Thousands of algal species live as phytoplankton in both freshwater and marine ecosystems.

- Picoplankton: less than 2 micrometres (µm) in diameter.
- Nanoplankton are intermediate sized microalgae and range in size from 2-20 µm.
- Microplankton are more than 20 µm in diameter.

2.3.3. Aerial and Sub-aerial algae: They grow on substrata like the trunks of trees, walls, fencing wires, rocks, etc. that are exposed to open atmosphere to varying degrees. They may be further classified as follows

- Epiphyllphytes: Such algae grow epiphytically upon leaves of plants.
- Corticolous: Algae like *Trentepohlia* which inhabit the bark of trees.
- Epiphloephytes: Algae grow on the bark of trees mixed with many mosses and liverworts. e.g.: *Scytonema* and *Schizothrix*.
- Epizoophytes: Algae are found even on the bodies of land animals. Eg. Chaetophorales are found even on the hairs of sloth.
- Lithophytes: Algae growing on the rocks and walls. Species of *Nostoc* grow on the wet walls or rocks in rainy season.
- Epilithic algae: They grow on a wide range of hard surfaces and artificial substrates such as glass and plastic. Eg. *Chamaesiphon* sp., and *Gongrosira incrustan*.

2.3.4. Terrestrial algae: These algae are found growing in the moist surfaces on land. The soil flora is collectively known as edaphophytes (Prescott 1969).
Those algae like Mesotaenium, Botrydium, Protosiphon, etc., growing on the soil surface are described as saphophytes or epiterranean algae and those like Nostoc, Anabaena and Euglena grow inside the soil are called cryptophytes or subterranean algae. Some of the subterranean algae become partially heteromorphic in darkness. Some algae may grow upto a depth of more than one metre and they need proper moisture, illumination and nutrition. Friedmann and Ocampo (1976) observed small sized algae in desert soils and classified the desert soil algae into 5 categories

- Endedaphic: Algae living in the desert soil.
- Epidaphic: on the surface of desert soil.
- Hypolithic: living on the lower surface of stones on desert soil.
- Chasmolithic: Algae living in the rock fissures in desert soil.
- Endolithic: Algae which penetrate rock as they grow.

2.4. HISTORY OF PHYCOLOGY

The study of Algae is called Phycology. Reference to algae one finds in the early Chinese, Roman and Greek literatures. Phykos was the Greek word for an alga. The Romans called it Fucus and the Chinese named it Tsao. The ancient Hawanians used algae as food and called them Limu. On the north coast of France the algae were used as manure as early as in the 12th century (Hoek et al. 1995, Tharakan 2009). The real progress in our scientific knowledge of the algae, however, began with the invention and development of the microscope in the middle of the 17th century. Even in the last quarter of the 18th century only four genera or groups, Fucus, Ulva, Conferva and Corallina were named. All others were placed under one of these four. De Jussieu (1789) was the first scientist to delimit the Algae as known to us at present.
In the beginning of 19th century when microscope was developed into a workable tool, many European biologists started taking interest in the study of algae. Roth (1800) discovered and described Hydrodictyon, Batrachospermum and Rivularia. Turner (1809) described fertilization in Fucus. Borge (1913) worked out the fresh water algae of Sweden. West and West (1902) reported and described 45 species of desmids from Singapore. Later published descriptions of 7 species of Red algae, 49 species of diatoms, 33 species of blue-green algae, 246 species of desmids and 84 species of green algae from Ceylon and again in 1907 reported 84 species of diatoms and 148 species of desmids. Fritsch (1907) published an account of the sub-aerial and fresh water algae of Ceylon.

The beginning of 20th century witnessed Oltmann’s work (1904) on morphology of algae. The studies of fresh water algae of Britain by West and West are classical. West’s book on “Algae” gave an excellent account of structure and reproduction of algae. Fritsch and Rich (1927) investigated the fresh water algae of South Africa. Cotton (1914) worked out the marine algal ecology of Clare Island. The investigations of Czurda (1932) and (1937) on the morphology, physiology and cytology of Zygnemaceae are of great importance. Physiological and biochemical studies in algae made considerable advance with the works of Kylin, Kniep and Herder, Transleau’s work on the Zygnemaceae and of Tiffany on Oedogoniales are of particular significance. With the introduction of new techniques and the invention of electron microscope our knowledge of algal-cell, cell-wall, cell sap, nuclear division and structure of flagella and eye spot has greatly increased since 1930 (Vashishta 1990).
2.4.1. History of algal studies in India

The macroscopic forms were the first to attract attention. Montagne (1849) described *Calothrix indica* from India. An army officer Wallich (1860) studied and published a paper on the desmids of lower Bengal. A memoir of the East India fresh water algae was published by Turner (1892-93). Boergesen (1935) laid the basic foundations of the systematic study of Indian marine algae. Among the Indian phycologists, Ghose (1919, 1920, 1923, 1926, 1927a,b,c, 1931) was the pioneer worker. He studied the blue-green algae of Burma and Punjab. Iyengar assisted by his students Balakrishnan, Desikachary, Kanthamma, Ramanathan and Subrahmanian, described a number of new species and genera and worked out life histories of many Indian algae. Along with Subrahmanian he investigated meiosis and auxospore formation of *Cyclotella meneghiniana*. His discovery of *Fritschiella tuberosa*, a terrestrial alga, is of particular significance.

M.O.P. Iyengar, known as the ‘Father of Indian Phycology’, started his work with the aim of obtaining a comprehensive knowledge of India’s algal wealth, its diversity and ecology in 1920 and has made significant contributions to enrich the algal literature of India. Iyengar and his co-researchers established a number of new genera and species. Iyengar (1920) reported two species of *Botrydium* from India. Bhardwaja (1928, 1930, 1933a, b, 1934, 1935) established a school of algology at Banaras Hindu University and made a significant contribution to the knowledge of Cyanophyceae in Uttar Pradesh. Randhawa (1936, 1959) worked on Zygnemaceae, Oedogoniales and Vaucheriaceae of Punjab and Uttar Pradesh. Iyengar and Vimala Bai (1941) investigated the South Indian desmids and gave many new reports. Iyengar and Subrahmanyan (1944) explored the diatoms of Jammu and Kashmir.
Rao (1948) described the genus *Volvox* in North India. Pringsheim (1948, 1953, and 1956) studied the taxonomic problems of the class and contributed towards a monograph on Euglena. Biswas (1949) enumerated some members of Xanthophyceae. Gupta (1950) studied the myxophycean algae from Chamba state. Mitra (1951) reported new members of Volvocales from Indian soils. Balakrishnan (1954) conducted a cyto-taxonomical investigation of Indian members of the order Cladophorales.

Krishnamurthy (1954) contributed to the diatom flora of South India. Gandhi (1955) investigated the flora of Rajastan. Desikachary and Allen (1955) studied on the diatoms of Tamil Nadu. Randhawa (1959) published a monograph on Zygnemaceae and explored many new species from Kumaon, Himalayas. The monograph titled as ‘Cyanophyta’ was published by Desikachary (1959) and it became an important resource on the taxonomy of blue green algae. Pal et al. (1960) published a monograph on Charophyta. Ramanathan (1962) reported the zygospore formation in some South Indian desmids. Gandhi (1967) studied the diatoms of Gujarat. Munawar (1970) have described the diatoms of Andhra Pradesh.

Gandhi (1970) has conducted a study on the planktonic and periphytic flora of Kerala. Singh and Tiwari (1970) studied heterocyst germination in *Gloeotrichia* sp. Desikachary and Rao (1972) conducted studies on the diatoms of Tamil Nadu. Seenayya (1972) studied the blue-green algae of ponds of Hyderabad. Dixit (1973) described some species of *Pithophora* from Maharashtra and Uttar Pradesh. Sreenivasan et al. (1973) studied on the diatoms of Tamil Nadu. Sarma and Khan (1980) have enumerated the algal flora of India in their book entitled "Algal Taxonomy in India" and they reported 4269 species of algae belonging to 653 genera. Out of the 4269
species, 3023 species are freshwater forms and 1222 are from marine habitats. 24 species are reported as common to both habitats.


Srivastava et al. (1982) described Vaucheria crenulata, a new report from India. Nair (1988) gave reports on some Cladophorales of Kerala. Anand (1989) reported 174 species of blue green algae from the rice fields of South India. Nirmala et al. (1990) reported many species of Stigeoclonium from Tamil Nadu. Prasad and Misra (1992), studied some species from Andaman and Nicobar Islands. Habib et al. (1992a) gave an enumeration of Chlorococcales from Rampur district (Uttar Pradesh) and described the forms of Chlorococcales from Andaman and Nicobar Islands.

contributed much to the species diversity of the genera of Oedogoniales from Kerala.

Gupta (1995) published a checklist and key for the identification of Euglenoids and studied the blue-green algal flora of rice fields of Raipur, reported many species of Spirogyra from Bihar. Shaji et al. (1995) reported many species of the genera Phacus and Trachelomonas from Kerala. Sahu et al. (1996), studied the blue-green algal flora, especially from paddy fields of Orissa.


Perumal and Anand (2009) published a Manual of Freshwater Algae of Tamil Nadu. Arulmurugan et al. (2010) studied the biodiversity of fresh water algae from temple tanks of Kerala and reported 61 species of microalgae. Rehakova et al. (2011) studied the algal diversity of the dry mountains in
Ladakh, NW Himalaya, with reference to various ecological parameters like the habitat, altitude and vegetation. Patil et al. 2011, reported the occurrence of 591 taxa of freshwater algae in Jalgaon district of Maharashtra. Karthick and Kociolek (2011) reported the detailed structure of four centric diatoms from the Western Ghats. Karthick et al. 2013 reported diatoms from Indian peninsula.

2.5. ALGAL TAXONOMY

It was Linnaeus who coined the term “algae” for one of the four orders of class Cryptogamia of the plant kingdom in the year 1753. Fritsch in 1935 commented “unless purely artificial limits are drawn, the designation alga must include all halophytic organisms (as well as their numerous colourless derivatives) that fail to reach the higher level of differentiation characteristic of the archegoniate plants.” Smith (1955) defined algae as “simple plants with autotrophic mode of nutrition”. Prescott (1969) rightly stated that "the algae are those chlorophyll bearing organisms (and their colourless derivatives) which are thalloid, i.e., having no roots, stems and leaves or leaf like organs." Bold and Wynne (1978) were of the opinion that "sometimes even the professional botanist and biologist find algae embarrassingly elusive of definition."

H. Harvey (1836) classified algae into three main groups like Chlorospermae (green algae), Melanospermae (brown algae) and Rhodospermae (red algae) on the basis of their pigmentation. Eichler (1886) proposed a 5-group system of classification for algae and divided them into Cyanophyceae, Diatomeae, Chlorophyceae, Phaeophyceae and Rhodophyceae. Round (1973) classified algae into two major groups, Cyanophyta in Prokaryota and the rest in Eukaryota. Bold and Wynne (1978) followed the suggestions of Pappenfuss (1946) and proposed nine divisions of algae.
Cyanochloronta, Chlorophycophyta, Charophyta, Euglenophycophyta, Phaeophycophyta, Chrysophycophyta, Pyrrhophycophyta, Cryptophycophyta and Rhodophycophyta The advancement in algal studies especially on morphology, structure and reproduction, life cycle, marine algal ecology, physiology and cytology began during the early years of 20\textsuperscript{th} century.

2.5.1. Nucleotide sequence analysis and DNA barcoding in Algal Taxonomy

Many reports of molecular data have suggested that there are lots of taxonomic problems in their traditional classification system based on the morphology (Komarek and Anagostidis 1986, Kurger et al. 1995). The traditional generic concept, based predominantly on morphological characters, has often been challenged (Krienitz et al. 2001, Krienitz et al. 2004, Luo et al. 2006). However, for reliable identification and to differentiate the algae to species level molecular techniques can be used (Fawley et al. 2002). The ribosomal RNA unit (especially small subunit) has substantiated to be an invaluable tool in molecular evolution (Woese 1987, Sogin 1989, Doolittle and Brown 1994). Analysis of small sub unit ribosomal RNA (rRNA) sequence data has facilitated classification of phylogenetic relationship among micro algae (Gunderson et al. 1987, Wilcox 1989, Doolittle and Brown 1994). However, molecular based phylogenetic analysis of micro algal species is very much limited in India as most of the diversity analysis relies on the morphological characterization. The effect of these identification methods can be evaluated by more number of research papers from many scientific journals, and the widespread acceptance of sequence studies as the method for phylogenetic speculation.
Regardless of the impressive results obtained from molecular methods, attention is needed in the interpretation of their phylogenetic significance. The relationships of one group to other groups are difficult to distinguish. Bhattachary (2011) provided an introduction to the methods used in phylogenetic studies of the algae. Like all plants, algae are also classified in accordance with the recommendations and prescriptions of the International Code of Botanical Nomenclature. This code recognizes the individual organism as belonging to a species, the species to a genus, the genus to a family, the family to an order, the order to a class and the class to a division. Many systems of classification of algae are proposed by different workers over the years. Phycologists differ from each other with regard to the exact number of algal divisions.

2.6. DNA BARCODING

DNA barcoding is the process of using a predefined minimal section of an organism’s DNA to identify it. Such standard short gene sequences from a uniform locality on the genome used for identification of a species are called DNA Barcodes. DNA sequences are being used as powerful tool in systematics and molecular phylogeny of green algae and have given new insights about the evolution of this group of organism. Different segments of DNA are as the barcodes in various groups of organisms. A identified set of primers for the Barcode regions serves as the first step. These primers facilitate the amplification of the barcode region using PCR.

Morphologically identical specimens could be genetically distinct. For genetic analysis of environmental DNA samples from natural sources and to characterize the mixture of genomes or genome fragments new protocols and
bioinformatics methods were developed. This would eventually replace the many of the laborious microscopical counts made during ecological and applied research on microalgal communities. Furthermore, if a barcode system established for algae, in which barcode sequences were linked to correctly named specimens, samples could be accurately identified by anyone with a basic training in DNA technology even without the knowledge of the algal taxonomy. Although DNA barcoding will not immediately solve all the taxonomic problems, it reduces the need of referring to physical type specimens to a great extent, provided the barcodes are linked to type specimens and made effective in nomenclature. A barcode, sequence-essentially a molecular type is unambiguous and can be communicated easily, unlike the morphology of a physical specimen.

The taxonomic identification of an organism has become more reliable as it is confirmed by the construction of systems that employ DNA sequences as taxon 'barcodes'. The CBOL (Consortium for the Barcode of Life) launched in 2004, is an international initiative devoted to developing DNA barcoding as a global standard for the identification of biological species. Studies have shown that the $rbcL$ gene can be used for whole community analysis of marine phytoplankton (Paul et al. 2000) and phytoplankton found in the effluent from wastewater treatment plants.

2.7. APPLIED ASPECTS OF ALGAE - PHYCOREMEDIATION

Olguin (2003) defines phycoremediation in a much broader sense as the use of macroalgae or microalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and carbon dioxide from waste air. Of late among bioremediation systems,
Phycoremediation has gained importance because the technology is both eco-centric as well as econo-centric. Research on algae for wastewater treatment has been investigated over 60 years, when Oswald and his co-workers (1957) described about this application. The use of microalgae for the treatment of municipal wastewater has been a subject of research and development for several decades (Oswald 1963, 1988).

The primary conjecture is that the microalgae will transform toxic contaminants to non-hazardous materials and the treated water can then be reused for cultivation or safely discharged (Oswald 1988). Unicellular green algae such as *Chlorella* sp. and *Scenedesmus* sp. have been widely used in wastewater treatment as they often colonize the ponds naturally and have fast growth rates and high nutrient removal capabilities. In last few decades use of microalgae for nutrients removal from different wastewaters has been described in many research articles (Shelef et al. 1969, Lukavski 1986, Oswald 1988b, Gantar et al. 1991). Apart from these, microalgae were also reported to remove heavy metals from industrial wastewater and found to be competitive compared to other treatment methods (Kratochvil and Volesky 1998). They can effectively remove or specifically uptake metal ions with higher efficiency (Canizares-Villanueva 2000).

Due to the presence of proteins, polysaccharides or lipids on the surface of algae cell wall, they possess high metal binding capacities. Studies have reported that presence of functional groups like hydroxyl, amino, carboxyl and sulphate on the cell surface act as metal binding sites in algae (Ramelow et al. 1992, Holan and Volesky 1995). The cell wall of green algae constitutes 10 – 70% of proteins and heteropolysaccharides which provide amino, carboxyl and sulphate groups on the cell wall matrix (Andrade and Rollemberg 2005).
The biosorption of heavy metals from aqueous solutions involves four major steps:

(a) entrapment by cellular components,
(b) active transport across the cell membrane,
(c) cation exchange or complexation, and
(d) adsorption.

2.8. NUTRIENTS REQUIREMENT IN ALGAL CULTIVATION

Microalgae require essential macro and micronutrients for its growth. Macro nutrients were supplied in the form of carbon, nitrogen and phosphorous to the culture media in bioavailable form for its efficient uptake (Grobbelaar 2006). Being a photosynthetic microorganisms microalgae uptake carbon inform of inorganic CO$_2$ dissolved in the medium. Supplementation of inorganic C in the form of soluble bicarbonates which supply the equilibrium of carbon ions in the solution have been successfully evaluated and reported in several studies (Gardner et al. 2012, Gris et al. 2014, Eduardo et al. 2016). Nitrogen is taken up in the inorganic forms of NO$_3^-$ or NH$_4^+$, the latter one is the inorganic N form prevailing in most waste streams. Microalgae preferred ammonia form of N, being the most reduced form and it requires less energy to be assimilated. However, supplementation of ammonium to the cultivation medium may lead toxic to microalgal cells because in solution it is in chemical equilibrium with free ammonia (Uggetti et al. 2014, Markou et al. 2014). This drawback can indeed be controlled by either regulating the pH and/or using proper concentrations.

Phosphorus, in the form of orthophosphate is taken up by microalgal cells from the medium, while other inorganic or organic forms of P required to
be first mineralized and converted to orthophosphates in order to be assimilated (Markou et al. 2014). Besides these macronutrients, microalgae require micronutrients like magnesium, potassium, sulphate, iron etc. in the cultivation medium which are essential components for biomass.

2.8.1. Seawater and wastewaters as nutrient sources

The use of alternative water sources in place of freshwater for large scale algal cultivation has been largely encouraged. This would be advantageous both in terms of nutrient supply and water footprint. Seawater contains more amount of potassium, magnesium and sulphur which are required for algal growth as micronutrients and also contains little amounts of nitrogen, phosphorus, as well as $\text{CO}_2$ absorbed from the atmosphere (Yang et al. 2011, Kim et al. 2015, Park and Lee 2016). In any conditions, the concentration of N and P in saline water is not sufficient to obtain significant algal growth, it must be increased by a suitable technique in order to achieve substantial biomass productivities.

Also, wastewaters which are generally rich in N and P were used as cultivation medium (Markou et al. 2014). The possibility of growing algae in wastewaters has gained a lot of attention because of its dual advantage of wastewater treatments and simultaneous production of valuable biomass (Markou et al. 2014). Depending on the source of wastewater, compositions and characteristics of wastewater differs, which determine their suitability for microalgae cultivation and influences the biomass productivity.
2.8.2. Municipal wastewaters

Municipal wastewaters have been extensively investigated as feasible nutrients sources for growth of microalgae (Singh et al. 2011, Ahmad et al. 2013, Ramos Tercero et al. 2014, Umamaheswari and Shanthakumar, 2016). In both batch and continuous mode of cultivation, microalgae have been shown efficient in uptake of N and P from the wastewater (Martinez 2000, Ramos Tercero et al. 2014). The nutrient concentrations in municipal wastewaters depend on the stage of the depuration process (primary or secondary treatment) of wastewater treatment. Chlorella protothecoides (Ramos Tercero et al. 2014) and Scenedesmus obliquus (Martinez 2000), showed a remarkable growth rate but the final biomass production was limited and found not more than 0.5 g/L due to relative low nutrient concentration of N (20-40 mg/L) and P (3-10 mg/L).

2.8.3. Agro-industrial wastewaters

Industrial processes generate huge amounts of polluted, nutrients rich wastewaters. Depending on the industry sector, these wastewaters have different components, which include heavy metals. Besides textile, leather processing and carpet industries, the agro-food industry sector represents the major source of effluents rich in organic compounds, phosphates and nitrogen (ammonium and nitrate). Microalgae showed efficient growth and lipid productivities (60 mg/L/d) when brewery effluents are used as nutrient medium (Raposo et al. 2010, Farooq et al. 2013). Also findings from the dairy industry is quite interesting showed S. obliquus as efficient mixotrophic algal growth using cheese whey permeate and obtained final biomass concentrations of 4.9 g/L and lipid productivities of 40 mg/L/d (Girard et al. 2014).
2.9. RECOVERY OF ALGAL BIOMASS

The recovery of biomass is a challenging phase of the algal biomass production which generally requires more than one solid–liquid separation steps, and the process accounts for 20–30% of the total costs of production. The downstream processes include centrifugation, filtration, and flotation, some of which are highly energy intensive.

2.9.1. Harvesting techniques

Harvesting is defined as a sequence of process for eliminating water content from the algal culture growth using various downstream processes (Show et al. 2013). By taking consideration of the cost of extraction and production process, the choice of selection on downstream process plays a crucial role. Generally, the most widespread harvesting processes include screening, coagulation, flocculation, flotation, sedimentation, filtration, and centrifugation (Brennan and Owende 2010). Though conventional harvesting techniques like coagulation, filtration, flocculation sedimentation and centrifugation are being studied, scientist turned their attention towards techniques that should be energy efficiency and cost factor. An efficient harvesting technique should take into consideration various parameters of algae like size and density, in order to achieve a higher yield of biomass, with less operating cost (Chen et al. 2011a). However, while designing an efficient harvesting technique following points should be considered as essential.

- The choice of microalgae and the desired products
- A complete cell separation process for efficient recycling that contributes to the low cost of down streaming processing.
The harvesting process involves two steps

a) Bulk harvesting: A bulk suspension of biomass undergoes sedimentation.

b) Thickening: This process is done to separate the biomass and to concentrate the slurry matter with the help of filtration and centrifugation.

Many of the current harvesting techniques have numerous drawbacks which have an impact on the cost and quality of the products.

2.9.2. Centrifugation

Centrifugation is a method which can be used to separate and concentrate microalgal cells. It involves in the application of a centrifugal field to a liquid. This force causes relatively dense materials to settle more rapidly than they would under normal gravitational force. Many research has been done on harvesting using centrifugation technique and interesting results were found (Huang et al. 2010). Heasman et al. (2000) stated that 90–100% harvesting effectiveness can be attained via centrifugation. The process is rapid and energy intensive, biomass recovery depends on the settling characteristics of the cells, slurry residence time in the centrifuge, and settling depth (Lee et al. 2010). The disadvantages of the centrifugation process include high energy costs and potentially higher maintenance requirements due to freely moving parts (Feng et al. 2011).
2.9.3. Flocculation

Microalgae are covered by extracellular polysaccharides (EPS), which give them a negatively charged surface. The negatively charged surface allows flocculation or aggregation of the cells using cationic metals or other flocculating agents. Thus, flocculation was proposed as a means of recovering microalgae at a low cost prior to centrifugation and has therefore been specifically proposed for harvesting microalgae in wastewater treatment (Oswald 1988). This method is an extensively used technique in diverse activities ranging from brewing to wastewater treatment and mining etc. (Molina Grima et al. 2003). In recent years various flocculation techniques have been explored ranging from chemical flocculation to the latest bioflocculation.

2.9.4. Bioflocculation

Bio-flocculation using microalgae species with self-aggregation properties was described a few decades ago as a potential harvesting procedure (Olguin 2003). In a previous study (Borowitzka 1988), a cyanobacterium of the genus *Phormidium* was isolated because of its capacity to release a bioflocculant. This cyanobacterium secreted a large molecular weight polymer that contained polysaccharide, fatty acid and protein moieties. In another work, a filamentous bioflocculating microalga of the genus *Chlorhormidium* was used to successfully treat secondary municipal effluents (Serodes et al. 1991). There is still limited knowledge of the factors that influence bioflocculation, and more research is needed to apply this approach on a larger scale. The literature in this field is limited, indicating that this area offers a very promising niche for research and development. A recent report on this subject used a combination
of chemical flocculants together with a bio-flocculant polymer that was naturally produced by a bacterium (*Paenibacillus polymyxa*).

### 2.9.5. Auto flocculation

This process automatically occurs at basic pH due to CO$_2$ depletion. The major precipitates which are formed in the auto-flocculation process are Ca and Mg precipitates (Salim et al. 2011). Since these Ca surfaces are positively charged they can interact easily with the negatively charged surfaces of the algal cells which results in the reserves for the phosphate sources and making the surfaces of the algal cells active. Hence microalgae can be used in the treatment of wastewater for the removal of excess phosphate (Lee et al. 2009). When the pH is high flocculation is caused due to the formation of inorganic precipitates, thus after harvesting biomass consists of the excess amount of minerals. This process is performed by the addition of metallic salts, an alkaline compound, or polyelectrolytes. The alkaline compounds such as sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide (Ca(OH)$_2$), or magnesium hydroxide (Mg (OH)$_2$) cause biomass accumulation (Ummalyma et al. 2017).

### 2.9.6. Electrolytic process

In this process, the movement of microalgae is subject to the movement of charges from the anode to cathode and they form aggregates (Poelman et al. 1997, Vandamme et al. 2011). This process basically involves three different mechanisms:

- Coagulants production by the electrolytic oxidation of the sacrificial electrode.
• Deterioration of particulate suspension and breaking of the emulsion.
• Destabilized phase accumulation which can form flocs.

Based on the existing literature (Poelman et al. 1997), it was shown that when there is electrolytic flocculation applied there is an approximately 80–95% of algae is removed (Vandamme et al. 2011).

2.9.7. Gravity sedimentation

Technically this is a method where the heavier algal particles settle down to the bottom. Hence we can easily detect the lighter and the heavier algal particles separately. Gravity sedimentation is the most common harvesting technique for algae biomass in wastewater treatment because of the large volumes treated and the low value of the biomass generated. The major factors which decide and influence the gravity sedimentation are the radius and density of the algal cells. Edzwald (1993) revealed the fact that the low mass microalgal units do not resolve well and are not efficiently separated by the settling process.

2.9.8. Filtration

It is a mechanical or physical process which is used for separating solids from liquids or gasses by interposing a medium through which fluid can only pass (Brennan and Owende 2010). This method involves continuous passing of the broth with the microalga across a filter on which algal cells will concentrate constantly until it reaches a certain thickness. Due to the small size of C. vulgaris, conventional filtration is not an adequate method to be applied. Instead, ultrafiltration or microfiltration is more efficient. Fouling generated by soluble compounds like exopolysaccharides of some microalgae such as
Porphyridium is one of the major limitations during the ultrafiltration process, but with Chlorella this phenomenon is negligible, and thus its structure provides more important permeation flux without the need of an additional unit operation like swirling while filtering. Other filtration processes, like vacuum filtration, dead-end filtration, pressure filtration, and tangential flow filtration (TFF) are also studied (Petrusevski et al. 1995). According to the recent studies, it is shown that TFF and pressure filtration can be used effectively since they consume less energy. The major drawback of this filtration is the frequent change of filters and membranes which makes the process economically not viable (Mohn 1980).

2.10. APPLICATIONS OF MICROALGAE

The main components of algae feedstock are carbohydrates, lipids, proteins and other valuable components like pigment, anti-oxidants, fatty acids, vitamins etc. Nowadays there is a variety of commercial as well as industrial applications of the algae have been noticed. With the genetic modification of the genes in potent organisms, we can achieve new products (Spolaore et al. 2006). The protein and carbohydrate contents in various strains of microalgae are high, up to 50% of its dry weight. The maximum lipid contents in microalgae are also around 40% on wt. basis, which is reasonably good. All these factors make microalgae a potential source for bio-oil production. Other than biofuel, microalgae have also been proven to produce many important bioproducts like pigments, polyunsaturated fatty acids, antioxidants, carbohydrates, pharmaceuticals, natural colourants etc., (Brennan and Owende 2010, Trivedi et al. 2015). These products range from the food supplements and nutrients for human, livestock feed, fine organic chemicals for pharmaceuticals, pigments and various other applications, e.g. chlorophyll,
biobutanol and acetone etc., Brief account of the various bioproducts from various algae feedstocks were discussed under following subsections.

2.10.1. Fuel sources

In the research on biofuel production microalgae have emerged as a suitable candidate in last few decades. Many researchers are utilizing this opportunity to increase the lipid inside the tiny algae for conversion of oil and utilizing for biofuel production. Now researchers turned their attention on the native environmental species which have high lipid content. The viability of microalgae for biodiesel production has been studied by a number of authors. A comprehensive review on it has been presented by Mata et al. (2010). Khan et al. (2009) has also reported a critical evaluation on prospects of biodiesel production microalgae. They have emphasized the need to explore the possibilities of producing biodiesel from microalgae, as it will not compete with the land and cereal crops. Microalgae are potential to be used as a raw material for biodiesel production, as it meets all of these requirements. They possess high growth rate and provide lipids fraction for biodiesel production Song et al. (2008). Microalgal lipids are mostly neutral lipids with lower degree of unsaturation. This makes microalgal lipids a potential replacement for fossil fuel.

2.10.2. Wastewater treatment

The wastewater released into many water bodies is also a major concern and algae are proved to be efficient in wastewater treatment in several studies (Mohan and Chandrasekhar 2011a, 2011b, Reddy et al. 2011, Chandrasekhar and Venkata Mohan 2012, Pandit et al. 2017). Research has
demonstrated that algae can survive in high level of toxic compounds in the wastewater (Pittman et al. 2011). Algae utilise these toxic substance as nutrients for its growth and in turn the efficient species have shown to produce increased biomass and lipid content thereby serving as a dual benefits (Gomez-Villa et al. 2005, Sawayama et al. 1995). Once the wastewater treatment infrastructure is developed in a full-fledged manner, it provides an opportunity for biofilm technologies which is also another key factor in the treatment of wastewater (Hodaifa et al. 2008). The biomass formed from the wastewater treatment and simultaneous cultivation could be used as a potential source for the human as well as animal feed.

**2.10.3. Bioelectricity**

Nowadays there is a great crisis of power supply affecting the daily activity. More power is needed to empower gas turbines in generating the required current (Chandrasekhar et al. 2015a). Due to the continuous generation of current from these sources, there is a heavy noise pollution and a lot of heat generation during the process. Since these microalgae are very efficient converters of solar power they can be used in producing the electricity through the biotic system as well as enhancing biomass (Saratale et al. 2017). Since it is still an emerging process more exploration needs to be carried out in the field of bioelectricity to make a full-fledged algal-based microbial fuel cell system to cut short the power crisis for the generations to come (Kakarla and Min 2014a, Kakarla and Min 2014b, Kakarla et al. 2015, Saratale et al. 2017).
2.10.4. Algal biohydrogen

Bio-Hydrogen is considered as a potential alternative source of energy. Researchers have found microalgae as the easily available source for the production of bio-H₂ (Chandrasekhar et al. 2015b). *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* are the most studied microalgae to produce biohydrogen. Hydrogenase is the main enzyme to catalyze these reactions. Photobiological reaction involves oxidation of ferredoxin by hydrogenase enzyme in electron transfer chain, which liberates hydrogen. The research in modification of hydrogen production pathways in algae by genetic manipulations to increase biohydrogen yield is finding its way (Saifuddin and Puvunathan 2016, Batyrova and Hallenbeck 2017).

2.10.5. Algal biogas

Research in biogas production from microalgae has recently increased due to their advantages over other feedstocks. Microalgal biomass is a suitable substrate for anaerobic digestion since mineral composition of algae cells fits the nutrient demands of anaerobic bacteria (Sialve et al. 2009). The solar energy stored in algal cells is converted into usable energy by burning the methane liberated in the anaerobic digestion process (Golueke et al. 1957). The first study on anaerobic digestion of microalgae was published by Golueke et al. (1957) who made a comparison between anaerobic digestion of green microalgae and raw sewage resulting in a similar methane yield. After this, many studies have been published focused mainly on the anaerobic digestion of green algae (Mussgnug et al. 2010, Prajapati et al. 2014) and cyanobacteria (Prajapati et al. 2013, Grimm et al. 2015).
2.10.6. Algae in human nutrition

Microalgae are intensively investigated in the last few years on human health benefits especially after the introduction of probiotic supplements (Barrow and Shahidi 2007). Microalgae biomass offer a better quality of protein than the vegetables, rice, and wheat but lower than the animal protein such as milk and meat. Microalgae produce sterols which are used to cure cardiovascular diseases. *Spirulina* sp is reported to produce clionasterol, which aid in disease prevention of vascular cells (Barrow and Shahidi 2007). Similarly, many antioxidant compounds are produced by microalgae that have the ability to protect from oxidative stress.

2.10.7. High-value molecules

Microalgae are capable of producing various high-value chemical compounds, for example, antioxidants, pigments, fatty acids, β-carotenes, polysaccharides, triglycerides, which are used in the production of different industrial products such as biofuel, cosmetics, pharmaceuticals, nutraceuticals and functional food industries. Microalgae also produce agar, algal hydrocolloids alginate, and carrageenan which are generally used as a viscosity-modifying agent in pharmaceutical and food industry (Barrow and Shahidi 2007).

2.10.8. Bioactive Compounds

Microalgae are important sources of bioactive natural substances. Many metabolites isolated from these microorganisms have shown biological activities and potential health benefits (Pangestuti and Kim 2011). Microalgae
accumulate specific secondary metabolites (such as pigments and vitamins) which are high value products that have applications in the cosmetic, food, or pharmaceutical industries (Markou and Nerantzis 2013, Skjanes et al. 2013). Microalgae are known to produce various therapeutically effective biocompounds that can be obtained from the biomass or released extracellularly into the medium (Harun et al. 2010). These microorganisms contain many bioactive compounds, such as proteins, polysaccharides, lipids, vitamins, enzymes, sterols, and other high-value compounds with pharmaceutical and nutritional importance that can be employed for commercial use.

2.11. CONCLUDING REMARKS FROM LITERATURE

From the literature survey, we conclude that an integrated approach on evaluation of identified microalgae samples from local regions are yet to explore. Each isolated strains from native regions where identified either microscopically or rRNA based identification were attempted or field evaluation were studied. Detailed comprehensive study on isolation, identification, characterization, evaluation and metabolic profiling will help in development of scientific data with commercial application. Hence our study was formulated with above said objectives as mentioned in Chapter 1.4.