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Services of Algae to the Environment

RAI, LAL CHAND[1]*, HAR DARSHAN KUMAR[1], FRIEDER HELMUT MOHN[2], AND CARL JOHANNAS SOEDER[2]

[1]*Department of Botany, Laboratory of Algal Biology, Banaras Hindu University, Varanasi - 221 005, India*
[2]*Forschungszentrum, Jülich, Institut für Chemie und Dynamik der Geosphäre (ICG-6), Postfach 1913, D-52425, Jülich, Germany*

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Abstract Being autotrophic, algae occupy a strategic place in the biosphere. They produce oxygen both directly and indirectly through the chloroplasts of all green plants. The chloroplasts are believed to have originated from archaic prokaryotic algae through endosymbiosis with primitive eukaryotic cells. Phytoplankton and other algae regulate the global environment not only by releasing oxygen but also by fixing carbon dioxide. They affect water quality, help in the treatment of sewage, and produce biomass. They can be used to produce hydrogen which is a clean fuel, and biodiesel, and fix N_2 for use as a biofertilizer. Some other services of algae to the environment include restoration of metal damaged ecosystems, reducing the atmospheric CO_2 load and mitigating global warming, reclamation of saline-alkaline unfertile lands, and production of dimethyl sulphide (DMS) and oxides of nitrogen (NOx) involved in the regulation of UV radiation, ozone concentration, and global warming. Algae can be valuable in understanding and resolving certain environmental issues.

Key words: Algae, DMS, NOx, UV-B, biomonitoring, global warming, sewage treatment, biodiesel, N_2-fixation, biomass

The algae are a heterogeneous group of simple photosynthetic organisms found in all kinds of marine, freshwater, and terrestrial habitats where some moisture and light are available. Some of these organisms are good model systems for understanding problems of phylogeny, evolution, physiology, genetics, molecular biology, biotechnology, environmental pollution, and others. The recent upsurge of world-wide interest in several aspects of environmental phycology [11, 32, 105] not only attests to their profound importance in local, regional, and global environmental problems but has also contributed to a much better appreciation of the algal services to aquatic and atmospheric environments besides, of course, the well-known role of the blue-green algae (cyanobacteria) in the nitrogen economy of paddy fields [116, 131]. The most important of these services relate to the photosynthetic carbon fixation and oxygen evolution by oceanic phytoplankton [60]. Besides the terrestrial forests, the marine phytoplankton is the major sink for the greenhouse gas carbon dioxide. Any decrease or loss of phytoplankton caused, say, by the increased incidence of ultraviolet-B radiation resulting from stratospheric ozone depletion, may have adverse impacts not only on the world's fish harvests (a food chain effect) but also on global warming [44]. According to Fogg [31], global warming might produce some drastic rearrangements of flora, fauna, and fishing grounds in some sea areas but the overall oceanic productivity may not change significantly. Although algae have been widely used in understanding the environmental problems in a variety of ways (the damage caused by algae in the form of blooms, toxins, eutrophication, biofouling, etc. are not discussed here), the information related to their environmental role is highly scattered. It was, therefore, decided to review some of the information, albeit in an abridged form.

O_2 Production and Primary Productivity

Although algae are primarily aquatic in nature, they are widely and ubiquitously distributed on our planet, even in some harsh environments. Algae constitute the first link in the aquatic food chain due primarily to their autotrophic nature. As a group, they range from extremely small picoplankton to the giant seaweeds (up to 70 meters long). Approximately 50% of the primary productivity in aquatic bodies comes from algae and phytoplankton [143]. Aquatic ecosystems contribute almost the same amount of biomass as the terrestrial systems and are assumed to incorporate 90–100 gigatons (1 Gt=10^9 tons) of atmospheric CO_2 annually [114]. This biomass in turn determines the fish production and climatological processes associated with marine productivity. Some estimates of biomass productivity have suggested that a 5% decrease in

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*Corresponding author
Phone 91-542-367520; Fax: 91-542-317074;
E-mail: lcrai@banaras.ernet.in

phytoplankton productivity would lead to a 7% decrease in fishery and aquaculture, which equals a loss of about 7 million tons of fish per year [84].

Algae provide most of the oxygen required for aerobic organisms. According to Oswald [88], taking NH_4^+ as the source of nitrogen, carbon dioxide as the source of carbon, and phosphate of phosphorus, 1.5 g of oxygen is released from water during the synthesis of 1 g of algal biomass. Grobbelaar *et al.* [42] reported production of over 1.9 g of oxygen per gram of algal biomass produced. In view of the evolutionary significance, the prokaryotes (including cyanobacteria, eubacteria, and archaea) are undoubtedly the most abundant, widely distributed, and biochemically versatile organisms found on earth. These microorganisms lack a membrane bound nucleus and have been thriving on the planet for at least 3.9 billion years, since the Pre-Cambrian when there was no oxygen in the environment. Most are morphologically simple but have a rich and complex biological heritage. In the course of evolution, some became engulfed by their neighbors and transformed into subcellular organelles, the mitochondria and chloroplasts [24]. For quite some time, researchers have been, and are still interested in, establishing a linkage between cyanobacteria and the chloroplasts of higher plants. It is now widely believed that oxygenic photosynthetic prokaryotes are the most probable candidates to have given rise to the chloroplasts of eukaryotic plants by a process called endosymbiosis [59, 80] or a phyletic primary endocytobiosis (a symbiosis at the cellular level involving an intracellular symbiont) involving uptake of a prokaryote (symbiont) by a eukaryote (host) [9, 14, 70, 71]. According to Lewin [64], *Prochloron*, a member of the Prochlorophyta, may have been the first organism to have become endosymbiont within zooxanthellae, which later evolved into the chloroplasts of today. It needs mention that prochlorophytes make up the most abundant component of the oceanic phytoplankton. These are tiny-celled organisms (cells sized about 0.6 µm) which uniquely combine a prokaryotic cell structure with pigmentation (chlorophylls *a* and *b*) that is broadly reminiscent of eukaryotic green algae. They have been estimated to contribute between 10% and 80% of the total primary production [34].

It is worthwhile to mention that an enormous amount of molecular evidence has accumulated on the endosymbiotic origin of chloroplasts during the last few years [9, 14, 47, 70, 71, 123]. Nakamura *et al.* [82] generated data to provide a complete sequence of the cyanobacterial genome and plastid genomes of algae and higher plants. This vividly shows that plastids have originated from the endosymbiotic integration of a photosynthetic prokaryote into eukaryotic host cells. A strong indication in support of this event is that the organization of the ribosomal protein genes has been conserved from bacteria to plastids of higher plants. In view of the above situation, it may not be wrong to say that the photosynthetic or O_2 production potential of all green plants owes its origin to the photosynthetic prokaryotes such as cyanobacteria and prochlorophytes. Hence, all photosynthetic oxygen on the planet may be attributed ultimately to the prokaryotic algae.

Algae in CO_2 Uptake and Global Warming

Fossil fuels are the major source of energy all over the world. It is their combustion that has largely resulted in an increase in the atmospheric CO_2 concentration from 240 to 345 ppm during the last century; this has led to some global warming. A global increase in surface air temperature by about 1.5 to 4.5°C is expected to occur by the year 2080 when the present day CO_2 level would be doubled. This increase in temperature can lead to a 1 meter rise in the sea level [48]. It must be borne in mind that CO_2 release and uptake were balanced in the atmosphere before the advent of the industrial era. Due to this reason, the CO_2 concentration has remained fairly unchanged before the turn of the 19th century [95]. Accelerated inputs of CO_2 from fossil fuel burning and deforestation together account for about 7 Gt+ of carbon into the atmosphere. Long-term measurements have indicated that out of 7 Gt+, only 3 Gt+ is deposited in the atmosphere annually. It may be assumed that the remaining 4 Gt+ is removed from the global atmosphere by a net uptake by biological organisms in the ocean and by terrestrial autotrophs [128]. The desirability of reducing CO_2 emissions has been fully appreciated with the result that over 150 countries signed an agreement on climate change during the UN conference on Environment and Development, at Rio de Janeiro in 1992. This document exhorts developed nations to limit the emissions of CO_2 and other greenhouse gases so that they can be brought down to the 1990 emission levels by the year 2000 [140]. A clear message that emerged from this conference was the urgency to find out alternatives to fossil fuels. Ritschard [110] and Orr and Sarmiento [86] opined that the potential for mitigating global warming lies with the marine biological resources. If the fossil fuel burning were substituted by biomass burning, the global CO_2 emission of 5.4 Gt yr^{-1} of carbon produced could be brought down to the emission levels of 1985 by the year 2050 [35].

Approximately 500 Gt of carbon is estimated to be present in the land biota. The annual release of carbon from fossil fuel combustion is estimated to be 70 Gt [95]. The annual excess of carbon is approximately 9 Gt. However, viewing the present day atmospheric CO_2 concentration from the standpoint of plant growth and productivity, the ambient CO_2 levels are found to be suboptimal for plant growth as 1,000 ppm (1% volume) or approximately three times the present atmospheric level would be optimal for land plants. It is needless to state that photosynthesis has been removing a significant amount of

CO_2 and fixing it in plant biomass for a few billion years. The marine system remains the major sink of CO_2.

During the last few years, there has been an upsurge of interest in the use of algae in decreasing the CO_2 burden of the atmosphere. The selection of algae for reducing the CO_2 load of the atmosphere is based on properly documented scientific data. The productivity of large brown algae (e.g. *Macrocystis*, *Laminaria*, *Ecklonia*, *Sargassum*) ranges from 1,000 to 3,400 g m^{-2} yr^{-1} C or about 3,300- 11,300 g m^{-2} yr^{-1} dry wt. The red algae almost do the same job. The cultivated kelp *Laminaria japonica* produces more than 15,000 g m^{-2} dry wt (150 t ha^{-1}) for a 7 month growing season [18]. By comparison, sugarcane, the most productive of the land plants, produces 61 to 95 t ha^{-1} yr^{-1} fresh wt in the USA. This production can be increased to 180 t ha^{-1} yr^{-1} when seed cane is planted each year. Therefore, the production of uncultivated macroalgae is about 2.8 times higher than sugarcane, and that of cultivated *Laminaria japonica* is about 6.5 times of the projected yield of sugarcane. Obviously, the algae have an edge over land plants in sequestering CO_2. In view of the above, and also since maximum algal biomass is found in the sea, the latter becomes the major sink for CO_2.

The CO_2 released into the atmosphere comes from either point- or non-point sources. For the CO_2 diffused into the atmosphere, the land plants and marine biomass are assimilating it to a large extent. However, for fixation from point sources, three alternatives exist: (i) chemical binding with $Ca(OH)_2$, $Mg(OH)_2$, or NaOH, (ii) deposition in the earth's gas fields or in deep oceans, and (iii) biological processes including photosynthetic fixation or enzymatic reactions.

The chemical binding of CO_2 is already in practice and has the advantage of using waste hydroxides. However, the cost of transportation and storage for the quantity of hydroxide required to take up 2.5 Gt of CO_2 produced annually from the burning of fossil fuel alone, as estimated by Paul and Clark [95] for a global scenario, would be enormous. Another problem might be the prevention of dissociation of crystallized carbonates. For storage in gas fields, mines, and deep oceans, it would be extremely difficult to prevent leakage of CO_2 back into the system as CO_2 would be under tremendous pressure. In the deep ocean, these carbonates would affect the flora and fauna [98].

Benemann *et al.* [12] advocated development of an integrated ecosystem for wastewater treatment, CO_2 fixation, and biomass production. Mohn *et al.* [78] and J. U. Grobbelaar, F. H. Mohn, and C. J. Soeder (personal communication) brought out a good estimate for mitigating CO_2 concentration coming to the atmosphere from a point source, based on the emission of CO_2 from a 300 MW power plant run on lignite (Table 1).

The above data demonstrate that CO_2 generated from point sources can be fixed into usable biomass, but the cost of production for temperate countries like Germany seems prohibitive. However, the potential underlying the above studies may be equally relevant for some tropical climates.

A significant role is played by marine phytoplankton and marine macroalgae in removing CO_2 from the atmosphere. By comparison, contribution of freshwater algae (only 0.5%) to the total productivity is meagre. In seawater, bicarbonate (HCO_3^-) is the main bulk form of inorganic carbon (Ci), reaching upto 200-fold higher levels

Table 1. Algal biomass production using CO_2 generated from a power plant (basic data used for calculation).

	Climate, Growth Rate of Algae, and Culture Surface	Biomass production
1.1.1	Solar radiation	1,800.0 Joule · cm^{-2} · d^{-1}
1.1.2	Temperature	20.0 °C
1.1.3	Time of growth and gas input	12.0 h · d^{-1}
1.1.4	Growth rate of algae in	
1.1.4.1	open systems (raceways, pond etc.)	0.02 kg · m^{-2} · d^{-1}
1.1.4.2	closed systems	0.50 kg · m^{-2} · d^{-1}
1.2.	C- and CO_2-parts	
1.2.1	CO_2-weight	1.9643 kg · m^{-3}
1.2.2	C-parts in 1 kg CO_2	0.2727 kg · C
1.2.3	C-part in 1 kg algae	0.500 kg · C
1.2.4	Amount of algae per 1 kg CO_2 ($\eta = 100\%$)	0.5454 kg
2.2.1	Flue gas output, CO_2-amount (15 Vol. %), and algal biomass necessary to fix CO_2.	
2.2.2	Total volume per 12 h. d^{-1}	12,000,000 m^3 · d^{-1}
2.2.3	CO_2-weight	3,535,740 kg · d^{-1}
2.2.7	C-part in the CO_2 (27.3 %)	964,196 kg · d^{-1}
2.2.8	C-part of the algae	50 %
2.2.9	Amount of algae necessary to fix CO_2	1,928,393 kg · d^{-1}

than the CO_2 consumed by marine macroalgae. Two important strategies used for acquiring this Ci are (i) extracellular surface bound carbonic anhydrase (CA) mediated dehydration to form CO_2 prior to uptake, and (ii) direct uptake via a membrane transport protein [10]. The former mechanism is widely known for marine macroalgae. Since the C4 type of photosynthesis is rare in marine algae, HCO_3^- utilization appears to be the main route for CO_2 concentration. These organisms possess a high oxygen-insensitive photosynthesis which, unlike marine seagrasses, is not saturated by inorganic carbon (HCO_3^-).

Calcification by marine algae has recently attracted attention of environmental scientists, for trapping more and more of atmospheric CO_2 [124]. The mechanisms of calcification are known to be different in macrophytes and microalgae: it is mostly a species-specific phenomenon in algae. In the calcifying microalgae, crystals of $CaCO_3$ are surrounded by a complementary matrix, which together form a structure popularly known as coccoliths; when coccoliths are present in the intracellular vesicles, they are termed coccolithosomes. Coccolithophorids, the algae bearing coccoliths, use CO_2 for photosynthesis and HCO_3^- for calcification. Through this process, high levels of CO_2 are converted into carbonates of calcium and deposited into the oceanic ecosystem over time. This helps decrease the CO_2 load and hence the greenhouse effects. Any decrease in phytoplankton productivity would consequently reduce the sink capacity for the atmospheric CO_2, resulting in an enhancement of the greenhouse effect and global climate change [43].

Much research is currently underway for developing improved algal strains through the use of molecular techniques. This is aimed at producing strains efficient in fixing atmospheric CO_2. One such method could be the isolation of strains having carboxylase specific RuBisCO where the entire energy could be utilized for carbon fixation. Uemura *et al.* [129] demonstrated the occurrence of a highly carboxylase-specific ribulose-1,5-bisphosphate carboxylase/oxygenase in *Galdieria partita* and *Cyanidium caldarium*. These workers observed a significant difference (5.2 kcal mol^{-1}) in the activation energy between carboxylase and oxygenase in the RuBisCO of *Galdieria* and concluded that this difference was responsible for a strong carboxylase-specific activity.

DMS Production

Many algae are known to be endowed with defence mechanisms for survival in extremes of cold and salt stress. One such antifreeze and osmotic regulator is called dimethyl sulfoniopropionate (DMSP). This compound breaks down to release volatile dimethyl sulphide (DMS) and acrylic acid. DMS is enzymatically broken into sulphur, released into water from where it diffuses into the atmosphere. DMS is a key substance in the global sulphur cycle. The chief sources of DMSP in the natural environment are marine phytoplankton blooms where high levels of primary production and biomass occur on a time scale of weeks [67]. The Antarctic alga *Phaeocystis* is the principal producer of DMS, accounting for approximately 1% of the total sulphur released into the atmosphere but other algae also produce DMS. Oxidation of DMS gives rise to methane sulfonate and sulphate particles, which are the potential substrates, associated with the formation of cloud condensation nuclei (CCN). Approximately 40 million tons of gaseous sulphur is released into the atmosphere by anthropogenic activity and about 50% of this comes from DMS emission [37]. Cloud condensation nuclei play a significant role in cloud cover and surface temperature by cutting the UV-light. Any decrease in phytoplanktonic algae, especially the DMS producers, will decrease the sulphur content and thus affect the global climate. Keller *et al.* [54] reported synthesis by marine phytoplankton of DMSP, a sulfonium compound that seems to be the main biological precursor for DMS. DMSP is widespread among algal taxa but is most commonly found in selected groups of algae such as Dinophyceae and Prymnesiophyceae. DMS gas makes up about 90% of the biogenic sulphur emission from oceans and is an important agent in climate regulation [5]. DMS in seawater originates primarily via lyase degradation of DMSP, an osmolyte produced in high concentration by some marine phytoplanktons. DMS has been implicated in climate regulation by increasing the earth's reflectivity and also by increasing the acidity of precipitation over remote ocean areas [7]. Most studies on DMS production have been motivated by its climatic change potential. Culture studies have been mainly focused to pinpoint the factors which regulate DMS production. Likewise, field studies have attempted to look at algal blooms having high potential of DMS production [46]. It is interesting to note that axenic cultures of exponentially growing *Hymenomonas carterae* and *Phaeocystis panchetii* have never produced appreciable amounts of DMS [121, 130]. It is also not known if all algae synthesizing DMSP can convert this to DMS. The production of DMS by nonaxenic cultures of phytoplankton, and especially its production during microzooplankton grazing, generated the view that bacteria may also be associated in DMS production. The role of physical grazing in inducing DMS production was confirmed when it was found that the algal DMS lyases are activated during grazing [134].

It is interesting to mention that, until recently, DMS production was found exclusively in marine phytoplankton and therefore DMS formation was considered almost insignificant or even non-existent for freshwater algae. However, studies conducted by Ginzburg *et al.* [38] demonstrated that freshwater algae also produced DMS. The breakdown products of DMS are known to produce some odor in drinking and recreational waters. *Peridinium* *gatunense*, a freshwater dinoflagellate in lakes of Israel has been reported to produce up to 5.5 pg/cell of DMSP during the winter-spring season. The production was found to be maximum during the stationary and declining phases of the algae. A thorough study of DMS production by algae from different habitats would help in developing a global sulphur budget and reveal its impact on global warming.

NOx Production

With the recent upsurge of interest in stratospheric ozone depletion and the consequent increase in UV-B radiation, research effort has been directed at identifying the factors associated in the formation and/or depletion of ozone in the environment. During the course of this search, many land plants and microbes have been screened and found to produce nitrous and nitric oxides (NOx). Algae, especially cyanobacteria, play a pivotal role in the nitrogen economy of the ecosystem primarily through nitrogen fixation. Many eukaryotic green algae and some cyanobacteria, like *Nostoc* and *Aphanocapsa*, have been reported to produce nitrous oxide (N_2O) from NO_2^- through the involvement of two nitrite reductases (NiR), one associated with ammonia and the other with nitrous oxide production [137, 138]. The results obtained by these workers were quite in tune with those reported for *Paracoccus halodenitrificans* by Grant and Hochstein [39]. Nitrous oxide production by photosynthetic algae and cyanobacteria is a novel phenomenon with implications in the nitrogen economy where NO_2^- may be converted to N_2O rather than ammonia. This process may have positive implication in high nitrate waste management systems where the nitrite produced by microbial denitrification may lead to nitrosamine production [53]. Use of heterotrophic nitrite-reducing cyanobacteria such as *Aphanocapsa* 6714 [109] could prevent production of undesirable nitrosamines.

This reactive trace gas is currently attracting the attention of environmental scientists and geochemists and its significance in atmospheric chemistry is being appreciated. NO in living cells is produced from arginine via the enzyme nitric oxide synthase (NOS). However, in the atmosphere it comes from several sources including the activity of soil microbes, fixation of NO by lightning discharges, intrusion from stratosphere, oxidation of ammonia, and photolytic processes in marine ecosystems [139]. Significance of NO in atmospheric chemistry stems from the fact that this gas has a dual role: at high concentration (>8 ppb), it degrades ozone, but at low concentration (<8 ppb), it catalyzes the formation of ozone. Although some higher plants have been found to produce NO [139], their global significance has not yet been appreciated. Likewise, the role of algae which contribute >50% of the total carbon fixed in the biosphere in NO production has remained unexplored.

To redress this, efforts have been made, for the first time, by C. J. Soeder and associates [79, 107, 120]. Their findings have shown that eukaryotic microalgae such as *Scenedesmus* and *Chlorella* have a high potential, comparable to higher plants, for NO production. Work underway at the ICG-6, Forschungszentrum Jülich has further revealed that NO production is not confined to the above eukaryotic algae but also to natural populations of *Cladophora* and the prokaryotic *Anabaena doliolum* and *Synechoccocus* [68]. These workers demonstrated that nitrite produced either through nitrate reductase or supplemented to the culture medium exogenously, elicited NO production by algae. Their studies involving the use of inhibitors such as DCMU, diplosan, DCCD, arsenate, and the uncoupler 2-4 DNP, demonstrate that inhibition or blocking of the activity of NiR stimulates NO production. These workers are also trying to pinpoint the role of NR in catalyzing NO formation. It is worthwhile to emphasize that NO production by algae has always been accompanied by an increase in the nitrite concentration in the external medium. Although globally significant, the quantitative role of NO produced by algae in climate change is not yet very clear.

Production of Liquid Fuels and Hydrogen

Some algae have been used for photobiological production of hydrogen which is a good energy source and a clean, nonpolluting fuel [83]. The turnover times and pool sizes of photosynthetic hydrogen production by green algae have proved promising for solar energy conversion and storage [40]. Further research in this area not only clearly demonstrated the involvement of two photosystems (PS I and PS II) but also of the nuclear genes in processes essential for photoproduction of hydrogen [45]. Using *Chlamydomonas reinhardtii*, Miura *et al.* [77] demonstrated a two-stage biophotolysis system for hydrogen production. In an alternating light and dark cycle, the algal cells accumulated starch in light. When these cells were incubated microaerophilically in the dark, hydrogenase activity with appreciable lag time was induced and thus H_2 production was observed for >10 h. Brand *et al.* [17] screened the potential of eukaryotic algae and found that the orders Chlorellales and Volvocales have the highest H_2 production potential. In a marine strain of *Chlamydomonas* sp. MGA 161, Miura *et al.* [76] found stimulation of hydrogen production at high (15%) CO_2 concentration and low temperature. Skulberg [119] suggested that the biophotolysis of water is achieved by the activity of the chlorophyll-containing reaction centres coupled to hydrogenase and nitrogenase in blue-green algae. He further opined that the microalgae belonging to the classes Chlorophyceae and Cyanophyceae can produce molecular hydrogen by splitting water using solar energy. Kumar and his associates [6, 57, 58, 133] have demonstrated a relationship between nitrogenase and hydrogen production in cyanobacteria.

These workers further demonstrated production of hydrogen both by symbiotic and free living cyanobacteria. They also studied the effect of various environmental factors and inhibitors on hydrogen production by blue-green algae [58].

The biggest hurdle associated with photoproduction of hydrogen is the sensitivity of the enzyme hydrogenase to both oxygen and high light intensity. Some exploratory efforts have been made by Ghirardi *et al.* [36] to produce variants of *Chlamydomonas reinhardtii* capable of tolerating high O_2 on the one hand and producing hydrogen on the other. Cyanobacteria, generally speaking, are efficient biocatalysts in solar energy conversion. Cyanobacterial photobioreactors have been used for the production of (i) hydrogen gas, (ii) assimilation of CO_2 with production of algal biomass, (iii) excretion of ammonia, and (iv) removal of NO_3^-, PO_4^{3-}, and heavy metals from contaminated waters. Cyanobacteria such as *Anabaena, Synechococcus*, and *Oscillatoria* sp. have been widely studied for photoproduction of H_2. Immobilized *A. cylindrica* can produce H_2 continually for a year [83]. Increased H_2 productivity was found for *Hup*$^-$ mutant of *A. variabilis. Synechococcus* sp. has a high potential for H_2 production in fermentors and outdoor cultures [83]. Although the conditions required for H_2 production in the laboratory (anaerobiosis, *Hup*$^-$ strains etc.) are difficult to obtain for large-scale H_2 production in nature, it appears plausible that, if algae are suitably modified through modern molecular techniques to produce more hydrogen, one may obtain a pollution-free energy source.

Benemann [11] reported that microalgae cultures can be used to directly utilize power plant flue-gas CO_2 for production of biomass which could be harvested and converted into substitutes for fossil fuels, in particular biodiesel (vegetable oil methylesters). Researches being carried out in U.S.A., Europe, and Japan have focused on cutting down the production cost by way of attaining high productivity in simple cultivation systems. This may involve increasing photosynthetic efficiency above current levels. However, on a short-term basis, algae can be successfully employed for production of lipids high in Ω-3 fatty acids, aquaculture feeds, and especially animal feeds. Natural photosynthesis may be adapted to an advantage in the development of clean energy technologies [22].

Biomonitoring of Water Quality

The ubiquitous presence of algae in aquatic habitats and their short generation time, and ease of cultivation and handling are some of the attributes that make them good experimental materials. Since algae are primarily aquatic in nature, undesirable changes in water quality may be caused by the algae or may affect the algae. For this reason, algae have been preferred over other organisms (besides fish) in biomonitoring of water quality. Algal bioassays provide a methodology for ascertaining the factors that limit or inhibit algal growth and productivity. While explaining the utility of algae as a bioassay tool, Rodhe [111] quoted Goethe "Gott gibt die Nüsse, aber er beißt sie nicht auf (Nature provides the nuts, and we have to crack them)". Rodhe explained that 'experimental use of algal cultures offers excellent implements for cracking the problems concerning algae as well as the interplay between biota and the environment in natural or disturbed water'. Work done on algae has provided some data and ideas on how to crack Nature's nuts in the context of aquatic monitoring.

Two German limnologists, Kolkwitz and Marsson [56], opined that the mere presence or absence of an alga can point to the quality of water; this prompted the **indicator species concept**. The use of algae as indicators of water quality and monitors of organic pollution has been widely appreciated by many scientists [91, 92, 94]. Some users of this approach questioned the validity of the indicator species concept as many organisms were also found in those aquatic systems having different sets of water characteristics. The hypothesis proposed by Kolkwitz and Marsson was termed the 'polysaprobic approach' to indicate the levels of pollution popularly known as polysaprobic zones. These zones are highly complex, harbor a whole array of nutrients, and support the growth of selected algae, bacteria, fungi, and others. Many proposals were made to classify organically polluted waters, using algae and other organisms as test systems. However, the proposal of Fjerdingstad [29], which is broadly an extension of the Kolkwitz-Marsson proposal, attracted wider acceptance and application. Using algae as a tool, he classified organically polluted water into several zones of contamination (Table 2).

Keeping in view the change in the nature of pollutants as a result of increasing industrial activities, newer approaches were initiated much earlier than those of Fjerdingstad. Ruth Patrick of the Academy of Natural Sciences of Philadelphia, U.S.A., used the diatom species diversity approach to determine the level of contamination in a large number of American rivers [94]. The diatometer technique developed by her group was widely acclaimed and still holds good for pollution monitoring in natural aquatic ecosystems. Diatometers can be simultaneously placed in healthy as well as contaminated waters and algal diversity can be analyzed and correlated with water quality.

One major drawback of the approach developed by Patrick and associates was that it does not provide a toxicant- and dose-specific effect on algae. To address this limitation, a **single species test** was devised. While appreciating the utility of single species tests in bioassay of the U.S.A. pollutants, eight national laboratories and universities of U.S.A. joined hands to recommend the use of the green alga *Selenastrum capricornutum* (*Monoraphidium capricornutum*) as the most sensitive organism for monitoring the contamination as well as eutrophication

Table 2. Classification of organically polluted water bodies suggested by Fjerdingstad [29, 30].

Zone I	The Caprozoic zone, Algae absent, a=Bacteria community. b=*Bodo* community c=Both
Zone II	The α-Polysaprobic zone 1. *Euglena*, 2. Rhodothiobacteria community 3. Pure *Chlorobacterium* community
Zone III	The β-Polysaprobic zone 1. *Beggiatoa*, 2. *Thiothrix*, 3. *Euglena*
Zone IV	The γ-Polysaprobic zone 1. *Oscillatoria chlorina* 2. *Sphaerotilus natans*
Zone V	The α-Mesosaprobic zone a=*Ulothrix*, b=*Oscillatoria* c=*Stigeoclonium*
Zone VI	The β-Mesosaprobic zone a=*Cladophora*, b=*Phormidium*
Zone VII	The γ-Mesosaprobic zone a=Rhodophyta (*Batrachospermum, Laminaria*) b=Chlorophyta (*Cladophora* , *Ulothrix*)
Zone VIII	The Oligosaprobic zone a. Chlorophyta (*Draparnaldia*) b. *Meridian*, c. Rhodophyta
Zone IX	The Katharobic zone. a. Chlorophyta, b. Rhodophyta c. Encrusting algae (*Calothrix, Chamaesiphon*)

a, b, c=as alternatives (any one of these may occur at a time): 1, 2, 3=differences in degree.

Table 3. Experimental design for algal assay potential bottle test (AAP:BT) to estimate maximal standing crop (mg dry weight per liter).

Treatment
Control
Control + 1.00 mg N l^{-1}
Control + 0.05 mg P l^{-1}
Control + 1.00 mg Na_2 EDTA l^{-1}
Control + 1.00 mg Na_2 EDTA + 0.05 mg P l^{-1}
Control + 1.00 mg Na_2 EDTA + 1.00 mg N l^{-1}
Control + 1.00 mg Na_2EDTA + 0.05 mg P+1.00 mg N l^{-1}

potential of aquatic habitats. This approach is popularly known as the Algal Bioassay or Algal Assay Potential Bottle Test (AAP:BT) [75] (Table 3). For monitoring the presence of heavy metals or nutrients limiting the growth of algae, the natural water may be spiked with N, P, and EDTA either separately or in combinations, and maximum yield of the alga is measured.

Users of this approach realized that single species tests are useful for laboratory based experiments. However, the data do not hold true for natural ecosystems, where the algae or toxicants do not exist in isolation but rather in multispecies assemblages. Cairns *et al.* [19, 20] preferred the use of multispecies/microcosm or synthetic microcosm tests. Multispecies microcosm tests serve as a useful intermediate between the simplicity of the single species toxicity test and the complexity of the natural ecosystem [127]. Employing a synthetic microcosm, Rai and Mallick [102] studied the effects of Cu, Ni, and Fe on the species diversity of algae, protozoa, and crustaceans present in the system as well as on the functional variables of algae. This study showed that reduction in species diversity was brought about by the inhibition of physiological variables of algae. The most interesting point that emerged from the work of Rai and Mallick [102] was that algal variables such as ^{14}C uptake and O_2 evolution were not only quite sensitive for toxicity monitoring in single species test but were equally sensitive and useful in the synthetic microcosm. It is worth mentioning that several laboratory and field based methods have been developed to monitor the pollution of water by algae. Some widely acclaimed ones are listed in Table 4.

Controlled ecosystem pollution experiments (CEPEX) have been widely used in marine ecosystems to monitor the quality of water using algae [20]. Rai *et al.* [104] and Singh and Rai [117] for the first time used CEPEX enclosures to study the structural and functional responses of algae of the river Ganga in relation to Cu, Cr, Zn, Hg, and Cd toxicity. Chlorophycean algae were found to be the least sensitive, and cyanophytes the most sensitive, to Hg supplementation. In contrast, cyanophytes were the least sensitive to, and bacillariophytes the most sensitive to, Zn. ^{14}C uptake was found to be a highly sensitive and reliable parameter for monitoring toxicity of heavy metals in natural ecosystems.

Many scientists have advocated the multispecies microbial approach for bioassay of environmental quality in the belief that many problems cited for multispecies toxicity early in their evolution for hazard assessment have been either refuted or overcome. Hence, natural microbial communities are excellent systems for hazard assessment and evaluation.

A very interesting and reliable system of biomonitoring heavy metal toxicity vis-a-vis water quality using algae has been developed and widely used by Jensen and Sicko [49], Jensen *et al.* [50], Rachlin *et al.* [101], and others [103, 141]. This technique is based on the morphometric and X-ray energy dispersive microanalysis (EDAX) of algal cells exposed to heavy metals. This approach measures reductional changes in cell dimensions, thylakoid surface area, numbers and volumes of polyhedral bodies, polyphosphate bodies,

Table 4. Field and laboratory variables used for toxicity assessment by algae.

Method	Description/comments	Reference
1. Controlled Ecosystem Pollution Experiment (CEPEX)	Response of the algal community to pollutants is studied by enclosing natural populations in big polythene enclosures placed in natural conditions	Menzel and Case [72] Rai *et al.* [104]
2. Dialysis Bags	Dialysis bags enclosing algal populations are suspended in natural conditions, thus avoiding artificial laboratory studies	Powers *et al.* [99] Eide *et al.* [25]
3. Phytoplankton Cages	Algae are enclosed in small cages, two surfaces of which are removable and are made up of membrane filters to facilitate the direct contact of algae with pollutants	Owens *et al.* [90]
4. Artificial substrates		
a. Diatometer	Plexiglas slides are attached in a floating rack and the growth of periphyton communities, particularly diatoms, is studied	Patrick *et al.* [94]
b. Concrete blocks	Concrete blocks measuring 9.2×39.0×19.3 cm prove to be better than plexiglas slides	Liaw and MacCrimmon [65]
c. Artificial islands	Polyurethane foam islands (5.1×7.6×7.6 cm) are placed in lakes by anchored and buoyant rope lines	Jones [51]
5. Algal Bioassay (Bottle test)	Specifically devised for measuring the eutrophication potential. Widely used for assaying toxicants. Response of test alga (or natural algal population) is correlated with chemical species present in water	EPA [26], Miller *et al.* [75]

cyanophycian granules, lipid bodies, membrane limited crystalline inclusions, and volumes and numbers of wall layers and mesosomes [103]. Although it is a powerful technique to measure intracellular localization and compartmentalization of metals, it cannot be recommended for routine bioassays because it is very expensive, time consuming, and highly sophisticated, and hence is beyond the reach of small institutions or small public undertakings, especially in developing countries.

Restoration of Metal Damaged Ecosystem

Accelerated inputs of toxicants including heavy metals released from a host of ever-increasing industrialization activities, have resulted in deterioration of many aquatic ecosystems. This increased abuse of aquatic resources is threatening the very survival of living beings due to an impending scarcity of potable water. The dimensions and consequences of this peril are serious because of the diversity of pollutants involved. This has necessitated a call for restoration of the pristine quality of existing aquatic resources. Several strategies, mostly chemical and some biological, have been put forward for restoration of metal damaged ecosystems. The chemical methods include adsorption, precipitation, oxidation/reduction, ion exchange, and evaporative recovery. Since most chemical processes are quite expensive, the application of microbial bioremediation has emerged as a potential alternative [81] for removal of heavy metals. Of the large number of organisms, viz., algae, bacteria, fungi, macrophytes, and higher plants employed for metal removal, algae assume special significance as biosorbents for removal of toxic metals. Algae as well as other microbes accumulate metals by (i) binding onto cell surface, (ii) entrapment in capsules, (iii) transport into the cytoplasm, and (iv) immobilization by extracellular enzymes.

Biosorption, the phenomenon of removal of cations from solution by live or dead biomass is considered to be a viable alternative to conventional methods of metal recovery [132]. The use of microbe based sorbent has emerged as a novel development in environmental biotechnology for the removal and recovery of precious and strategic metals from wastewaters. The work of Adams and Holmes [2] using tannin, resin, and bark of *Acacia mollissing* for removal of Ca and Mg may not only be considered as a major achievement of ion exchange chemistry but an early attempt to use biosorbent in metal removal. Strong biosorption ability of microbial biosorbents depends upon the reactive groups present in the microbial cells. Such active biosorbents are generally dead or inactive microbial cells. Biosorbents could be both broadrange (capable of sequestering a variety of metals), or specific for a given metal. These have their utility in situations where one may be interested in removing many metals at the same time, or a specific metal from a mixture of metals present in the solution.

An important consideration in metal removal through biosorbents is the availability of biomass used for development of biosorbent. Marine macroalgae (seaweeds), particularly *Ascophyllum nodosum*, *Sargassum natans*, *Ulva lactuca*, *Fucus vesiculosus*, and *Chondrus* constitute an expensive type of biomass that are widely used as biosorbents [61]. Besides freshwater algae such as *Chlorella pyrenoidosa*, *Cyanidium caldarium*, *Chlorella vulgaris*, *Spirulina platensis*, and *Scenedesmus obliquus*, are some common algae frequently used for metal removal [132]. It is worth stating that following biosorption of metals in the field, algae will have to be suitably harvested and incinerated. They should not be left on site to enter their biogeochemical cycle. Percival and McDowell [96] reported on the excellent biosorption ability of seaweeds and suggested that this ability was due to the presence of polysaccharide in their cell walls. Besides, there are a large number of substances present in phaeophycean and rhodophycean algae. Kloareg *et al.* [55] reported that the cell wall of brown algae, in addition to the cellulose chain, contains four other biopolymers (alginates, xylofucoglycans, xylofucoglycuronans, and homofucanans). The main substances found to biosorb metals in Rhodophyceae include carrageenans, agars, porphyrans, and furcellarans. The sulphate esters of galactans and xylans are found respectively in *Codium taylori* and *Ulva lactuca* of Chlorophyceae [21]. Green algae are also known to contain a complex of heteropolysaccharides. Galacturonic, glucuronic, guluronic, and mannuronic acids, collectively known as polyuronides, are found in Rhodo-, Phaeo-, and Chlorophyta. Fourest and Volesky [33] while investigating the physical properties of the alginate component in four different seaweeds (*Sargassum fluitans*, *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Laminaria japonica*), reported that the heavy metal binding capacities of the corresponding seaweeds were directly proportional to their respective total carboxyl group contents and related to the electronegativity of the elements, Ca, Zn, Cd, Cu, and Pb, investigated.

The presence of mucopolysaccharide, otherwise known as capsule, is a characteristic feature of the cyanobacterium *Microcystis* which grows abundantly in village ponds and recreational waters of tropical countries including India. Prompted by the role of this polysaccharide (which contains galacturonic acid) in metal biosorption, as known for capsulated bacteria [13], considerable attention has been given by Rai and his associates [93, 100, 106, 118] for using this cyanobacterium as a metal biosorbent. The external polysaccharide of the *Microcystis* and other cyanobacteria contains many functional groups such as carboxylate, hydroxyl, sulphate, phosphate, and amino, which can interact coordinately with heavy metal ions [28, 97]. In several experiments on laboratory cultures and field samples of *Microcystis*, Rai and his associates demonstrated the superiority of natural populations over laboratory-cultured *Microcystis* in biosorption of Fe, Ni, Cd, Cu, and Zn. On comparing capsulated and decapsulated strains of *Microcystis* for their metal biosorption potential, capsulated ones were found to be superior to the non-capsulated organisms [106, 118]. This demonstrates a role of capsular polysaccharide in metal binding and removal. It was further observed that in a mixture of Cu and Fe, the test cyanobacterium showed greater preference for Fe than Cu [118]. Attempts have been made to ascertain if this cyanobacterium could be used in column packing for large-scale application and, if so, whether it would be affected by environmental variables while alive or dead. Biosorption was found to be influenced by pH and temperature. Interestingly, however, heat-killed, formaldehyde-treated, and metabolically-inactive (DCMU-treated) cells had the same biosorption potential as the metabolically-active cells. This suggested that even the dead biomass could be equally useful in metal removal. Application of mathematical models (Langmuir, Freundlich, and BET isotherms) demonstrated a monolayer binding for some (Fe, Cu) and multilayer binding for other metals (Ni, Cd) tested. These experiments collectively demonstrated the suitability of naturally-occurring blooms of *Microcystis* in metal biosorption. Our group is currently working on the feasibility of packing columns with dried dead algal biomass, as well as dried and alginate immobilized biomass for removing metals from single and multiple metal solutions. Attempts are currently underway to optimize the environmental (pH, temperature) as well as other (flow rate, metal concentration) conditions for large-scale application of *Microcystis*, an algasorb, for metal removal. Thus, algae appear to hold great potential in environmental clean-up and restoration programmes.

Microalgae in Wastewater Treatment

Discharge of agricultural, industrial, and domestic effluents, collectively known as wastewater, is a problem fully realized and comprehended by environmental scientists the world over. Addition of these substances lowers the O_2 content of the receiving water on the one hand and increases DOC, BOD, COD, N, P, and suspended solid content on the other. These eventually result in eutrophication of the receiving water, thereby impairing its quality and producing undesirable changes in the community composition of aquatic organisms. The intensity of eutrophication depends on the type of the effluent. One of the worst kinds of organic pollutants is swine manure which can increase the N and P content by 20,000-fold over that of natural water [62]. Since the very inception of sewage treatment technology, the objective behind wastewater treatment has been to reduce the amount of organic matter and pathogenic organisms. In view of the environmental standards for water quality put forward by WHO and others, the primary and secondary treatments are not enough to meet such standards. Although considerable reduction in BOD_5 and COD values has been observed in effluents subjected to secondary treatment, treated waters so obtained still contain large amounts of eutrophication triggering nutrients (e.g. NO_3^-, PO_4^{3-}, NH_4^+). In such a situation, tertiary treatments have been or are still being used to reduce the concentration of some undesirable elements by such processes as chemical coagulation, break-point chlorination, NH_3 stripping, filtration, and reverse osmosis. However, the prohibitive operating cost and use of undesirable chemicals such as

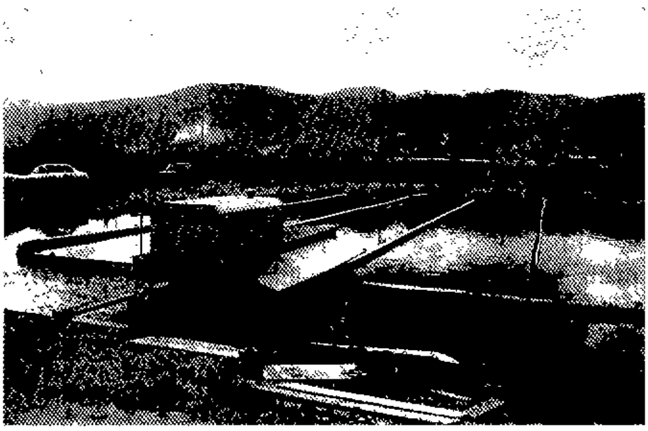
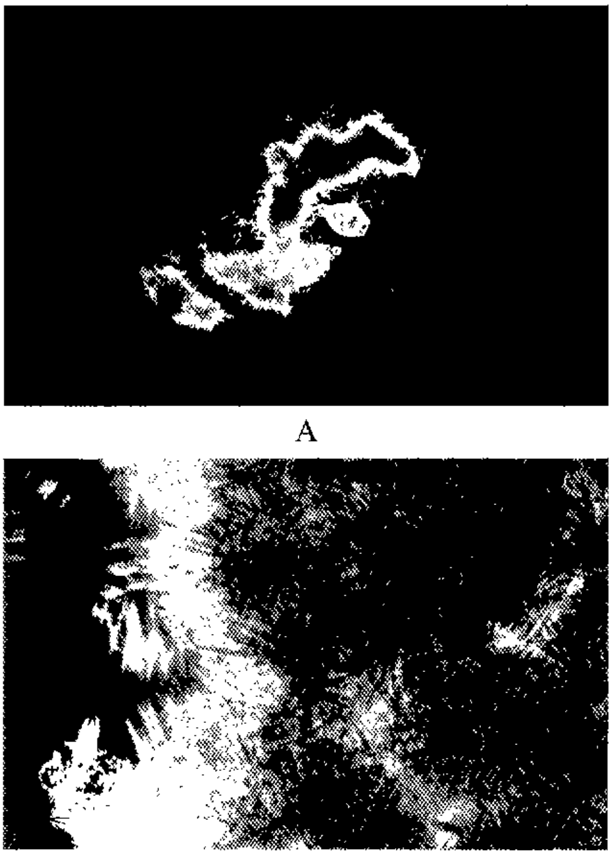


Fig. 1. Photomicrograph of an outdoor Yagur pond, Haifa, Israel. Courtsey of J. Groeneweg, Juelich, Germany.

aluminium salts limit the applicability of this process. Moreover, these chemicals are highly selective and do not work equally efficiently for other ions present in the system. The treatment of wastewater is largely dependent on the intended use of the treated water, that is, whether the treated water is to be used for irrigation, industry, domestic, recreational, or other purposes. The complete treatment of water is a multistep process [62, 89].

Two types of ponds where algae are used for wastewater treatment are (i) facultative ponds which are generally more than one meter deep (Fig. 1), anoxygenic at the bottom, and where algae are normally found on the surface quite often in the form of flocs (Figs. 2A and 2B), and (ii) high rate algal ponds (HRAP) which are less than 1 meter deep, oxygenated properly and continuously mixed by gentle stirring, and contain algae throughout. While growing in organically rich water, algae undergo a mutualistic relationship with bacteria. The algae produce oxygen during the process of photosynthesis, and the bacteria utilize the oxygen to oxidize the substances thereby releasing inorganic nutrients and CO_2 required for the growth of algae [1, 41, 69, 87, 88, 108]. It is worth emphasizing that by employment of algae in sewage, while fulfilling the primary objective of sewage treatment by way of reducing the nutrient elements responsible for eutrophication, it is possible to produce low-cost algal biomass [142]. Oswald [88, 89] provided data on the production of oxygen by algae growing in sewage. That algae are more appropriately suited for the removal of N and P from sewage is apparent from the fact that nitrogen and phosphorus are the major constituents of their cells, where nitrogen is bound to proteins, constituting approximately 50-60% of the dry weight, and phosphorus is associated with phospholipids, nucleic acids, and ester phosphates [113].

Another pressing consideration in favor of the use of algae in wastewater treatment is their mixotrophic growth, in addition to the normal autotrophic growth of all photosynthesizing organisms. Mixotrophic growth equals the sum of autotrophic and heterotrophic growths. Employing *Chlorella* as the test organism, Lalucat *et al.* [63] demonstrated that its mixotrophic growth was much greater than the sum of autotrophic and heterotrophic growths. In addition to this, algae yield more biomass at low light intensity in photomixotrophic than in photoautotrophic growth. Besides the presence of many essential inorganic nutrients, sewage contains other organic substances such as formic, valeric, propionic, acetic, butyric, fatty, and amino acids, and carbohydrates [122] which are used by mixotrophically growing algae.



A

B

Fig. 2. A. Photomicrograph showing algae, bacteria, and zooplankton flocs from the Juelich indoor pond. B. Magnified view of 2A showing different types of algae.

Algae are known to remove a variety of heavy metals such as Cd, Zn, Ni, and Pb found in wastewater. The biomass produced on metal-contaminated wastes may not be useful for animal feed, and algae may be used to remove heavy metals from such wastewater. Some phycologists mistakenly believe that the algae contaminated with heavy metals in sewage systems are unsuitable for further use [8, 23, 126]. Metal removal has been found to be more efficient (approx. 70-90%) by the natural algal flora of contaminated waters as compared to algae collected from non-contaminated habitats [27]. Thus, wastewater treatment with microalgae is an environmentally-friendly technology and one that does not cause secondary pollution [85]. Microalgal technology is being widely used in Taiwan, China, Malaysia, and some other countries. Hopefully, this technology may be gainfully used in temperate countries too. While working on an outdoor system, Grobbelaar *et al.* [41] demonstrated that algal technology can be successfully used for sewage treatment for approximately 7 months of a year even in temperate countries such as Germany. Tang *et al.* [125], using forty-nine strains of filamentous, mat-forming cyanobacteria isolated from Arctic, subarctic, and Antarctic environments, showed that polar cyanobacteria (e.g. *Phormidium* strain E18) were effective in wastewater treatment in cold climates during spring and autumn. A comparison with green algal assemblages revealed that the latter fared better at high temperature.

Algae in the Enrichment of Soil Fertility

Fast-dwindling natural reserves of petroleum oil and coal coupled with increasing demand of fertilizers for increased crop yields to meet the increasing demand of food in the developed and, more so in the third world, countries have been compelling considerations in the quest for some alternatives to nitrogenous fertilizers. Of the various options, such as chemical fertilizer, organic manure, animal dung, and green manure, for increasing the fertility of soil, the use of algae has proved fairly useful in some countries. Soil algae are at the forefront in the context of their significance as biofertilizers. It is unfortunate that, as compared to the ecology of algae from freshwater and marine systems, the soil system has remained less explored. Nevertheless, it is now known that different classes of algae, viz., Chlorophyceae, Cyanophyceae, Bacillariophyceae, Xanthophyceae, and Euglenophyceae are well represented in soils. Metting [73] assigned to algae three major functions in soil: soil conditioners, biofertilizers, and plant growth regulators. Algae are known to interact with aggregates of soil particulates, other substances such as plant growth regulators, and with different organisms present in soil. However, standard methodologies are not available to quantify these interactions. In contrast, the situation is comparatively better for the measurement of nitrogen fixation where reliable methods are available.

Meeting the global needs, which could approach 200 million tons of fixed nitrogen by the turn of the century, would use up nearly 10% of the earth's known natural gas reserves. The manufacturing of 1 kg of nitrogenous fertilizer consumes nearly 2 m^3 of nitrogen. The biological N_2 fixation accounts for nearly two-thirds of the N needs of crops world-wide. Although most of the biologically fixed nitrogen comes from *Rhizobium*-legume symbiosis, the role played by cyanobacteria is also significant. Nitrogen-fixation by cyanobacteria is primarily restricted to the heterocystous filamentous species. Heterocysts not only house the concerned enzyme nitrogenase (N_2ase) but also provide the optimal conditions, especially an anaerobic environment, for the functioning of this enzyme. The N_2 fixation is known to be stimulated by light [15]. In addition to heterocystous cyanobacteria, some unicellular cyanobacteria, e.g. *Gloeocapsa* and *Aphanothece*, are also known to fix dinitrogen. These organisms are surrounded by a mucilaginous material (exopolysaccharide) which provides the microaerophilic condition essential for the activity of the nitrogenase.

A large variety of cyanobacterial species growing in tropical soils serve as major sources of fixed N for rice cultivation. Encouraging the growth of blue-green algae in paddy fields of tropical countries is a common practice. Two methods popularly employed for increasing soil fertility through algae are: algalization and green manuring. Algalization uses mixed cultures of free living cyanobacteria [112, 131]. Green manuring is usually with the water fern *Azolla* whose leaf cavities harbor the cyanobacterium *Anabaena azollae*. Water-logged paddy fields of India and other tropical countries harbor luxuriant algal growths of several species. In contrast to this, *Azolla* is the preferred green manure for subtropical regions where it grows well even during the shorter summers. The report of Roger and Kulasooriya [112] on the biomass distribution of blue-green algae from different continents suggests that their number ranges from 10^3 to 10^7 g^{-1} dry soil. The value of standing crops ranges from a few hundred to 16,000 kg fresh wt ha^{-1}. A comprehensive report on the successional trend of dominant blue-green algae was first recorded and documented by Singh [115] for Indian soils. He observed strong seasonal changes in algal communities in the field. Natural blooms of algae in paddy fields most often observed by Singh [115] included *Aulosira fertilissima*, *Anabaenopsis circularis*, *Anabaena* sp., *Cylindrospermum licheniforme*, *C. gorakhporense*, *Gloeotrichia natans*, *Hapalosiphon welwitschii*, *Nostoc calcicola*, *N. muscorum*, *Porphyrosiphon notarisii*, *Scytonema* sp., *Tolypothrix tenuis*, and *Wöllea bharadwajae*. Various estimates for nitrogen fixation have been reported for different species of edaphic blue-green algae. *Cylindrospermum licheniforme* (18,000,000 filaments g^{-1} of soil) was found to fix 80.25 pounds of nitrogen in one acre of land in 75 days. *Aulosira fertilissima* fixed some 47.6 pounds of nitrogen per acre in one paddy season and caused an increase in crop yield by 114.8%. However, in pot experiments, only a 36.8% increase in yield was recorded. Watanabe [135, 136] demonstrated high nitrogen fixation by *Tolypothrix tenuis*; he could mass culture this alga for distribution to farmers. Mian [74] demonstrated that nitrogen fixed by free living cyanobacteria becomes immediately available to the crop.

Algalization practices have been more widely used in China where they cover an area of approximately 25,000 ha.

The cyanobacteria popularly used there include *Anabaena azotica* and *Nostoc sphaeroides*. In India, algalization has been promoted by an All India Co-ordinated Project on Algae covering an area of approximately 200 million ha. Prominent cyanobacteria used in this project were species of *Anabaena*, *Scytonema*, *Aulosira*, *Plectonema*, *Nostoc*, and *Tolypothrix*. The mixed inoculum is prepared in a soil based mixture and distributed to farmers through agriculture extension programmes. The starter cultures are either raised in small ponds to produce more inoculum or directly applied to paddy fields during the season. The cost saving achieved through algalization in paddy can amount to approximately one-third of the cost of fertilizer [112]. Algalization has been reported to increase rice yield by approximately 14% over animal manure and 16% over unmanured soil.

It has been demonstrated that blue-greens also play an important role in the nitrogen economy of other crops such as maize and sugarcane. Some important blue-greens include *Cylindrospermum licheniforme* and *Camptylonema lahorense*. Thus, the above data clearly indicate that blue-green algae contribute a substantial amount of nitrogen to the field and help save the burning of fossil fuels required for the chemical synthesis of these fertilizers, thereby serving the cause of the environment.

Reclamation of Saline-Alkaline Soils by Blue-Green Algae

Infertile sodic soils with high alkalinity (usar lands) cover vast areas in some parts of India. They are generally regarded as wastelands. If such soils are properly reclaimed and made suitable for agricultural usage, they can contribute significantly to the total grain production of India. Singh [116] did pioneering work on the reclamation of usar soils, using blue-green algae. He reported that the Usar soils in India, after the first shower of the rainy season, support good growths of blue-green algae. Later, when these soils become water logged, there develops a mat of blue-green algae, which floats on the surface of water. As a result of a few repeated cycles of growth of such algae, the alkaline sodium soil is converted into a fertile calcium soil, especially after irrigation is stopped. However, this approach does not offer a permanent relief of soil salinity.

Another method of usar land reclamation involves the creation of earthen embankments around the field so that rain water can accumulate. This is conducive to the growth of such N_2-fixing blue-green algae as *Aulosira fertilissima*, *Anabaena ambigua*, and *Nostoc commune*. The algal growth in these water logged fields lowers the soil pH, exchangeable sodium and sodium-calcium ratio, and increases the nitrogen, phosphorus, organic matter, and water holding capacity of the soil. Blue-green algae are the first colonizers of several soils and form a thick mat on the surface when some moisture is available, thereby helping in preventing soil erosion. Some more widely acclaimed algae include *Microcoleus chthonoplastes*, which is a good soil binder in barren and highly dispersed soils, and *Porphyrosiphon notarisii* which aids the control and prevention of soil erosion [116]. Booth [16] demonstrated that algae grow actively in barren soils of Oklahoma and the algal mat so produced does not affect the water permeation during the rainy season. Besides this, the mucilage producing algae have a significant role in minimizing soil erosion and maintaining the moisture content of the soil. Sadly, microbiologists have not paid sufficient attention to microbes in general, and algae in particular, for their role in conservation of eroded lands which cover vast areas in some tropical and subtropical countries. Beneath the canopy of plants and grasses, many blue-greens such as *Nostoc commune* and *Cylindrospermum licheniforme* enrich the soil in nitrogen. The role of these algae as soil binders and protectors of soil erosion becomes more significant in saline coastal areas of Bombay and Kathiawar. Being loose and sandy, grassland soils require a good soil cover to check erosion, and *Porphyrosiphon notarisii* along with other algae plays a significant role there [116]. Increasing the soil fertility by means of N_2-fixing algae and spreading the bottom sediments of ponds and lakes, where algal blooms die, decay, and add organic matter to the soil, is a common practice followed by farmers in many tropical countries. Many shallow ponds, having blooms of the non-nitrogen fixer *Microcystis* and other algae, dry up in the summer and add lots of organic matter to the bottom mud, which acts as a good fertilizer.

Algae as Ecosystem Engineers

A large number of organisms including algae play a major role in the creation, modification, and maintenance of habitats-activities that do not involve direct trophic interactions between species but are nevertheless important and quite common. These organisms are called ecosystem engineers [52]. They directly or indirectly modulate the activity and the resource availability to other organisms by causing physical changes, both in biotic and abiotic materials [52].

In deserts, several species of cyanobacteria secrete long-chain sugars that bind soil and sand into a black crust, which protects their damp colonies from the searing heat. The slimy exudates change the infiltration, percolation, retention, and evaporation of water and reduce soil erosion [66]. This is not only a survival strategy but also benefits other species [3]. In the latter context, the cyanobacteria act as ecosystem engineers which, by modifying habitats to suit their own needs, alter the availability of energy, food, water, or sunlight, and thereby influence the fates of other species. Blooms of marine phytoplankton scatter and absorb light in upper layers of the ocean. This enhances warming of surface waters that can initiate the development of a thermocline. Freshwater phytoplankton in lakes also intercepts light in the upper water column, smaller algae being more effective as compared to larger species. Light interception leads to shallower mixing depth, lower metalimnetic temperatures, and lower heat content of the water column [52]. Indeed, the importance of ecosystem engineers in affecting species diversity, distribution, and survival, is now being widely appreciated by ecologists.

Ecosystem engineers indirectly control the flow of energy within an ecosystem. These species can have as much influence on an ecosystem as keystone species or top predators. Ecosystem engineers modify habitats in two major ways. Autogenic engineers transform ecosystems by their own growth and are integral to the altered environment. Corals, for example, build reefs for their own needs that also serve many other species. This is amply demonstrated by crustose coralline algae such as *Lithophyllum* found in coral reefs. This alga overgrows and cements together detritus particles on the outer algal ridge of the barrier reef. This tends to break the force of water and protects corals against strong wave action [4].

Allogenic engineers change the environment and then move on, leaving structures behind. Beavers, for instance, transform streams into ponds by building dams that obstruct stream flow. The pooling water submerges grasses and shrubs but provides marsh for herons and other species; crustaceans colonize debris from beaver dams. The desert cyanobacteria are good examples of allogenic engineers.

CONCLUSIONS

Algae are now known to offer a host of services to the environment and considerable amount of information exists on their role in helpring to understand several environmental perturbations. It is, however, pertinent to mention that much research is still required to provide remedies to many unresolved issues of the environment. Some areas requiring urgent attention include global warming, restoration of metal damaged ecosystem, and production of biomass for fuel.

Algae have great potential for biomass production and reduction of CO_2 load from the atmosphere. Some countries like Japan are actively promoting green technologies which are expected to find great demand worldwide in the near future. CO_2-induced global warming is an international political issue. There is an urgent need to provide genetically engineered strains capable of decreasing CO_2 load from the atmosphere. Algae biomass stands a good chance to become an economic and environment friendly alternative to fossil fuel.

Keeping in mind the significance of DMS in global warming, the study of DMS production is another areas of interest. It is not clear if all algae synthesizing DMSP can convert this to DMS. It would be interesting to find out the algal classes other than Dinophyceae and Prymnesiophyceae which could produce DMSP. In view of the work of Ginzburg *et al.* [38], there is an urgent need to screen some freshwater algae that produce DMSP, as well as the environmental factors which regulate the production of DMS.

Although NO production by microalgae has been demonstrated, it is not yet clear if NO production is an enzymatic process in algae. What the global scenario of NO produced by algae is and how far this will help in global warming requires further investigation. Likewise, the H_2 production by cyanobacteria appears to have greater potential over other H_2 producers in terms of the stoichiometry and cost of production. However, it requires a complicated technology for commercial application. In view of an increasing dependence on fossil fuel for energy, and in view of an everincreasing threat to the environment, research efforts are required for developing suitable strains of algae which could be used for sustained production of hydrogen.

Algae appear to be excellent organisms for removal of nitrate, phosphate, and heavy metals from contaminated aquatic ecosystems. The ongoing research on macro- and microalgae has demonstrated that dead biomass immobilized in alginate or other suitable matrix may be used to develop columns (Algasorb) that remove metals over repeated cycles. The algal biomass seems to be a good alternative to chemical and physical adsorbents. However, further work is needed to develop metal specific columns and to optimize conditions that would give the best performance of these biosorbents.

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