

Industrial Wastewater Treatment Using Phycoremediation Technologies and Co-Production of Value-Added Products

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Abstract

Algae are a diverse group of photosynthetic organisms with profound bioremediation potential and industrial applications that can reduce the cost of expenditure on energy and fuels. Algae have been applied to various applications from fuels and medicines to wastewater treatment. Potential applications of phycoremediation have instigated laboratories to reinforce the development of algal-based technologies for better exploitation of their bioremediation potential and by-product generation ability. Many species of algae have been studied and utilized for their function in wastewater treatment. Certain technological drawbacks need to be addressed to manage the shortcomings of algae for industrial usage. This review mainly focuses on the state of the art applications of phycotechnology for wastewater remediation. Many problems related to algal technology have also been exemplified and discussed such as contamination, decreasing the resilience time and increasing the biomass of the algal culture. Possible solutions of these bottlenecks have been suggested to better apply phycotechnology for wastewater remediation in industries considering the environmental issues. Recently, the concept of genetic engineering is found very useful that has increased the potential of phycotechnology reducing the resilience time considerably. On the contrary, the negative impact of the algal-based technologies on the environment and economy has also been deliberated in order to effectively utilize and manage this important organism with utmost benefit. Lastly, the review illuminates the scope and options of future research in the area of phycoremediation technologies.

Keywords: Algae; Phycoremediation; Phycotechnology; Wastewater treatment; Industrial effluents

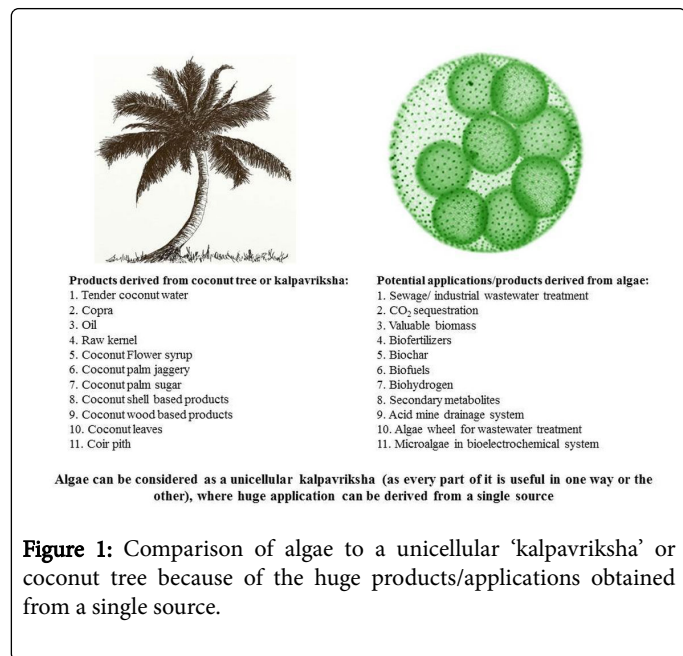
Introduction

Algae (“phyc” Greek for “alga”) are heterogeneous, predominantly eukaryotic, aquatic organisms that vary from single cells to highly differentiated plants. Algae can use sunlight to fix carbon from carbon dioxide to release oxygen into the atmosphere [1]. Algae are important for the planet as they contribute more than 50% of the total photosynthetic activity thereby forming the foundation of the food chain [2]. Algae produce oxygen that is utilized to degrade organic substances and released carbon dioxide can be utilized by algae for their own growth. In the absence of sunlight, the rate of photosynthesis in algae is reduced and so is their role in removing nutrients from the organic sources. Phycotechnology signifies the technological relevance of algae (including cyanobacteria, micro-algae and macro-algae) for industrial products or process development.

After the massive industrialization operating around the world throughout the year what remains back is the waste material generated from the above. Phycoremediation signifies the bioremediation or removal of wastes from the environment utilizing algae and has many advantages over other conventional remediation techniques that are costly, involves huge energy and produces a large amount of waste. Phycoremediation has been used as an environment friendly method of removing waste from the environment. A wide range of non-pathogenic species including *Chlorella*, *Scenedesmus*, *Spirulina*, *Chlamydomonas*, *Nostoc* and *Oscillatoria* have been used for phycoremediation purposes [3].

Algae can be compared to a unicellular ‘kalpavriksha’ or coconut tree (every part of it is useful in one way or the other) as huge applications can be derived from a single source as shown in Figure 1. Algal cells can be effectively used for wastewater treatment as they have an intrinsic property of nutrient, metal and organic compound removal from the wastewater [4]. Algae can be effectively employed for the CO₂ mitigation from the atmosphere due to its ability of CO₂ sequestration. In 1960 for the first time the utilization of algae for wastewater treatment was described along with the potential of the system for energy generation from the algal biomass [5]. Oxidation ponds have been employed in various countries for sewage water treatment where the photosynthetic bacteria and algae are grown to oxidize the wastewater with the liberation of O₂ and fixation of CO₂ [6]. Large-scale cultivation of algae for waste removal from the polluted air, wastewater and industrial establishment can lead to increased biomass that has high calorific value and can be used as a substitute of coal and can be utilized for other applications such as biochar and biofertilizers. Various alternatives of biofuels such as bioethanol, biohydrogen and biodiesel are produced from algae [7]. Hydrothermal liquefaction (HTL) is a very useful thermochemical method of producing bio-oil from crude microalgae that can be used as potent biofuel in the coming years [8]. The cellular structure of algae possesses the ability to synthesize macromolecules such as lipids and active metabolites that can be used as colourant, preservatives and medicines. Additionally, through the concept of genetic engineering, the algal cellular factories can be manipulated to enhance the production of compounds thereby achieving greater technological advancement [9]. Algae have also been utilized as bioindicators that pose a constant check on the contaminant level that helps to keep the impurities within limits in the environment [10]. Algal-based biofilms have been found as an alternative method

for increasing the biomass production of the algae. Moreover, algae have also been harnessed to produce electricity and energy for their ability to behave as an anode in MFCs [11].



Although major prospects exist to channelize the photosynthetic efficiency of algae for waste management and generating products in diverse areas, the technique is challenged by the the space required by the algal species for their growth and the time required for the waste removal from the contaminated site [12]. To successfully implement phycoremediation technology in future our primary focus should be on minimizing the usage of land, energy, nutrients and water involved in the process.

The present review is an outcome of an exhaustive literature survey on the utility of algal-based technology in wastewater remediation, few real instances wherein the technique is applied, the technological limitations, the challenges imposed and the scope of future research in this area. The limitations discussed in this review range from inadequate understanding of the phycoremediation technology to the scale-up of the technique in the pilot scale plant. The analyses presented in this review is to help update the readers about the possibility of effective research in phycotechnology for the benefit of mankind and the environment. Moreover, all this understanding shall pave the way for effective walking through the phycoremediation technology in the future.

Benefits of Phycoremediation Technologies in Diverse Areas Like Wastewater Treatment, CO₂ Sequestration and Value-Added Products

Sewage and industrial wastewater treatment

Phycoremediation is a valuable technique for improving the physicochemical properties of wastewater as it does not involve harmful chemicals and huge energy unlike other convention methods [3]. Phycoremediation has been successfully applied for wastewater treatment owing to its eco-friendly characteristics. Few algal species like *Scenedesmus*, *Chlamydomonas*, *Chlorella*, *Pediastrum*, *Nitzschia*,

Cosmarium and *Botryococcus* is used for phycoremediation of wastewater and biofuel production [13,14](Hoh et al., 2016). Algae grown on wastewater for remediation along with the production of value-added by-products exemplifies the concept of environmental sustainability [15].

In the present scenario, shortage of suitable water supply for drinking, household and industrial purposes is a major challenge throughout the world. To overcome this challenge, it is essential to develop an appropriate method of wastewater treatment. Wastewater includes two general categories of used water like domestic wastewater and industrial wastewater [16]. Treated wastewater can be used for cleaning and gardening purposes so that fresh municipal water can be used for drinking, cooking and bathing purposes. The method generally employed for waste water treatment are expensive, utilize harmful chemicals and high energy. The method employed for water treatment should necessarily be economic and environment friendly [17]. The above-mentioned short-comings of the presently used methods has instigated academics and industries to develop a cost-effective and environmentally friendly technique for the treatment of wastewater. Bioremediation refers to the usage of naturally present biological entities or methods to eliminate environmental pollutants at a given site [18]. Bioremediation has been applied to clean up water, soil, atmosphere or any other contaminated site in the environment. The biological agents commonly used for remediating the environment can be of microbial, algal or fungal sources [19].

Nitrogen and phosphorus are essential components for the growth of algae and are commonly present in wastewater [20]. This suggests that algae can be beneficial in removing the excess nitrogen and phosphorus present in wastewater through absorption and sequestration mechanisms. Another application of algae can be the removal of toxic metals from industrial wastewater. Therefore, the phenomenon of bioremediation employing algae or phycoremediation can help us solve the problem of wastewater treatment. To reinforce the wastewater treatment potential of algae genetically engineered algal strains can also be developed and employed for better wastewater management.

For the development of suitable genetically engineered algal strains that would be effective in increased uptake of nitrogen and phosphorus, we need to search genome databases like NCBI and GenBank for the selection of suitable genes with increased nitrogen and phosphorus uptake capacity [21]. Such genes, when overexpressed under the influence of suitable promoter system will lead to increased uptake of nitrogen and phosphorus in the transformed algal strain and removal of such organic compounds from wastewater. Therefore, development of genetically transformed algal strains with enhanced absorption and metabolism characteristics can help us control of problem of wastewater treatment. In the present study, physicochemical properties of wastewater will be assessed and compared after treating it with various control and genetically modified algal strains. This would help us to know the algal species and their genes which are effective in the treatment of wastewater that would ultimately lead to improved treatment of wastewater. This approach will be helpful in strategizing the future wastewater treatment plans.

Treatment of industrial effluents

Except a few industries algal technology is an intervention at a proper stage to improve the efficiency by avoiding chemicals and reduction in energy input and cost of operation. Biochemical

parameters of the water that are generally improved through the phycoremediation technology are:

1. pH correction.
2. Colour and odour removal.
3. Sludge reduction.
4. BOD and COD reduction.
5. Nutrient removal including ammonia.
6. Better management of R/O rejects.
7. Reduction of operation costs (90%).
8. Flue gas/carbon mitigation.

Restoration of polluted water bodies like lakes, ponds and rivers

Large volume of water in our planet has become polluted due to human activities and discharge of industrial effluents. PERC (Phycospectrum Environmental Research Centre, India) is focussed on controlling water pollution through phycoremediation of sewage water and industrial effluents. Polluted water of the lakes and rivers due to sewage and industrial wastes have been restored using algal technology initiated by PERC. Recently, Mainath lake in Uttar Pradesh has been treated where the contamination level was 50% more than the contamination level of raw sewage. PERC had employed a local microalgae system to control the pollution level within short time wherein major parameters like BOD, COD and coliform bacteria were reduced by 90% resulting in transparent water. Malegaon, in Maharashtra is another example where no sewage plant is present and the entire waste of a textile industry is discharged in river Mousam. PERC together with a textile industry in Malegaon had set up a phycoremediation plant for treating the effluent of the industry. The pH was reduced to 8 from 13 without adding chemicals and the COD was reduced to less than 300 ppm. The microalgae harvested after the treatment was stabilized by adding specific micronutrients and finally discharged into the river. The quantitative parameters like pH, COD, ammonia, concentration of *E. coli* and coliform had improved after three weeks of micro-algal treatment. Moreover, a significant drop in the smell was observed in the entire Malegaon city.

CO₂ sequestration

Algae have been potentially used in wastewater treatment for its ability to sequester during their growth. They can utilize the excess of organic contents present in wastewater during photosynthesis. The pH of the wastewater increases during the growth of algae in wastewater due to CO₂ fixation. There is no need to added caustic soda or lime to correct the pH in areas where the pH is very low. Amongst different methods of carbon capture, algae-based carbon capture method is the best method to capture the emission of CO₂ from the atmosphere [22]. Microalgal species commonly used for CO₂ sequestration include *Anabaena* sp., *Chlamydomonas reinhardtii*, *Chlorella* sp., *Scenedesmus* sp., and *Spirulina* sp. [23]. The *Chlorella* culture when exposed to flue gas from coke industry absorb the CO₂, NO and SO₂ thereby utilizing them for increasing the biomass content. Up to 60% of CO₂ has been removed on-site from the flue gas when it is exposed to *Chlorella* sp. [24]. The growth of algae in closed photobioreactors faces problems of sunlight and CO₂ exposure but the development of open photobioreactors utilizing special membrane has removed this short-

coming thereby resulting in effective CO₂ sequestration [25]. Interestingly, it has been found that increasing the production of carbonic anhydrase in *Chlamydomonas* in the presence of calcium carbonate can increase the CO₂ mitigation ability of this organism [26].

Valuable biomass

Utilization of valuable biomass for developing aqua culture feed, biofertilizer, compost, bio diesel, bio ethanol bio gas, bio char etc. Dried biomass with high calorific value is used as coal substitute. The different valuable substances obtained from the algal biomass are illustrated below:

Production of biofertilizers: Microalgae have been extensively used to in agriculture for soil reclamation purposes. Microalgae have a unique property to fix atmospheric nitrogen in soil and is therefore used for increasing the fertility of soil and as biofertilizer. Microalgae when added to soil can result in improving its physico-chemical properties like pH, electrical conductivity, presence of important elemental content in the soil like potassium, phosphorus and nitrogen [27]. Members of blue-green algae like *Nostoc*, *Anabaena*, *Gloeotrichia*, *Aulosira* and *Spirulina* have been used as biofertilizers to fix atmospheric nitrogen in the soil [28]. Microalgae have been found very important for rice cultivation in paddy fields due to their intrinsic ability of nitrogen fixation, high yield, eco-friendliness and low cost [29]. *Spirulina platensis* was able to absorb ammonia and nitrate from fish water and used as biofertilizer to promote the growth of leafy vegetables such as *Eruca sativa*, *Amaranthus gangeticus* and *Brassica rapa*. The performance of *Spirulina platensis* as fertilizer was found to be comparable to chemical based fertilizers [30].

Production of biochar: In the present scenario of energy crisis algal biomass is very important for its sustainability view point. Algal biomass is considered an important energy source because of its easy availability, huge growth and efficient CO₂ fixation. Algal biomass is converted to biochar through slow pyrolysis to form a carbon rich product that has high pH that can improve the pH of acidic soils. Additionally, the high nutrient content of nitrogen, phosphorus and inorganic elements can improve the soil fertility for agricultural purposes [31]. Due to the presence of various functional groups and inorganic elements algal biochar can also be used as an absorbent surface for wastewater remediation purposes [32]. Some specific functional group on the surface of microalgal biochar can make it good biosorbents for organic molecules to be separated from wastewater. Biochar has gained concern for its widespread use in agriculture and wastewater treatment.

Production of biofuels: The world demands more of energy for a larger population and high-class living. The key problems related to the use of fossil fuels is global warming and change in the climate conditions [33]. This increasing demand for energy has directed researchers to seek alternative energy sources such as algal-based biofuels. The most efficient energy sources of biofuels like the photosynthetic algae and the cyanobacteria which can be used efficiently for the synthesis of bioethanol, biodiesel, biohydrogen [34]. Biodiesel production from the micro-algae has received attention because of the sustainability point of view. Algae have the ability for biodiesel production because of their high photosynthetic efficiency and their ability to accumulate large quantities of lipids in their intracellular structure [35]. Microalgal species are widely used for their bioremediation potential of wastewater by removing the major proportion of inorganic constituents from wastewater. Nonetheless,

microalgae like *Chlorella vulgaris* have been majorly used to produce significant quantities of biomass and produce biofuel [36]. Microalgae have been intensively utilized to produce biodiesel owing to their high CO₂ metabolizing ability, their ability to abundantly utilize wastewater and produce intracellular lipids. However, the commercial production of biodiesel from algae is still not appreciable due to the low biomass content of algae and expensive down-stream process to procure biodiesel. An improvement in the production of biodiesel from algae has been made using microwave irradiation assisted trans esterification. Microwave irradiation involves less time and heat energy to obtain biodiesel from the algal biomass [37]. In another instance, nano Ca(OCH₃)₂ (Calcium methoxide) was developed and utilized to catalyze the trans esterification of crude oil obtained from algal species *Nannochloropsis* to produce biodiesel [33].

Production of bio-hydrogen from green algae: Microalgae can be used to produce H₂ gas. H₂ gas is a significant fuel as it is eco-friendly and contradictory to global warming. The main problem in using H₂ as a source of energy is the continuous production of H₂ gas in an environment-friendly manner [38]. Hydrogen as a source of energy is being used for its high energy content and lead to no pollution as water is generated as its by-product. A group of photosynthetic organisms has evolved to generate H₂ gas from water in the presence of sunlight. *Chlamydomonas reinhardtii*, a group of eukaryotic organisms are important hydrogen producers. The efficiency of this organism for H₂ production with the appropriate design of photo bioreactor can be utilized for large-scale production of H₂ gas [39].

Production of secondary metabolites from algae: Algae and cyanobacteria possess the ability to transfer the solar energy into vital secondary metabolites like food, feed, medicines, hormones, biocides and polyhydroxyalkanoates (PHA) [40]. Secondary metabolites obtained from algae have several industrial, cosmetic and pharmaceutical uses. Microalgae have been extensively used as protein source replacing fishmeal and different microalgae have been used for rearing different fishes [41]. The production of secondary metabolites can be possible by producing huge biomass of algae. Genetically modified algal strains have been utilized for producing secondary metabolites as high biomass can be obtained. However, we need to concentrate on downstream processing involved in producing the secondary metabolites such that we can reduce the cost involved [42]. Sustainable polymers like PHAs and PHBs are produced from algal biomass that is used as biodegradable plastics. These polymers can be converted into nanomaterials and successfully used as tissue engineering scaffolds because of their nano-size level and biocompatibility.

Acid Mine Drainage (AMD) system

The high acidic content of the effluent water from the industries may result in the release of high acidic substances in the environment. These acidic substances have very detrimental effects on the environment and result in environmental pollution. It is essential to control their devastating effect on the environment. Acid mine drainage tends to contain significant quantities of metal ions dissolved in it like lead, iron, zinc, aluminum and tin. The quantity of the presence of metals in these areas is large and so is the time required for their removal, as a result, there is an alarming need for the removal of these metals from the site using a cost-effective robust technology [43]. The acidic contaminants present in this system dissolve metal ions resulting in their ionization and solubilization in the liquid medium. Therefore, an economical and eco-friendly procedure is essential for

the elimination of heavy metals from the pollution site. Many algal strains such as *Spirulina*, *Chlorella*, *Anabaena*, *Oscillatoria* have been exploited in acid mine drainage systems. Algae can sequester the metals based on their property of biosorption and high pH is created that eliminates metals from acid mine drainage areas. The algal mass can be dried to recover the metal oxides and algal biomass can be stored to be used as biofuels [44]. Recently, a potential technique has been developed to recover metals from the algal biomass and to utilize the resulting biomass for the production of biofuels through the technique of hydrothermal liquefaction (HTL) [43]. On the other hand, acid mine drainage has been used as an economical flocculant to harvest *Scenedesmus obliquus* and *Chlorella vulgaris* for other applications like biochar or biofertilizers.

Algae wheel for wastewater treatment

Algae wheel is a very upcoming and innovative technology that integrates the algal biofilms to the existing biological wastewater treatment plants. The thick algal mats formed on the surface of the wheel that rotates water in the wastewater treatment plant utilizes the photosynthetic ability of the algae to enhance the performance of the existing systems. The algae wheel technology employs the symbiotic relationship between algae and bacteria wherein the oxygen released during the photosynthesis process is used by the bacteria for better performance in this integrated system [45,46]. It is a very effective method for wastewater treatment and controlling water pollution. This technology for wastewater treatment has several advantages such as being simple, cost-effective, can endure flow rate variability, low energy requirement, less skilled personnel requirement and no noise or odour related to the system.

Microalgae in bioelectrochemical systems

Owing to the huge energy demands of the current scenario investigators are in constant need to find alternative energy resources which will be clean, renewable, cost-effective and environment friendly. MFCs are one such energy generating systems that fulfill all the above characteristics. The employment of algae in MFCs is facilitated by its ability to photosynthesize atmospheric CO₂ generating biomass and O₂ thereby accelerating cathodic reaction [47]. These MFCs are immensely useful for power generation, wastewater treatment, algae biomass cultivation, and oxygen production [47]. Different types of MFCs utilizing different types of microalgae are already been developed [48]. In MFC the energy generated is considerable but the current flow is too low so they are mostly used as part of the sensors [49]. Similarly, biophotovoltaics considered as "living solar panels" generate electricity utilizing the solar energy of the sun. They function similar to bioelectrochemical system converting photosynthesis of algae to generate electricity. These play a significant role in power generation. These can generate power during daylight and consume it during the night time [50].

Algal biofilms

Biofilms denote the group of microbes that stick to each other or on the surface of the material where the microbes are growing. The microbial cells get surrounded by the slimy extracellular substances that are secreted by the cells and are known as extracellular polymeric substances (EPS) composed of extracellular proteins, DNA and polysaccharides secreted by the cells. Algal biofilms refer to biofilms formed by algae that are found in areas which are exposed to sunlight, moisture and nutrients [20]. Algal biofilms are important for

wastewater remediation and as low-cost biomass harvesting techniques. Algal biofilms are significant due to nutrient removal from wastewater due to their enhanced nitrogen (N) and phosphorus (P) metabolism ability. Algal biomass is also utilized for bioproduct formation like food, feed, fuels, nutraceuticals, fertilizers and bioplastics. An important role of algal biofilm system is that it has potential applications in wastewater treatment and algae harvesting. It avoids expensive harvesting techniques used in suspension cultivation like centrifugation and flocculation [51].

Large-scale production of algae using efficient photobioreactors

Large-scale production of algal biomass has gained utmost importance due to environment cleaning purposes, valuable food, feed, fuel and bioactive compounds [52]. A large number of efficient photobioreactors have been proposed but the one with efficient mass transfer rates have been proposed that are very advantageous for mass cultivation of algae [53]. From the economic point of view open photobioreactors are preferred but due to limited control over the growth conditions and risk of contamination, closed photobioreactors are used. Closed photobioreactors have better control over growth conditions like CO₂, water, temperature, light, pH, aeration, size of inoculum etc. Closed photobioreactors are employed for large-scale production but light and photosynthetic efficiency are very crucial to maximize the production of algae. In case of photobioreactors designing, optical engineering ensure that light is appropriately collected, channelized and distributed in large-scale photobioreactor for enhanced functioning of photobioreactors [54].

The Potential Advantages of Phycoremediation Technology

1. Algae possess the ability to tackle different problems together which is not possible by conventional chemical treatment. For instance, bioremediation can be performed by algae and the biomass obtained can be utilized to solve the energy crisis and obtain different bioactive products in the environmental friendly manner.

2. Algae can be employed according to the need of the hour. It is possible to operate the algae-based photobioreactor system in case-specific manner in batch-mode, semi-continuous mode or in continuous mode.

3. Lots of commercially valuable products can be obtained from algal biomass in the form of active secondary metabolites such as astaxanthin, β -carotene, eicosahexaenoic acid.

4. Algal technology is a very robust system and does not interfere much with the other industrial operations. Proper nutrient, water and land are the primary requisites of the algal based system.

5. Phycoremediation technology is an economic technique and does not involve lots of harmful chemicals or energy for its industrial operation

6. CO₂ sequestration ability of algae is a very potential solution for the threat of global warming. The oxygen released in turn can be helpful in purifying the environment.

7. Phycoremediation technology can tolerate fluctuations in the quality and the quantity of the material fed to it like the wastewater with different amounts of impurities present in it.

8. Phycotechnological operations are comparatively simple and do not require skilled personnel for its operation.

9. The technique is specific and removes only the unwanted materials from the site of contamination without disturbing the environment because of its environment friendliness

10. Lastly, phycoremediation technology can be explored further to unravel more advantages of this technique so that it can be employed successfully at the industrial level.

Drawbacks of the Present Day Phycoremediation Technologies

The important drawbacks of the present day phycoremediation technologies are the space requirement for the growth of the algae and the speed of the operation of the system. Algal species are known for the production of the huge amount of bioactive compounds which can be transformed into large quantity of pharmaceutical products for human usage. However, due to the production of low biomass, the above is never accomplished. Accordingly, the large biomass of genetically modified strains should be obtained, where a huge amount of bioactive compounds can be expressed which will meet the demand of these products in the market. Algal products possess huge applications and are required in large scale but the short-comings lies in the inadequate amount of these products being manufactured in the algal cells. Therefore, the key lies in algal cells behaving as cell factories to create such compounds in large quantities [55]. However, in open systems genetically modified algae are not permitted to grow for bioremediation purposes. In an open system, the algal strain gets adapted to the environmental conditions and continues to grow profoundly.

There is often information about bacterial contamination issues related to algal biomass production from the plant personnel. Contamination may not be a problem where symbiotic relationship between bacteria and algae is required for bioremediation in wastewater treatment plants. In an effluent treatment plant, in the presence of sunlight algae will predominate and bacteria is important for facilitating harvesting and flocculation of the algae biomass. The contamination problem can be solved by either using mild concentrations of antibiotics which may be otherwise an expensive affair. This can also be solved by maintaining a complete sterilized condition in the photobioreactor where the algal strains are being grown. The production of algae in efficient photobioreactor utilizes a huge amount of input like a constant supply of CO₂ in the photobioreactor chamber which needs huge efforts and is expensive. Closed and efficient photobioreactors are required with effective light, temperature, CO₂ etc. for the algal cultivation. Additionally, downstream processing parameters are very expensive like harvesting and recovery of secondary metabolites synthesized in the algal cells.

Algal growth for clearing up wastewater requires a large amount of algae to be grown in the water bodies etc. This may generally destabilize the ecosystem if animals feed a large amount of algae growing in the water bodies and lead to the death of such animals. When algae are used in large areas for clearing up the soil the pace is relatively slow which occupies it for a long time and thus inhibits economic development in the area.

Measures Adopted to Improve the Technical Bottlenecks Related to Phycoremediation Technologies

Genetic engineering

Algae generally involve applications where a trait can be introduced into the parent strain so that we can have the desired characteristics. The important challenge is to isolate or develop a strain which can have useful characteristics. Biotechnology offers the possibility to develop genetically engineered strains where the target gene carrying the property of interest can be introduced into the algal cells which will express further to manifest the desired property in the target algal cells [56]. Genetic manipulation of the native algal cells is possible through mutagenesis or by genetic recombination approach. Selective breeding has been performed using physical mutagens, chemical mutagens or through the random and specific insertion of foreign DNA. The genetically transformed algal cells are also known as genetically modified organisms (GMOs) that will fasten the formation of the desired products [57].

Biorefinery concept

Algae is a very potent resource and to construct a competent biorefinery it is important that connections are established between algae and other industries. Various input and output products will be required to establish a well-organized biorefinery. Also, various energy and non-energy resources will be obtained using algal biomass as raw material as depicted in Figure 2 [58]. The protein portion of the algal biomass can be used to produce food and feed. The lipid part can be used to produce biodiesel, kerosene, methane and syngas. The carbohydrate part can be used to produce acids and alcohols. Moreover, the algal biomass can be utilized to produce valuable compounds like pigments/carotenoids, antioxidants, fatty acids (PUFA), vitamins and other pharmaceutical products. Algae have potential to reduce dependence on petroleum-based fuels and greenhouse gas emission. Economic and environmental stability can only be obtained if biofuels are produced along with production of value-added products [59].

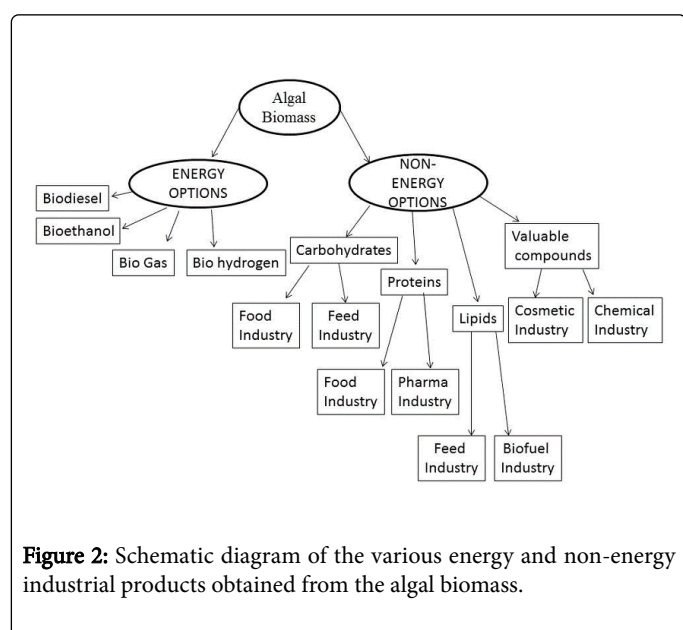


Figure 2: Schematic diagram of the various energy and non-energy industrial products obtained from the algal biomass.

Employment of an efficient photobioreactor system

The major drawback in the production of biofuels from the microalgae is the limited amount of algal biomass is produced. Microalgae is generally produced in open ponds but for better control of the physical parameters closed photobioreactor is considered. However, the usage of closed photobioreactor is expensive and consumes more energy. To overcome this issue semi continuous photobioreactor system is used [60]. An efficient photobioreactor system which can transport, distribute, capture and use light in the most efficient way should be employed for the development of algal biomass per area. The design of the photobioreactor should be such that sunlight is focused onto the photobioreactor, internally reflected using the glass and is eventually scattered in the photobioreactor chambers. Moreover, the bottom of the photobioreactor is sandblasted to obtain a uniform distribution of light in the entire photobioreactor. Every small precaution is taken to ensure uniform distribution of light in the photobioreactor system [61]. Among the various photobioreactor designs like H-shaped, X-shaped and serial column it has been found that the X-shaped airlift photobioreactor is most appropriate as it has the highest hydrodynamic properties for algal growth. Moreover, the biomass and lipid production in X-shaped airlift photobioreactor was considered more than in control photobioreactor [62]. For few photobioreactors which are lit up by artificial sources, wavelength of light being incident on the photobioreactor has to be considered for the optimum performance of photobioreactor for producing algal biomass [63].

Microalgal-bacterial aggregate system for wastewater treatment

Microalgal-bacterial aggregates (MABAs) have been successfully used for wastewater treatment from a long time due to less reaction time, easy nutrient uptake ability and easy resource recovery potential. However, the system is struggling with respect to the poor settlement of the algal biomass and harvesting problem. Thus, more emphasis should be provided to improving the harvesting potential and the resource recovery. Moreover, the microbiological aspects and the methanogenic potential of the MABAs should also be considered [64]. Formation and stability characteristics of MABAs under on-site conditions, effects of microalgae, bacteria and protozoa on the formation characteristics of MABAs should also be evaluated and the effects of the biomass generated in the process on the economic feasibility of the process should also be determined [64].

Continuous assessment and evaluation of the algal strains and their growth potential

In an open system continuous strain development is not required as the strains gets adapted to the environmental conditions and gets robust with time. However, in a photo bio-reactor based closed system, the algal strains used should be continuously evaluated for their growth potential in any growth experiment. The most important nutrient source used, the biomass, growth yield and growth kinetics should be evaluated continuously. The light energy absorbed by the cells. Genetically modified algal strains should also be used to compare them for their growth potential status. One should be able to constantly monitor the steps of development. There should always be human involvement.

Life cycle assessment of the algae

There has been a tremendous impact on the environment by the petroleum-based industries. Algae have been proposed as an alternative source of biofuels and many potential benefits of algae have been proposed over conventional fuel sources and other biofuel sources. Life cycle assessment studies are vital for establishing the environmental, economical and social performance of the system. Algae have been proved vital organisms for its application in different areas like food, feed, fuels, medicinal and cosmetics use [65]. Moreover, owing to the vast application of algae in different areas it is essential to perform its life cycle assessment.

Constant check on contamination

Algae have huge potential for technological advancement but the bulk production of algal biomass is hindered by contamination by the biological contaminants that inhibit the industrial process. Generally, algal culture is contaminated by bacteria and zooplankton so further research is imperative to understand the cohabitation of bacteria, zooplanktons and microalgae such that microalgae dominate [66]. Mostly algal contamination is caused by bacteria, zooplankton, other algae and virus. Among the zooplanktons mainly ciliates, cladophora and rotifers feed on microalgae in large-scale cultivation systems. Bacterial species like *Alteromonas* sp., *Flavobacterium* sp., *Bacillus* sp., and *Pseudomonas* sp., result in lysis of the microalgal cells [66]. The microalgae in the bioreactor can be contaminated by other microalgae due to direct cell contact or competition for nutrients. Viral species such as LPP virus and CCV virus were reported to infect cyanobacterial species and eukaryotic algae, respectively [66]. Such contamination by biological agents can be controlled by approaches such as filtration, utilizing chemical agents and altering culture conditions.

Even genetic engineering of the target algal strain is an important option wherein the target strain becomes resistant to the activity of the biological contaminants in the bioreactor. Developing a basic understanding of the interaction of the microalgal species with the contaminants may result in developing effective measures to control biological contaminants. Approaches such as developing genetically resistant algal species, specific inhibitor, altering contamination survival and continuously monitoring contamination presence in the system may lead to constant check on the contamination issue of the microalgal system such that they proliferate well in the bioreactor and the much sought after industrial development of phycotechnology can be visualized [66]. Moreover, it has been recently suggested that contamination due to microorganisms can be prevented by using the cationic biopolymer α -Poly-L-lysine (α -PLL). Due to the polymeric chain of this compound, harvesting was achieved quickly. Apart from efficient harvesting, the biological contamination of the algal biomass could be prevented because of the intrinsic anti-microbial activity of α -PLL. Thus α -PLL can be used to ease the harvesting and prevent contamination of the algal biomass [67].

Scope of Future Research in Phycoremediation Technology for About Two Decades from Now

Areas that require our focus in the near future is industrial wastewater treatment. Also, restoration of polluted water bodies like lakes, ponds, rivers for obtaining good quality water for human consumption. It is imperative that after the algal treatment water should be subjected to larvae toxicity such that after the treatment it

can be discharged safely to the water bodies in the open environment. Moreover, to develop the growth potential of algae, it is imperative to understand the pre-treatment, biosorption capacity, initial metal ion concentration, biomass concentration to develop engineered strains with better ion absorption and selection. This will lead to the development of inexpensive algal strains with improved potential towards bioremediation [68]. Growth optimization of *Spirulina* sp. has been done under different temperature and pH conditions and different media compositions [69].

1. Algae can be used as a convenient expression system for recombinant protein expression than compared to higher plants and animals. However, certain limitations are there which need to be addressed [70]. Since the last years, protocols are present for the genetic manipulation of the model organisms like the green algae *Chlamydomonas reinhardtii* and the diatom *Phaeodactylum tricorutum*. Recently, advancement has been made in the areas of algal transgenic expression, genome editing and identification of genetic elements in algal chromosomes. The above findings shall provide a basis to develop algal biotechnology [71].

2. Algae have been employed for the synthesis of silver nanoparticles with anti-fungal properties. Involvement of algae mediated synthesis enhances the biocompatibility of the silver nanoparticles for human usage. Further improvement can be done in this direction with respect to enhancing its biocompatibility and evaluating its anti-fungal activity against clinical pathogens that will help in providing a greater impetus to the work [72]. Algae have also been used for the synthesis of some metal oxide nanoparticles. The algae-based methods can significantly reduce environmental contamination and avoid the problems related to human health where toxic chemicals are employed for the synthesis of metal oxide nanoparticles [73]. Further research should be done in this area to improvise the work where algae are used for the synthesis of metal oxide nanoparticles.

3. There is a huge requirement to control on eutrophication level caused by the problematic algae *Ceratium hirudinella* so research attention is required in all aspects of this algae as well. There is a need for better understanding of eutrophication causing algae, their distribution, physiological properties, environmental role as well as the impact of eutrophication on the environment. There is huge need to evaluate the water quality and implement the standard methods for improving the quality of water. Also effective eutrophication management system within the area for reducing the eutrophication level in the community [74]. Effective training should be provided to the younger upcoming scientist and students for better understanding of the problem and to tackle it. There should be an assimilative approach to tackle the eutrophication problem by building a platform where new ideas, mechanisms, and methods should be continuously discussed and implemented to combat the problem of eutrophication and water management

4. Research on the coral reef, the threats and the problems and how to manage this fragile ecosystem [75].

5. Algae have an inherent ability to absorb CO₂ and thus decrease ocean acidification. More assessment should be done on ocean acidification as marine ecosystems have direct implications on human life. Human derives lots of advantages from the marine system and live them devastated. However, algal systems thrive on such ecosystems and decrease the acidification levels rejuvenating the marine ecology for the re-habitation of life. Since this area has a lot of economic utility

it much be considered an important area of research in the near future [76].

6. Energy and electricity generation from algae is a significant area as there is a huge burden on fossil fuel to meet the energy demand for petroleum-based vehicles. Algae to energy derived processes i.e., conversion of algae to biodiesel and bioelectricity for transport purposes and scope of further work. Both production and energy generated must be considered to come to an inference for the net energy generated from bioenergy and electricity production [77].

7. Algae as potent bioenergy resource must be compared to other bioenergy sources for harnessing maximum energy from the biological resources [78]. Though algae are significantly used for energy generation applications like biofuel production but the environmental impacts of such needs to be evaluated each time so that it may not adversely effect the environment in any way [79]. Wherever algae are to be used as a feedstock or for biofuel production it is very important to perform its life cycle assessment which will indicate whether the overall carbon or energy balance is favorable and will inform about the feasibility of the process [80]. For algae to be used economically for feed and biofuel resources it is imperative that the scope of future research should be on reducing the water, nutrient, energy and land usage for the algae-related production and processes [4].

8. Algae is a very good source of fuel and replacing it is not easy. However, one of its bottlenecks is economic feasibility from an industrial point of view (low biomass of algae, harvesting of biomass with high energy. We require high biomass and cost-effective low energy utilizing harvesting method. Therefore, advancement in downstream methodologies are required like state of the art harvesting methods, biorefinery ideas, futuristic photobioreactors for growing algae, will reduce the cost of algal production [81].

Conclusion

In the present scenario, huge challenges are imposed on phycoremediation technology, but we need to be optimistic to employ it for both remediation and industrial applications. Phycoremediation has proven to be a very beneficial method circumventing the problem of wastewater treatment utilizing expensive, environment degrading chemicals. Notwithstanding, phycoremediation technology involves challenges with respect to space requirement, nutrient requirement, algal strain requirement, and contamination from unwanted species. Further research is required for obtaining huge algal biomass such that it can be applied for large-scale industrial applications. There is a need for continuous assessment of algal based technology in industrial and academic settings such that there is more of awareness and discussions amongst industrialists, researchers and students so that this technique can be implemented in the most beneficial way for the overall development of mankind without harming the environment.

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