



## THE ROLE OF MICROALGAE IN TEXTILE DYE INDUSTRIAL WASTE WATER RECYCLE (PHYCOREMEDIATION)

S. ELUMALAI\* AND G. K. SARAVANAN

*\*PG and Research, Department of Biotechnology, University of Madras (Guindy Campus), Chennai, Tamil Nadu, India.  
PG and Research, Department of Plant Biology & Plant Biotechnology, Presidency College (Autonomous), Chennai, Tamil Nadu, India.*

### ABSTRACT

Many industrial areas in the world, show increases in generating of wastewater nowadays. Industrialization is considered as the key factor for the development of countries in economic terms. The recognition that environmental pollution is a worldwide threat to public health has given rise to new initiatives for environmental restoration for both economic and ecological reasons. The best methods to treat the textile dye industrial waste water by using a biological treatment method of cultivation of microalgae. Microalgae have gained a great deal of attention as remove contaminants from wastewater, potential biodiesel raw material because of their high growth rates and ability to accumulate oil and bind carbon dioxide. The normal primary and secondary treatment processes of these wastewaters have been introduced in a growing number of places, in order to eliminate the easily settled materials and to oxidize the organic material present in wastewater. Microalgal culture offers an interesting step for wastewater treatments, because they provide a tertiary biotreatment coupled with the production of potentially valuable biomass, which can be used for several purposes. The final result is a clear, apparently clean effluent which is discharged into natural water bodies. In the review, we have highlighted on the role of micro-algae in the treatment of wastewater and Biomass production. The biobased technology otherwise called phycoremediation of waste water.

**KEYWORDS:** Waste water, Microalgae and Biomass.



S. ELUMALAI

*\*PG and Research, Department of Biotechnology, University of Madras  
(Guindy Campus), Chennai, Tamil Nadu, India.*

## INTRODUCTION

The control of water pollution has become of increasing importance in recent years. The degradation of the environment due to the discharge of polluting wastewater from industrial sources is a real problem in several countries. There has been a dramatic increasing concern about environmental protection. Today, many industries in the world produce huge amount of wastewater from their processes such as; food industry, iron and steel industry, nuclear industry, olive oil mill industry and textile industry. This situation is even worse in developing countries like India, where little or no treatment is carried out before the discharge. However, discharge of a huge amount of contamination is produced from industrial processes<sup>1</sup>. The release of these contaminants in the environment from wastewater effluents have the potential health effects on humans and may also affect aquatic organisms in an unpredictable way<sup>2,3</sup>. Heavy metals have been considered as one of the most hazardous environmental pollutants<sup>4,5</sup>. Discharge of effluents containing heavy metals may affect all natural resources along with living organisms in the receiving water<sup>4,5,6,7</sup>. The risk of heavy metals in the environment is mostly due to their non-degradability, biological effects, and possible accumulation in the food chain<sup>6,7,8</sup>. Dyes are one of the most important groups of chemicals widely used in paper, textiles, pharmaceutical, rubber, plastics, leather, cosmetics, and food industries, in order to color products. Intensive research on effective water treatment has resulted in several technologies, which have been employed with varying degree of success for the removal of toxic pollutants from wastewater. The textile dyes are man – made organic compounds used to colour fabrics. Due to the escalation of fabric industries, the dyes have been and being contaminating the environment. Thus, the global textile industries are realizing the hamper situation against the production of high quality coloured fabrics along with eco-friendly manufacturing methods in order to withstand in their global business. Dyeing is the process of colouring the fabrics which needs more than thousand liters of water each day and which simultaneously paved the way for the redundant generation of environmental polluting waste water to drain in to the ecosystem and it needs an immediate action. The textile dyeing industries are the top polluters with very large quantity of useless effluent production from textile processing. There are several conventional and biological methods are available for the efficient and powerful treatment of waste water rather both of them have their own excellent as well as suppressed action due to characteristics of the dyes present in the waste water. Textile industries are found in most countries and their numbers have increased. These industries have shown a significant increase in the use of synthetic complex organic dyes as coloring material. The annual world production of textile is about 30 million tonnes requiring 700,000 tonnes of different dyes<sup>9</sup> which causes considerable environmental pollution problems. The textile waste water consists of untreated dyestuffs and other chemicals that are used in different stages of dyeing, fixing, washing and other processing. The textile waste water is characterized by high Chemical Oxygen Demand (COD), strong color,

high salinity, large amount of dissolved solids and suspended solids. The value of these parameters i.e., Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Total Suspended Solids (TSS), Total Dissolved Solids (TDS). The thin layer of discharged dyes, formed over the surface, decreases the amount of dissolved oxygen and photosynthetic activities due to reduced light penetration which badly affects the aquatic flora and fauna. Furthermore, most synthetic azo dyes are toxic, carcinogenic and mutagenic and pose a potential hazard to human health<sup>10</sup>. TDS is difficult to be treated with conventional treatment systems. The distribution of the world consumption of dyes. Dyes can be classified into different types depending on their chemical compositions and properties. Therefore, the usage of dyes varies from industry to industry depending on the fabrics they manufacture. The different types of dyes used in the textile industries are highly obvious even in a very low quantity present in effluent and which requires a strong remedial treatment before eluting in to the environment. The waste dye effluents released in to the aquatic ecosystem prevents the transmission of light which inhibit the proliferation of photosynthetically aquatic micro and macro organisms and elevate the levels of biological oxygen demand (BOD); both the factor directly and indirectly prevent the progression of aquatic zooplanktons and fishes<sup>11</sup>. According to a report by Jin,<sup>12</sup> about 2, 80,000 tons of textile effluents are generated annually worldwide. The textile industries alone engulf a major part of the economy of the developing countries. In India alone approximately 80 % of dyes are consumed which is the largest when compared to other countries and this was close to 80000 tons annually. When compared globally, India ranked second largest exporter of dyes next to China and approximately 10<sup>6</sup> tons of synthetic dyes are generated in a year, in which 1 to 1.5 × 10<sup>5</sup> tons of raw useless effluent are drained directly in to the environment as waste water<sup>9</sup>. The plethora amounts of waste water holds highly reactive dyes, though the dyes are not totally taken up by the fabric during dyeing process. Despite the various types of dyes used in the textile industries, the percentage intake of dyes in to the fabric may vary from 98 % to 50 % and rest of them are eluted in the form of waste water. Such highly reactive dyes results in the pollution of surface and ground water<sup>13</sup>. There are different classes of dyes manufactured globally for fabric dyeing which are azo, indigoid, xanthenes, phthalocyanine based, arylmethane and anthraquinone. Among them, the azo based dyes are the major choice employed in the textile industries. Some commercial choice of dyes are Acid Blue 120 (26400), Reactive Red 4 (18105) and Mordant Yellow 10 (14010). The azo dyes are the largely used dyes and implemented to generate 50 % of production every year<sup>9</sup>. Therefore, the textile effluent consists of large amounts of azo based dyes which are left out in to the environment<sup>14,15</sup>. The azo dyes can cause severe and proven health defects such as splenic sarcomas, hepatocarcinomas and bladder cancer with nuclear anomalies in animal models and chromosomal aberrations in cultured mammalian tissue. The existence of bladder cancer has been reported to occur in the labors working in the dye industries<sup>16</sup>. The characteristics of textile effluents vary and depend on the type of textile

manufactured and the chemicals used. The textile wastewater effluent contains high amounts of agents causing damage to the environment and human health, including suspended and dissolved solids, biological oxygen demand (BOD), chemical oxygen demand (COD), chemicals, odour and color. Most of the BOD/COD ratios are found to be around 1:4, indicating the presence of non-biodegradable substances. Typical characteristics of textile effluent the possible pollutant and the nature of effluents released from each step of the wet process. The textile effluents contain trace metals like Cr, As, Cu and Zn, which are capable of harming the environment. Dyes in water give out a bad color and can cause diseases like hemorrhage, ulceration of skin, nausea, severe irritation of skin and dermatitis. The proposed target of treating dye effluents should not only result to eradicate the colour of the effluents but also could detoxify the treated effluent. There are various technologies that were well developed for the treatment of synthetic dye effluent to minimize their environmental influence. The physical processes including membrane-filtration processes (nano-filtration, reverse osmosis electro-dialysis) and sorption techniques; the chemical processes including coagulation or flocculation along with floatation and filtration and precipitation flocculation with Fe (IT) / Ca(OH)<sub>2</sub>, electrokinetic coagulation, electro-floatation, conventional oxidation methods (e.g., with ozone), irradiation or electrochemical methods; and the biological processes which are aerobic and anaerobic methods of degradation and by using purified enzymes. The biological degradation of synthetic dyes from textile effluents is an arising and auspicious technology among other available techniques. The conventional methods of waste water treatment have shown retarded levels of remediation when compared with biological treatment. The enzyme production from microbes plays a key role against the degradation of synthetic dyes while bioremediation and should be studied much in the future for successive and eco-friendly approaches<sup>16,17</sup>. Wastewater treatment is an important measure to reduce the pollutant and other contaminants present in wastewater. The first step in wastewater treatment method is primary treatment which removes the solids, oil, and grease from wastewater. Secondary treatment or biological treatment is the second step, which exploits microorganisms to eliminate the chemicals present in wastewater. Final step is the tertiary treatment; which eliminates the heavy metals from wastewater before discharging into the river<sup>18</sup>. The effluent produced from the secondary treatment plant contains more amounts of nutrients (nitrogen and phosphorus) and if these effluents are discharged into water bodies; it causes eutrophication and affects the entire ecosystem. To remove these nutrients, several processes are used, but the disadvantages of this type of treatment are high cost and increased sludge production<sup>19</sup>.

## CONVENTIONAL TREATMENTS OF TEXTILE EFFLUENTS

In the wastewater treatment system, the removal of biochemical oxygen demand (BOD), suspended solids, nutrient coliform bacteria, and toxicity are the main goal

for getting purified wastewater. BOD exploits the ability of microorganisms to oxidize organic material to CO<sub>2</sub> and water using molecular oxygen as an oxidizing agent. Therefore, the BOD can deplete the dissolved oxygen of receiving water leading to fish kills and anaerobiosis, hence its removal is a primary aim of wastewater treatment. Suspended solids are removed principally by physical sedimentation. Wastewater treatment systems designed to remove nutrients, mainly dissolved nitrogen and phosphorus, are becoming an important step of treatment. Effluents discharged from the textile industries undergo various physico-chemical treatments such as flocculation, coagulation and ozonation and biological treatment for the removal of nitrogen, organics, phosphorus and metal removal. The disadvantages of the physico-chemical treatment process are: (a) the formation and disposal of sludge and (b) the required space. The disadvantages of biological treatment processes are: (a) the presence of toxic heavy metals in the effluent which affects the growth of microorganism (b) most of the dyes used are a non-biodegradable in nature and (c) the long time required for treating the effluent. Treatment of textile effluents involves three treatment processes: primary, secondary and tertiary treatments.<sup>20</sup>

## PRETREATMENT

The preliminary treatment of waste water removes larger solid materials delivered by sewers that could obstruct flow through the plant or damage equipment. These materials are composed of floating objects such as rags, wood, fecal material and heavier grit particles. Large floating objects can be removed by passing the wastewater through the bars spaced at 20–60 mm; the retained material is raked from the bars at regular intervals<sup>21</sup>. Grit is removed by reducing the flow velocity to a range at which grit and silt will settle, but leave organic matter in suspension; this is usually in the velocity range of 0.2–0.4 m/s<sup>22</sup>.

## PRIMARY TREATMENT

Primary treatment consists of temporarily holding the waste water in a quiescent basin where heavy solids can settle to the bottom while oil, grease and lighter solids float to the surface<sup>23</sup>. The first step in wastewater treatment is the removal of suspended solids, excessive quantities of oil and grease and gritty materials<sup>24</sup>. The effluent is first screened for coarse suspended materials such as yarns, lint, pieces of fabrics, fibers and rags using bar and fine screens<sup>25</sup>. The screened effluent then undergoes settling for the removal of the suspended particles. The floating particles are removed by mechanical scraping systems.

## SECONDARY TREATMENT

The Secondary treatment process is mainly carried out to reduce the BOD, phenol and oil contents in the wastewater and to control its color. This can be biologically done with the help of microorganisms under aerobic or anaerobic conditions. The secondary treatment removes dissolved and suspended biological

matter. Secondary treatment is typically performed by indigenous, waterborne microorganisms in a managed habitat. Secondary treatment may require a separation process to remove the microorganisms from the treated water prior to discharge or tertiary treatment. Wastewater treatment works use these same natural processes to break down and remove substances that might harm the environment, but speed them up within a controlled environment<sup>11</sup>.

## TERTIARY TREATMENT

Tertiary treatment process aims to remove all organic ions. It can be accomplished biologically using microalgae. The biological tertiary treatment process appears to perform well when compared to the chemical processes which are in general too costly to be implemented in most places and which may lead to secondary pollution. In addition, each additional treatment step in a wastewater system greatly increases the total cost<sup>26</sup>. A complete tertiary process aimed at removing ammonium, nitrate and phosphate are estimated to be about four times more expensive than primary treatment<sup>27</sup>. The final result is a clear, apparently clean effluent which is discharged into natural water bodies.

## BIOLOGICAL PROCESS OF WASTE WATER TREATMENT

The highlighted disadvantages in the physio-chemical process of waste water treatment are commercially high cost, minimum efficiency, requires specialized equipments, and handling of waste generated after the treatment<sup>28,29</sup>. The biological process of waste water treatment can overcome all the above said disadvantages and are considered as eco-friendly, because such process can completely mineralize all the organic pollutants such as P (Phosphorous) and N (Nitrogen)<sup>30</sup>. The microbial degradation of azo dyes involves two different mechanisms of action which are Biosorption and Enzymatic degradation or both combines to act<sup>31</sup>.

## BIOSORPTION

The biosorption is a process of electrostatic affinity between different functional groups such as amino, carboxyl, hydroxyl, phosphate and other charged groups of polysaccharides and lipid components of the cell wall and the azo dyes<sup>32</sup>. The microorganisms like bacteria, yeast, fungi and algae are using the biosorption process for dye degradation including macro algae<sup>33</sup>.

## ENZYMATIC DEGRADATION

The azoreductase is a significant enzyme involved in the enzymatic degradation of azo dyes to break the bond of azo linkage<sup>34</sup>. The azo dyes have azo linkage (-NN) and sulphonic (SO<sub>3</sub><sup>-</sup>) which makes the azo dyes an electron deficient and less reactive against microbial degradation<sup>35</sup>. During such conditions, the microorganisms synthesize enzyme reductases during degradation of

azo dyes<sup>36</sup>. The production of azoreductase are grouped by various types of genes and associated with more than one reductase which depends on the microorganism and culture conditions<sup>37</sup>. Rather than azoreductase, many other types of enzymes also involved in the cleavage of azo linkages which are NADPH-dependent reductases<sup>38</sup>, NADH-dependent reductases<sup>39</sup>, NADH-DCIP reductases<sup>40</sup>, FMN-dependent reductases<sup>41</sup> and FMN-independent reductases<sup>42</sup>. The syntheses of the azoreductases have been reported in many groups of microbes including yeast<sup>43</sup>, bacteria and algae<sup>44,45</sup> respectively.

## MICROORGANISMS USED FOR AZO DYE EFFLUENT TREATMENT

### *Bacteria*

Most frequently, bacteria are the applied microorganisms used to treat waste water rich in azo dyes due to rapid growth, cheap labour and most importantly grow in aerobic and anaerobic conditions even as facultative<sup>46</sup>. The most advantageous features are the bacteria can survive in extreme conditions of temperature, salinity due to the production of various Oxidoreductases. Some of the Oxidoreductases are laccase, tyrosinase and veratryl alcohol peroxidase.

### *Fungi*

The mycelious fungi implemented in the bioremediation of azo dyes is an efficient process with low cost, survival of their metabolism towards many different carbon and nitrogen sources and total mineralization of the dyes<sup>47</sup>. As micro algae and yeast, fungi also use either adsorption or enzymatic degradation to degrade the dye. The electrostatic attraction caused due to lowering the pH between 2 to 3 and which induces the adsorption on the cell surfaces of fungi against the dyes<sup>48</sup>. Dye adsorption is dependent on the pH, Temperature, Dye concentration, Carbon and Nitrogen supplies, additives and salts<sup>49</sup>. Enzymatic degradation includes enzymes such as peroxidases and phenloxidases<sup>50</sup>.

### *Yeast*

The huge absorption of dyes and heavy metals makes the yeast a good candidature for bioremediation of azo dyes<sup>51</sup>. Nor like fungi, the yeast can decolourize the dyes faster from the effluents and can survive even in unsupported conditions<sup>52</sup>. The enzymatic degradation and adsorption or the combinations of both are the mechanisms involved in the removal of azo dyes by both yeast and micro algae. The two azo dyes Remazol Blue and Reactive Red can be effectively removed up to 94 % and 44 % respectively by using *Candida tropicalis*<sup>8</sup>.

### *Algae*

The coloured azo dyes occurs in the aquatic ecosystem can cause severe toxic effect to the aquatic life forms but it will not affects the algal forms which can be proliferate in the eutrophicated conditions. The growth of photosynthetic algae can be seen even in the industrial effluents and therefore they are the potential candidate for waste water treatment<sup>53</sup>. The algae are photoautotrophs and they do not require carbon source

despite from the atmospheric air for the waste water treatment but in the case of bacteria and fungi it is reciprocal. Some of the diazotrophs (filamentous Cyanobacteria) can fix atmospheric nitrogen and thus the utilization of algae for waste water treatment is an efficient and cheap cost process<sup>54</sup>.

### Consortia of microorganisms

The presence of many different compounds of chemical in the dye effluents may affect the microbial degradation of a single species of microorganisms and thus using different groups of microorganisms as a consortium can be efficient. The microbial consortia are a mixed group of microbes involve many different mechanisms and even various types of enzymes to degrade the dye components completely<sup>55</sup>. The achievement of this method are the degradation of azo dyes using different microbes which degrade using different mechanisms at different positions or levels to mineralize azo dyes<sup>50</sup>. For instance, the bacteria *Bacillus cereus*, *Pseudomonas putida*, *Stenotrophomonas acidaminiphila* and *Pseudomonas fluorescens* as a consortium degrades the dyes up to three fold increase when compared with single microbe treatment<sup>57</sup>. The *Exiguobacterium* sp. is a bacterial strain with least effective in the degradation of azo dyes and the fungal strain *Penicillium* sp. is much effective when compared to the bacterial strain; but as in consortium both the strains more efficiently degrades the azo dyes comparatively with single strain treatment<sup>58</sup>.

## PHYCOREMEDIATION

Phycoremediation may be defined in a broad sense as the use of macroalgae or microalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO<sub>2</sub> from waste air with concomitant biomass propagation. There are numerous processes of treating water, industrial effluents and solid wastes using microalgae aerobically as well as anaerobically. Remediation is generally subject to an array of regulatory requirements, and also can be based on assessments of human health and ecological risks where no legislative standards exist. As an alternative to the conventional treatment methods, microalgae are suggested to remove the nutrients from wastewater<sup>1</sup>. The use of microalgae or macroalgae (seaweeds) to remove pollutants and nutrients from the wastewater is called phycoremediation. Microalgae wastewater treatment is Eco friendly and offers the

advantage of a cost effective way of nutrient removal and biomass production<sup>59</sup>. The microalgae grown in wastewater can be used as an energy source, fertilizer and fine chemicals production and as feed to animals<sup>60,61</sup>.

### Microalgae used in phycoremediation

Algae are phototrophic organisms of a wide diversity; over 30 thousand species are identified, and it is assumed that the actual number is substantially larger. Algae are found as eukaryotes in the kingdoms of Protozoa, Fungi, Chromista and Plantae<sup>62</sup>. Microalgae are unicellular or filamentous phototrophic organisms originating from a eukaryotic heterotrophy engulfing a cyanobacterium. The organism thus gained the ability of photosynthesis and these cells later evolved into plants<sup>63</sup>. Microalgae contain different pigments which make them green, red, brown or golden colored, and are commonly divided into groups according to these colors<sup>64</sup>. Green algae exist in freshwater, saline water or in soil or lichens<sup>49</sup>. The microalgae included in this study are all green algae, also called chlorophytes, which are eukaryotic organisms of the plant kingdom<sup>62</sup>. The most important common feature of all eukaryotic microalgae, and cyanobacteria is that they have oxygen-evolving photosynthesis and that they use inorganic nutrients and carbon. Microalgae are one of the most important Bioresources that are currently receiving a lot of attention due to a multiplicity of reasons. The growth of microalgae is indicative of water pollution since they respond typically too many ions and toxins. Blue-green algae are ideally suited to play a dual role of treating wastewater in the process of effective utilization of different constituents essential for growth leading to enhanced biomass production. Many species of microalgae are able to effectively grow in wastewater conditions through their ability to utilize abundant organic carbon and inorganic N and P in the wastewater. The use of microalgae in wastewater treatment has been long promoted<sup>65</sup>, however, chemical processing of waste or the generation of activated sludge is the conventional treatment method. Although the application of microalgae in the wastewater industry is still fairly limited, algae are used throughout the world for wastewater treatment albeit on a relatively minor scale. Successful treatment of wastewater with microalgae requires good growth, and understanding of the factors that affect growth is therefore essential. The growth rate of algae and cyanobacteria is influenced by physical, chemical and biological factors.

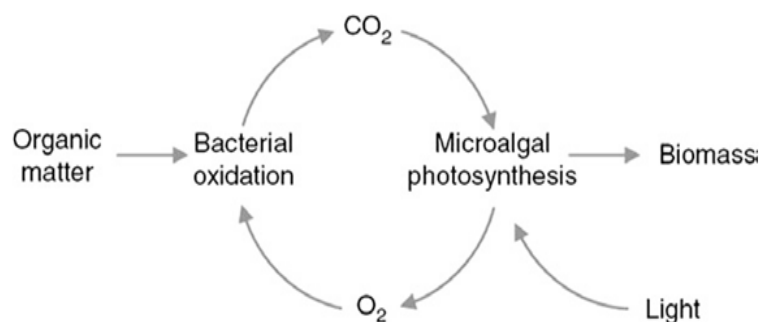


Figure 1  
Principle of photosynthetic oxygenation in BOD removal process

The use of microalgae is desirable since they are able to serve a dual role of bioremediation of wastewater as well as generating biomass for biofuels production with concomitant carbon dioxide sequestration. In addition, wastewater remediation by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling. Microalgae have excellent heavy metal scavenging property besides being cost effective and easy to handle<sup>66-72</sup>.

### **Multiple Roles of Microalgae**

There is several utilization of algae or microalgae by which we can sort out the environment problems like; the major problem of global warming is CO<sub>2</sub> in the atmosphere which creates green house effects, so far the growth of microalgae the utilization of CO<sub>2</sub> is very essential, and to generate around 1 kg algal biomass requires 1kg of CO<sub>2</sub> which is better to sort out this problem, it can reduce many heavy or toxic metals from waste water and this process called Phycoremediation. It can be used as biofuel to reduce the effect of our conventional fuel, which is going to be finish day by day because the algae have the potential to produce biofuel in the form of lipid which is further processed by the transesterification process get the biodiesel, which has the properties same as the our conventional diesel and it can be used for the many cosmetics, food and man use in the field of pharmaceuticals.

### **Role of algal photosynthesis in phycoremediation**

Photosynthesis is the most basic and important way in which living organisms obtain their energy and nutrients, directly and indirectly. Algal photosynthesis is a unique process by which solar energy is converted into chemical energy that is stored in organic carbon matter through the cycling of atmospheric CO<sub>2</sub>. Photosynthesis takes place in specialized organelles called chloroplasts of eukaryotic species, and also in a membrane-bound sac known as a thylakoid of the *cyanobacterium* due to lack of defined chloroplast structure in the prokaryotic organism. Photosynthetic aeration is therefore especially interesting to reduce operation costs and limit the risks for pollutant volatilization under mechanical aeration and recent studies have shown that microalgae can indeed support the aerobic degradation of various hazardous contaminants<sup>73,74</sup> (Fig. 1). Phycoremediation is the process of employing algae for improving water quality. Micro algae are superior in remediation processes as a wide range of toxic and other wastes can be treated with algae and they are non pathogenic. Algae can fix carbon dioxide by photosynthesis and remove excess nutrients effectively at minimal cost. The risk of accidental release of pollutants into the atmosphere causing health safety and environmental problems are avoided when algae are employed for remediation. Algae utilize the wastes as nutritional sources and enzymatically degrade the pollutants. The xenobiotics and heavy metals are known to be detoxified/ transformed/or volatilized by algal metabolism. They have the ability to take up various kinds of nutrients like nitrogen and phosphorus<sup>75</sup>. They can utilize various organic compounds containing nitrogen and phosphorus from their carbon sources. The mechanisms involved in microalgae nutrient removal from industrial wastewaters are similar to that from

domestic wastewaters treatment. Wastewater remediation by microalgae is an eco-friendly process with no secondary pollution as long as the biomass produced is reused and allows efficient nutrient recycling. Many researchers have studied micro algae as possible solution for environmental problems<sup>76-77</sup>. Algal growth can keep the water clean and make natural waters more suitable for human consumption.

### **Reduction of both chemical and biochemical oxygen demand**

As mentioned before, there are many compounds and microorganisms could be detected in wastewater, which is capable of causing the pollution of a water-course. Pollution of wastewater may be manifested in three broad categories, namely organic materials, inorganic materials in addition to microbial contents. The organic compounds in wastewater comprise a large number of compounds, which all have at least one carbon atom. These carbon atoms may be oxidized both chemically and biologically to yield carbon dioxide. If biological oxidation is employed the test is termed the Biochemical Oxygen Demand (BOD), whereas for chemical oxidation, the test is termed Chemical Oxygen Demand (COD). Colak and Kaya<sup>78</sup> investigated the possibilities of biological wastewater treatment by algae. They found that, in dye industrial wastewater treatment, elimination of BOD and COD were 68.4% and 67.2%, respectively.

### **Removal of Nitrogen and Phosphorus**

The bio-treatment of wastewater with algae to remove nutrients such as nitrogen and phosphorus and to provide oxygen for aerobic bacteria was proposed over 50 years ago by Oswald and Gotaas<sup>65</sup>. Wastewater is mainly treated by aerobic or anaerobic biological degradation; however, the treated water still contains inorganic compounds such as nitrate, ammonium and phosphate ions, which leads to eutrophication in lakes and cause harmful microalgal blooms<sup>79</sup>. Microalgal culture offers a cost-effective approach to removing nutrients from wastewater (tertiary wastewater treatment)<sup>80</sup>. Microalgae have a high capacity for inorganic nutrient uptake<sup>81-82</sup> and they can be grown in mass culture in outdoor solar bio-reactors<sup>27</sup>. The interest in microalgal cultures stems from the fact that conventional treatment processes suffer from some important disadvantages: (a) variable efficiency depending upon the nutrient to be removed; (b) costly to operate; (c) the chemical processes often lead to secondary pollution; and (d) loss of valuable potential nutrients (N, P)<sup>27</sup>.

### **Heavy metals removal from wastewater**

Microalgae are known to sequester heavy metals<sup>83</sup>. Discharge of toxic pollutants to wastewater collection systems has increased concurrently with society's progressive industrialization. Microalgae are efficient absorbers of heavy metals. Bioaccumulation of metals by algae may create a feasible method to remediate wastewater, contaminated with metals<sup>84,85</sup>. On the other hand advantages of algae are that it may be grown in ponds with little nutritional input or maintenance.

### **Advantages of phycoremediation**

Microalgae play an important role during the tertiary treatment of Textile effluent wastewater in maturation

ponds or the treatment of small to middle-scale textile dye effluent wastewater in facultative or aerobic ponds. Nitrogen uptake could be increased if the microalgae were preconditioned by starvation<sup>27,86-89</sup>. These hyper concentrated algal cultures, called 'activated algae' were shown to decrease the land and space requirements for microalgal treatment of wastewaters. This process removed nitrogen and phosphorus within very short period of time, i.e., less than 1 h<sup>90</sup>. Microalgae can be efficiently used to remove significant amounts of nutrients because they require high amounts of nitrogen and phosphorus for protein (45–60% of microalgae dry weight), nucleic acid and phospholipid synthesis. Nutrient removal can also be further increased by NH<sub>3</sub> stripping or NH<sub>3</sub> precipitation due to the raise in the pH associated with photosynthesis<sup>91-94</sup>. This method is not appropriate for large scale wastewater treatment, therefore there is a need to improve the technology.

#### **Advantages of high rate algal pond treatment**

HRAPs are the most cost-effective reactors for liquid waste management and capture of solar energy, and are used to treat waste from pig farms. Algal biomass can be harvested from the HRAPs for animal feed, and can be seen as a component of integrated approaches to recycling of livestock wastes, in which algal wastewater treatment is a second step following an initial anaerobic treatment of high-strength organic wastewater<sup>68,25</sup>. Biofuel production in conjunction with wastewater treatment and fertilizer recycling is seen as a near-term application (5–10 years), since the algae are already used in wastewater treatment<sup>95</sup>. The HRAP showed remarkable performance and behaviour when compared to a series of three facultative ponds such as anaerobic, aerobic and alternative constructed facultative ponds on the same site by receiving the same sewage<sup>96,31</sup>. The HRAP is a photosynthetic reactor in which microscopic, photosynthetic algae live together with heterotrophic bacteria which degrade the sewage organic matter. This co-habitation was often called the HRAP symbiosis and represents the central idea of Oswald's concept using the HRAP as a combined secondary/tertiary system for sewage treatment.

#### **Factors affecting microbial degradation**

The dye degradation depends on the azo dye molecular structure, salinity, pH, temperature, concentration of the dye.

#### **Molecular structure of azo dye**

The molecular structure of the azo dye enhances the enzymatic degradation due to the presence of high electronic groups SO<sub>3</sub><sup>-</sup> and is easier in the presence of electron-releasing groups like –NH-triazine<sup>79</sup>. The azo dyes with electron-releasing groups revealed to show quicker degradation due to electrophilic nature<sup>97</sup>. Therefore, the use of microorganisms which produces extracellular oxidases and reductases can completely degrade the azo dyes and detoxify the contaminated water in textile industries<sup>46</sup>.

**Carbon and nitrogen sources** The azo dyes have much less concentration of carbon and for its degradation external carbon source is much required. In the case of micro algae, the carbon source is less

required or not required because they can fix atmospheric CO<sub>2</sub> as their sole carbon source. But in consortium, external carbon source yield high percentage degradation of azo dyes. Some of the azo dyes are rich in nitrogen source which can be utilized by the micro algae for example, *Oscillatoria curviceps* uptakes nitrogen from Acid Black<sup>98</sup>.

#### **Salinity**

Increase in salt concentration above 3000 ppm inhibits the degradation of azo dyes by micro algae but the marine micro algal forms can efficiently degrade the azo dyes. Some of the micro algae can survive in high concentrations of sodium or calcium carbonate which may induce the micro algae as an alternate source of carbon.

#### **Dye concentration**

The dye concentration can affect the process of degradation due to the eutrophication of the culture conditions, prevention of light transfer in to water and changes in pH etc. For example, the growth rate of the micro alga *Scenedesmus quadricauda* was decreased by increasing the dye concentration<sup>99</sup>. Especially, the prevention of light transfer can be solved by the diazotrophs. Because, the diazotrophs produce air vesicles thus floats on the surface of water to trap enough light.

#### **pH**

In algal cultures, pH usually increases due to the photosynthetic CO<sub>2</sub> assimilation. In high rate algal ponds, this pH increase can be compensated by respiration deeper in the ponds, and the pH can then be regulated by letting in more organic material and thereby enhancing the respiration. High pH may also induce flocculation of some algae, which in turn lead to reduced nutrient uptake and growth, but this flocculation can, on the other hand, facilitate harvesting<sup>100-101</sup>.

#### **Temperature**

The temperature can prevent the proliferation of microbes during effluent treatment and degradation of azo dyes. The temperature may affect the biosorption as well as enzymatic degradation because oxidases and reductases require an optimum temperature for their activity and increase in temperature may affect the stability of the enzymes. Temperature can affect the intake of phosphorous in micro algae by ionic speciation of phosphate<sup>102</sup>.

#### **Oxygen**

The presence of oxygen favours or either inhibits the degradation of azo dyes. The enzymes need optimum levels of oxygen concentration for their activity and which may also requires for the microbes for phosphorylation. At the same time increased concentration of oxygen may leads to generation of oxidative free radicals.

#### **Light intensity**

High intensity of light during incubation of waste water treatment can enhance the phosphate accumulation in micro algae and the rate of accumulation can be faster.

But in low light conditions the rate was retarded due to relatively slow growth of the micro algae<sup>103</sup>.

### **Biomass production – from wastewater effluent**

The mass production of algae has historically been in use as a food supplement or wastewater treatment<sup>12</sup>. The technology for production of biomass from wastewater has been present since the 1950s. Microalgae are efficient in the removal of nutrients from wastewater. Thus, many microalgae species proliferate in wastewater due to the abundance of carbon, nitrogen and phosphorus that act as nutrients for the algae. Unicellular algae have shown great efficiency in the uptake of nutrients and have been found to show dominance in oxidation ponds<sup>104</sup>. Application of using wastewater for the production of biomass, however, occurs only on a minor scale and generally in the form of waste stabilization ponds or high rate algal ponds for the treatment of wastewater<sup>98,55</sup>. It has been suggested that growth of algae should focus on biomass productivity rather than lipid productivity as is the current thrust in the algal biofuels sector<sup>105</sup>. Large amounts of biomass produced may improve the viability on conversion of biomass to alternate biofuels<sup>98</sup>. Laboratory studies have shown the potential for moderate lipid production (less than 10–30% DW) and high lipid productivity (up to 505 mg L<sup>-1</sup> day<sup>-1</sup>) of algae grown in wastewater. This suggests the potential of lowering the cost of algal biofuels production, which is currently not economically feasible.

### **Harvesting strategies**

Effective wastewater treatment and the production of biomass for biofuels require separation of biomass from water. The selection of harvesting method is of great importance to the economics of biofuels production as harvesting can make up 20–30% of the total cost of production. Selection of the harvesting method depends strongly upon the characteristics of the culture grown<sup>14</sup>. Algae that are suitable for the remediation of wastewater and production of biofuels tend to be of unicellular forms of low density. This makes economical biomass harvesting difficult and the cost of biomass recovery significant<sup>147,104,68</sup>. Methods of biomass recovery include filtration, centrifugation, sedimentation and flocculation and floatation<sup>107</sup>. Continuous centrifugation is the preferred method for biomass separation as it is rapid, efficient and universal<sup>107</sup>. This is not economically feasible for large scale harvesting due to its process being highly energy intensive<sup>104</sup>. Gravity sedimentation is a common method of harvesting biomass.

### **Lipid Extraction Method**

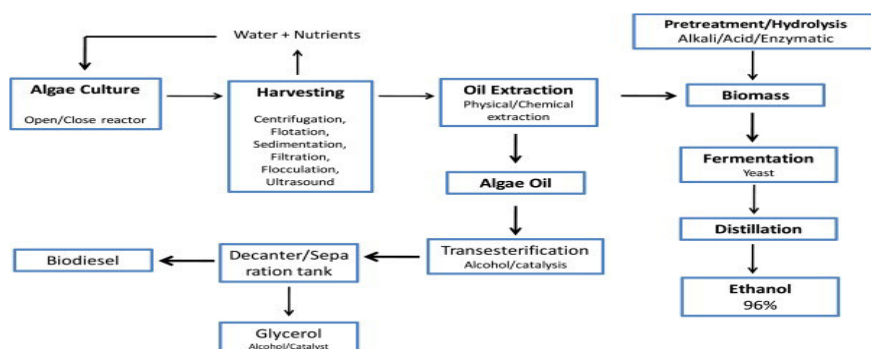
Extraction of microalgal lipid is central to the production of biodiesel from microalgae. Lipid extraction is performed by chemical methods in the form of solvent extractions, physical methods or a combination of the two. Extraction methods used should be fast, effective and non-damaging to lipids extracted and easily scaled up<sup>109</sup>. Extraction by using a modified Bligh and Dyer<sup>108</sup> method is the most commonly used<sup>107</sup>. Direct esterification, simultaneous extraction and transesterification of microalgal fatty acids can be performed on both wet and dry biomass making it a versatile method of biofuels production. The process is multistep and requires a combination of solvent extraction, ultrasonication, heating at high pressure (3.5 atm), filtration, density separation of liquids and solvent and oil recovery by evaporation to dryness<sup>110</sup>.

### **Lipid identification methods**

Algae storage lipids differ from strain to strain and even within a single culture under different growth conditions. It is necessary to identify lipids as the lipid fraction will dictate the properties of the biodiesel produced. Lipid qualification and quantification can be carried out by several means, including Nile red fluorescence microscopy, Nile red spectrofluorometry, Fourier transform infrared micro-spectroscopy (FTIR), Thin-layer chromatography (TLC), high pressure liquid chromatography (HPLC) or gas chromatography (GC) or any chromatography with mass spectrometry<sup>107,109</sup>.

### **Biorefinery approach and other biofuels**

The economics of biodiesel production can be significantly improved by using the biorefinery based production strategy where all the components of the biomass raw material are used to produce useful products<sup>68,10</sup>. Furthermore, it is recommended that a biorefinery approach is the best solution to combine and integrate the various processes to maximize economic and environmental benefits, while minimizing waste and pollution<sup>10,111</sup>. Despite the salient drawbacks of biofuels production from microalgae, various processes can be used to convert biomass to energy (Fig. 2). However, the spent biomass can be used as animal feed since it is rich in proteins, carbohydrates and other nutrients. Essentially, the biomass can be burned, transformed into a fuel gas through partial combustion, into a biogas through fermentation, into bioalcohol through biochemical processes, into biodiesel, into a bio-oil or into a syngas from which chemicals and fuels can be synthesized<sup>111</sup>.



**Figure 2**  
**Algae biofuels production approach. Biodiesel and bioethanol can be produced from microalgal biomass.**



**Bioethanol**

Generally, two methods are employed for the production of bioethanol from microalgal biomass, namely the fermentation (biochemical process) and gasification (thermo-chemical process)<sup>10</sup>. Microalgae are rich in carbohydrates and proteins which can be used as carbon sources for fermentation, therefore microalgae compete favourably with biomass derived from food crops such as sugar cane and maize. There is a moratorium on the use of food crops for the production of bioethanol in some countries due to issues pertaining to food security and agricultural land availability. Therefore microalgae are generating a lot of interest as biomass feedstock for bioethanol production<sup>11,12</sup>. Fermentation of the microalgal biomass is catalyzed by microbes such as bacteria, yeast and fungi and the main by-products are CO<sub>2</sub> and water. The spent biomass after fermentation is used in an anaerobic digestion process for methane, production so in essence, all the organic matter is accounted for<sup>10,11,12</sup>. However, research on bioethanol production from microalgal biomass is still in infancy and not yet commercialized, and to date work is ongoing to optimize conditions for improved bioethanol yield.

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**CONCLUSION**

Currently, the textile dyeing wastewater is one of the most important Root cause of sources of pollution. Many industries are producing non degradable pollutants and large scale production of wastewater. Each one has its own way of method in treatment by using microalgae. Microalgae can be used in wastewater treatment for a range of purposes, including; reduction of BOD, removal of N and P, inhibition of coliforms, removal of heavy metals. The high concentration of N and P in most wastewaters also means these wastewaters may possibly be used as cheap nutrient sources for algal biomass production. This microalgal biomass could be used for Biodiesel, Bioethanol, methane production, production of liquid fuels (pseudo-vegetable fuels), as animal feed or in aquaculture and production of fine chemicals.

**CONFLICT OF INTEREST**

Conflict of interest declared none.

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