



Use of microalgae for the removal of environmental pollutants

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Abstract

In this mini review it is to focus that how can pollutants be removed from the environment by utilizing microalgae which are deliberately increasing and causing hazardous effects to our environment. Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and high-value bioactives. Various types of pollutants continuously causing damage to the environment and after a long-term observation it is found that there is a best option of using microalgae in different techniques for reducing environmental pollutants. Since, versatile species of microalgae has been a part in reduction and removal of environmental pollutants as we observed in different bioremedial techniques such as in waste water treatment plants, heavy metal removal techniques, bio-degradation of azo-dyes, phenol and other organic aromatic compounds which are dangerous to the environment. It is reappraised that one of microalgae specie which is named as *Chlorella vulgaris* is found to be very effective in removing of heavy metals, waste water treatment and also in biodegradation of azo-dyes. This article basically explained the usefulness of using microalgae for the remediation of pollutants.

Keywords: *Microalgae; Pollution; Heavy Metal; Azo-Dyes; Biosorption; Microbial Consortium.*

1. Introduction

Pollution is the introduction of contaminants into the natural environment that causes adverse change, It is often classified as point source or nonpoint source pollution and release into the environment from both sources of pollution either point or non-point or both. A pollutant is a waste material that pollutes air, water or soil. Three factors determine the severity of a pollutant: its chemical nature, the concentration and the persistence. Through phytoremediation environmental pollutants can be removed, especially by using microalgae [61], [30], [3]. The algae also make large amounts of carbohydrates, which can be converted into bioethanol to fuel vehicles. The findings could have industrial applications as a cost-effective way to cut greenhouse gas pollution when paired with biofuel production. Although microalgae are not unique in their bioremoval capabilities, they offer advantages over other biological materials in some conceptual bioremoval process schemes. Selected microalgae strains, purposefully cultivated and processed for specific bioremoval applications, have the potential to provide significant improvements in dealing with the world-wide problems of metal pollution. Micro algal removal of environmental pollutants is an effective and cheapest option to get the goal of having clean environment.

Microalgae are also called Microphytes. It is found in freshwater and marine systems. They are unicellular species which exist individually, or in a chains or groups, depending on their species, their sizes can range from a few micrometers (μm) to a few hundreds of micrometers. They produce approximately half of the atmospheric oxygen [59]. They can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner [64]. It has been estimated that about 200,000-800,000 species exist of which about 50,000 species are described in which most of the species produce unique products like carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins and sterols [63]. Microalgae are used extensively in the treatment of waste waters because they can sequester large amounts of nutrients and other pollutants. At present, there are many municipal and industrial waste treatment plants that use microalgae in oxidation ponds to biodegrade wastes [35], [40] However, there is no control over the algal species and, with few exceptions, no harvesting of the algal biomass generated in these ponds.

Furthermore, there is now a commercial use of micro algal based ion exchange systems for the recovery of selected heavy metals and microalgae are a potential source of a very large variety of other high value products (bioactive) such as special chemicals, animal feeds, fuels, lubricants, antibiotics and enzyme inhibitors [63]. The microalgae species mostly used for biodiesel production are presented and their main advantages described in comparison with other available biodiesel feed stocks. Other potential applications and products from microalgae are also presented such as for biological sequestration of CO₂, wastewater treatment, in human health, as food additive, and for aquaculture [58].

2. Different species of microalgae

There are enormous genera found of microalgae such as Chlorella, Tetraselmis, Spirulina and Dunaliella, these species include Chlorella minutissima, Chlorococcum pinguidum, Chlamidomonos reinhardi, Tetraselmis cordiformis, Chlorella pyrenoidosa etc. Some of the characteristics of these species are described below.

- Chlorella: Species of chlorella can be grown under 20% CO₂ conditions [8]. Chlorella is a genus of single-cell green algae, belonging to the phylum Chlorophyta. It is spherical in shape, about 2 to 10µm in diameter, and is without flagella [51].
- Tetraselmis: It is a genus of phytoplankton. It has a very high lipid level, & is green, motile, and usually grows 10 µm long x 14 µm wide [52].
- Dunaliella: CO₂ tolerance of Dunaliella sp. also has been examined and the species has been used in the industrial production of b-carotene [52].
- Spirulina: It is a cyanobacterium that can be consumed by humans and animals and is made primarily from two species of cyanobacteria, Arthrospira platensis and Arthrospira maxima. It is used as a dietary supplement as well as a whole food; and is available in tablet & powder form. Another potential strategy to offset operational costs is to develop multi-functional systems such as waste treatment [46].

Table 1: Illustrates Some Species of Microalgae Which Are Used for Remove the Environmental Pollutants

Species	Pollutants	References
Chlorella vulgaris	Heavy metal removal, Waste water treatment & degradation of azo dyes	[61], [30], [32]
Scenedesmus acutus	Heavy metal removal	[61]
Nostoc muscorum	Heavy metal removal	[55]
Anabaena subcylindrica	Heavy metal removal	[55]
Chlorella sorokiniana	Waste water treatment	[30]
Euglena viridis	Waste water treatment	[19]
Ochromonas danica	Biodegradation of Phenol, Benzene & naphthalene	[16], [53]
Chlorella pyrenoidosa	Waste water treatment	[7]
Scenedesmus obliquus	Waste water treatment	[19]
Lyngbya lagerlerimi	Biodegradation of Phenol	[3]
Chlorella pyrenoidosa	Degradation of azo dyes	[32]

3. Environmental pollutants removing through microalgae

Environmental pollution is classified into various groups. For instance, pollution of air is termed as the atmospheric pollution, which causes effect on human and environment [49]. In which emission of different gases into the atmosphere included example NO_x, SO_x, CO, CO₂ that emit from industries & vehicles [25]. The pollution of hydrosphere or water is termed as water pollution, while pollution due to disposal of waste water is termed as industrial effluents pollution [5]. Biggest example of water pollution is oil spill & waste water of industry. The toxicity of heavy metals & pesticides are the big part of environmental pollutant which have a toxic effect to the human beings, animals, birds & for the other environment.

Removal of Heavy Metals: In the specific case of microalgae as living organism, traces of heavy metals are necessary as co-factors of enzymatic reactions, but high levels of heavy metals could result extremely toxic to them and, metabolic reactions can be inhibited. When heavy metals are released to the environment, they can create serious damage to the aquatic life. There are some aquatic organisms that can accumulate heavy metals into their protoplasmic structure without toxic effects. For example Euglena Gracilis could accumulate Zn ions until 5 mg/g dry weight [26], [31]. In other cases, toxic effects could inhibit the enzymatic system affecting the biochemical and physiological processes [13], [14], and [60].

Study 1: A study has been completed in which the effect of three heavy metals (Cadmium, Zinc and Chromium) on the microalgae growth using two different microalgae free cell's suspended culture (Scenedesmus acutus and Chlorella vulgaris) and to study the immobilization of microalgae cells (using kappa-carrageenan and polyurethane foam as supports) for heavy metals removal (Cd, Zn and Cr) [61]. Two strains of microalgae were used: Chlorella vulgaris LAM-C30 and Scenedesmus acutus. The first one was supplied by the Microbial Culture Collection & the second one was supplied by the Microalgae Collection [61]. The culture media were modified Bristol for Scenedesmus acutus and

C-30 with 2 % w/v agar addition for *Chlorella vulgaris* [9], [10]. For the experiments of heavy metals removal, two materials were tested for the selection of the optimal matrix for microalgae immobilization: Kappa-carrageenan and polyurethane foam. In the case of tolerance to Zn, Cr and Cd studies in the free cell's system, it was found that *Chlorella vulgaris* support zinc concentrations until 600mg.L⁻¹. For concentrations values between 100 and 400 mg.L⁻¹ no variations occurred (Table2). The pH ranged 6.8-7.2.

Table 2: Effect of the Zn Concentration on the Growth Rate of *Scenedesmus Acutus* and *Chlorella Vulgaris* [61].

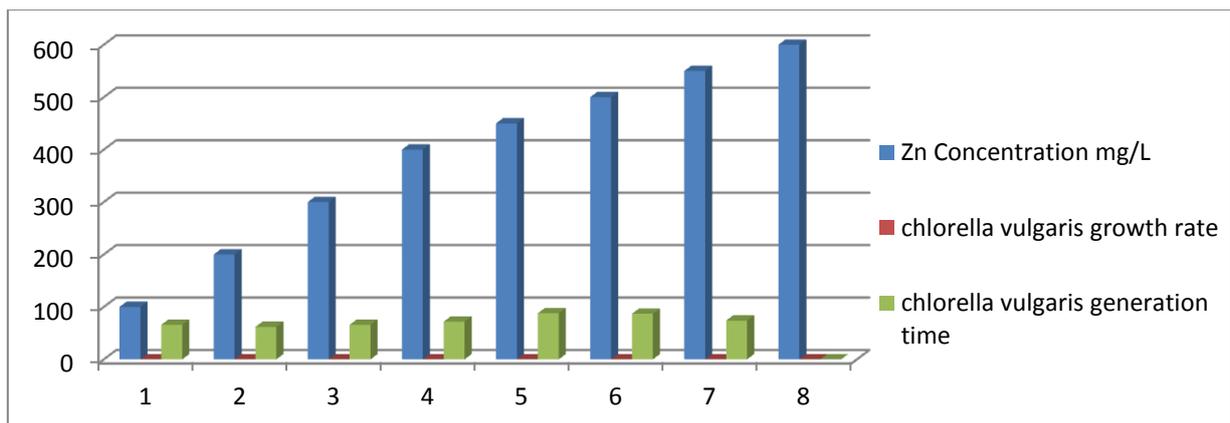
Chlorella vulgaris			Scenedesmus acutus		
Concentration mg/l	μ (h) ⁻¹	T(h)	Concentration mg/l	μ (h) ⁻¹	T(h)
100	0.0104	66	25	0.0123	56
200	0.0111	62	30	0.0132	53
300	0.0104	66	45	0.0136	51
400	0.0096	72	50	0.0142	48
450	0.0078	88	60	0.0134	51
500	0.0079	87	75	0.0129	53
550	0.0092	74	100	0.0124	54
600	0.0000	no growth	125	no growth	no growth
Control	0.0106	65	Control	0.0124	56

In table 2;

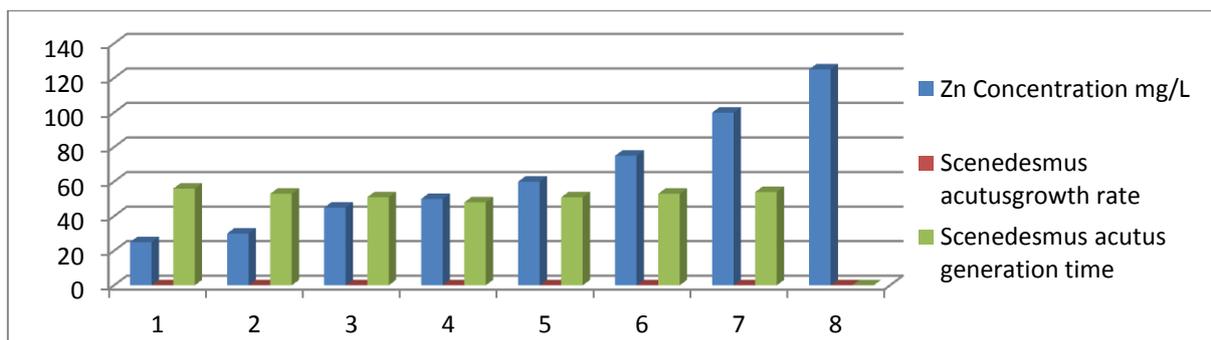
μ = microorganism specific growth rate

T= generation time

Scenedesmus acutus resists zinc concentrations of 100 mg.L⁻¹ maximum, and Cellular density diminishes when the concentration of zinc increase.



Graph 1: Shows the Generation Time and Growth Rate of *Chlorella Vulgaris* Against Zinc Concentration [61].



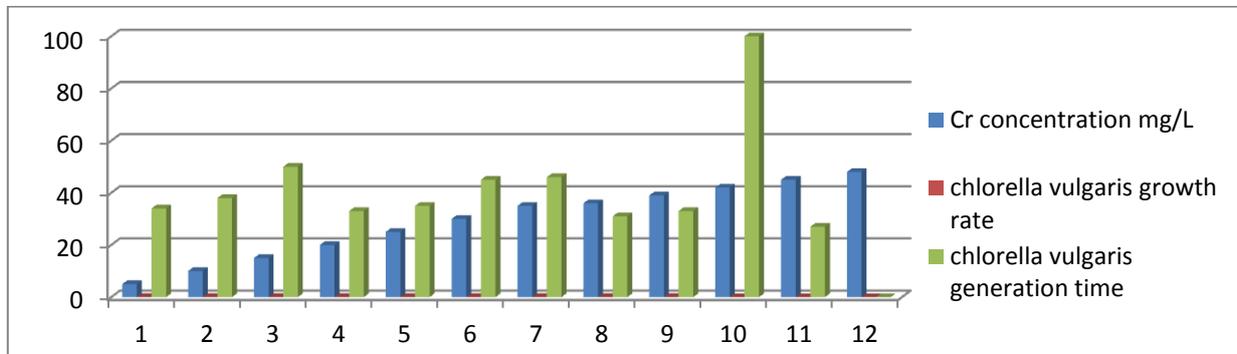
Graph 2: Shows the Generation Time and Growth Rate of *Scenedesmus Acutus* Against Zinc Concentration [61].

The effect of Cr concentration is shown in Table 3. The maximum Cr concentration that was resisted by *Scenedesmus acutus* was 15mg.L⁻¹. Over this concentration no growth was detected. A similar behavior occurs for *Chlorella vulgaris*. It only resists a maximum Cr concentration of 45mg.L⁻¹. Over these concentrations, an abnormal growth of the cells occurs. In the immobilized experiments, the packed columns, for both strains, had more efficient behavior than fluidized columns. This situation could be explained because of the absence of fixation system in *Chlorella vulgaris* and, in *Scenedesmus acutus*.

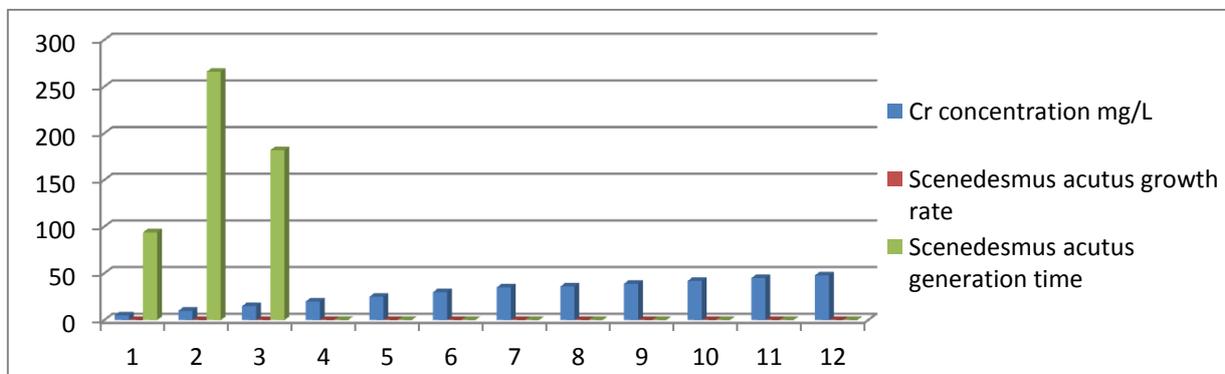
Table 3: Effect of the Cr Concentration on the Growth Rate of Scenedesmus Acutus and Chlorella Vulgaris [61].

Chlorella vulgaris			Scenedesmus acutus		
Concentration mg/l	$\mu(h)^{-1}$	T(h)	Concentration mg/l	$\mu(h)^{-1}$	T(h)
5	0.0200	34	5	0.0073	94
10	0.0181	38	10	0.0026	266
15	0.0137	50	15	0.0038	182
20	0.0206	33	20	no growth	no growth
25	0.0197	35	25	no growth	no growth
30	0.0154	45	30	no growth	no growth
35	0.0148	46	35	no growth	no growth
36	0.0220	31	36	no growth	no growth
39	0.0207	33	39	no growth	no growth
42	0.0069	100	42	no growth	no growth
45	0.0024	27	45	no growth	no growth
48	no growth	no growth	48	no growth	no growth
Control	0.0167	41	Control	0.0092	75

In table 3;
 μ = microorganism specific growth rate
 T= generation time



Graph 3: Shows the Generation Time and Growth Rate of Chlorella Vulgaris Against Chromium Concentration [61].



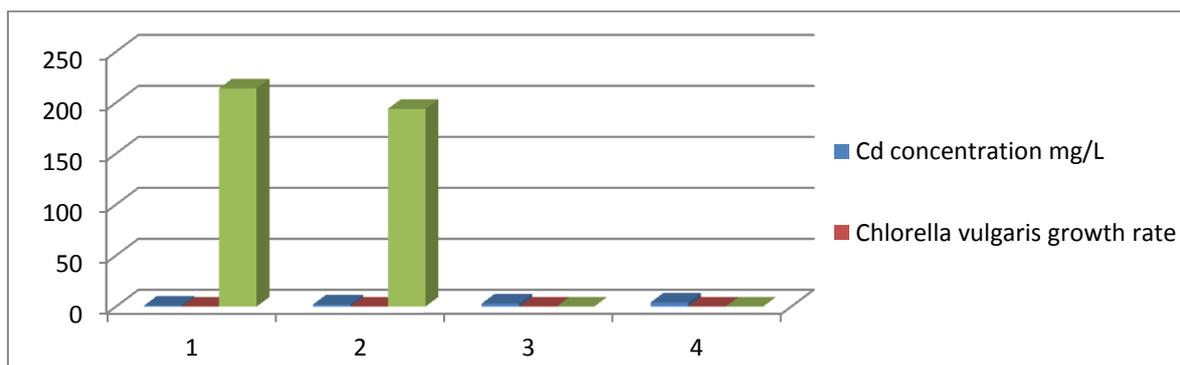
Graph 4: Shows the Generation Time and Growth Rate of Scenedesmus Acutus Against Chromium Concentration [61].

Concerning, cadmium, both strains presented morphological problems over concentrations of 2mg.L-1, but they remain with its metabolic characteristics and only the reproduction function was affected. The pH of the system range was 6.2-6.5

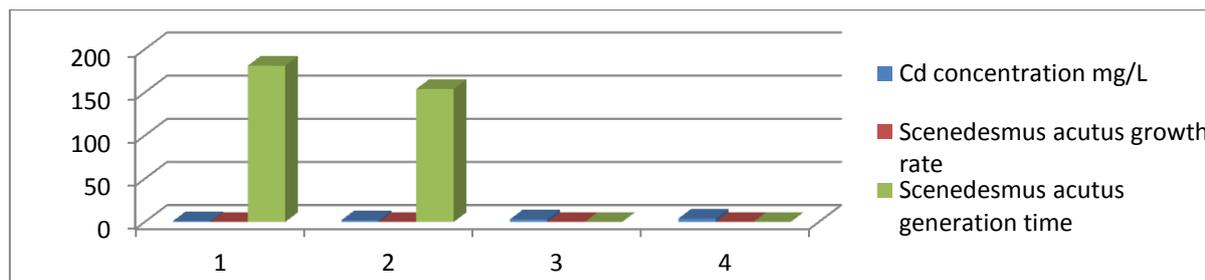
Table 4: Effect of the Cadmium Concentration on the Growth Rate of Scenedesmus Acutus and Chlorella Vulgaris [61].

Chlorella vulgaris			Scenedesmus acutus		
Concentration mg/l	$\mu(h)^{-1}$	T(h)	Concentration mg/l	$\mu(h)^{-1}$	T(h)
1	0.03	214	1	0.02	181
2	0.028	194	2	0.017	154
3	no growth	no growth	3	no growth	no growth
4	no growth	no growth	4	no growth	no growth
Control	0.03	216	Control	0.02	182

In table 4;
 μ = microorganism specific growth rate
 T= generation time



Graph 5: Shows the Generation Time and Growth Rate of Chlorella Vulgaris Against Cadmium Concentration [61].



Graph 6: Shows the Generation Time and Growth Rate of Scenedesmus Acutus Against Cadmium Concentration [61].

It was studied that microalgae *Scenedesmus acutus* and *Chlorella vulgaris* immobilized in polyurethane foam and Kappa-carrageenan gel, are tolerant to Cadmium, Chromium and Zinc concentrations over the normal concentration of these ions on industrial water. This fact implies a great possibility for this type of waste water treatment using immobilized microalgae.

Biosorption is an alternative technique over conventional methods to remove heavy metals from industrial waste and contaminated soil through microorganisms (bacteria, fungi, yeast, and algae). Biosorption is a term that describes the removal of heavy metals by the passive binding to non-living biomass from an aqueous solution [17].

It has been Studied the sorption and desorption of Cobalt by cyanobacteria such as *Oscillatoria angustissima* [38], and studied the growth and metal removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. *Spirulina platensis* [55] also showed considerable potential of adsorption. Biosorption of lead Pb (II), nickel Ni (II) and chromium Cr (VI) ions onto inactive *Saccharomyces cerevisiae* was investigated as function of initial pH, initial metal ion concentration and temperature by [45]. The sorption of Hg and Pb from mono-metal and bimetal solution using dried *Aspergillus niger* biomass was studied by [42] selected *Mucor rouxii* biomass immobilized in a polysulfone matrix and the biosorption column prepared was able to remove metal ions such as Pb, Cd, Ni and Zn not only from single-component metal solutions but also from multi-component metal solutions. Further, green algal species such as *Chlorella vulgaris*, *Scenedesmus quadricauda* [6] and *Chlorella homosphaera* [15] have also been studied apart from marine algae [41] for their biosorption capacities.

Furthermore studies have been done regarding bioremediation of soil so there is a newly study [20] to determine metal tolerance in soil. Interaction between *S.cerevisiae* and heavy metal were experimented as it has higher level of resistance and detoxification to heavy metals. The *S.cerevisiae*. showed maximum growth till 50ppm of Pb²⁺and scanty growth till 250ppm, whereas in case of Cd²⁺contaminated medium maximum growth till 250 ppm and scanty growth till 500ppm .It was reported that, *S.cerevisiae* shows high levels of tolerance to various metals and metalloids and hence associated in accumulating these metals to higher capacities compared other microorganisms. The high incidence of heavy metal resistance detected in this work indicates the potential of *S.cerevisiae* bioremediation agents. Thus *S.cerevisiae* has been chosen to biosorb heavy metals from contaminated soils in this study [28].

Table 5: Concentrations of Heavy Metals of Different Soil Samples [28].

Heavy Metals	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Cadmium	NIL	NIL	NIL	NIL	NIL
Cobalt	11	NIL	46.2	80.3	126.5
Chromium	39.6	15.4	44	33	31.9
Copper	28.6	55	18.7	15.4	NIL
Iron	141878	5533	>20000	15598	6378.9
Manganese	47.3	75.9	886.6	280.5	398.2
Nickel	NIL	NIL	NIL	NIL	0.11
Lead	23.1	NIL	14.3	25.3	12.1
Zinc	259.6	92.4	180.4	136.4	88

The *S.cerevisiae* cells were observed to have tolerance up to 250ppm of Pb^{2+} contaminated YPD agar media. At lower metal concentrations, these metals act as stimulants and maximize the growth of the microorganism.

Wastewater Treatment: Wastewater is a general term used to represent the water with poor quality that contains more amounts of pollutants and microbes. If wastewater is discharged into the nearby water bodies, it can cause serious environmental and health problems to human beings. 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 microbes from wastewater before discharging into the river [48]. 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 ecosystem. To remove these nutrients, several processes are used, but the disadvantages of this type of treatment are high cost and increased sludge production [65].

As an alternative to the conventional treatment methods, microalgae are suggested to remove the nutrients from wastewater [35]. The use of microalgae or macro algae (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 [40]. The microalgae grown in wastewater can be used as energy source, fertilizer, fine chemicals production and as feed to animals [62], [40]. To use microalgae for wastewater treatment is an old idea, and several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity. The nutrient removal is basically an effect of assimilation of nutrients as the algae grow, but other nutrient stripping phenomena also occur, e.g. ammonia volatilization and phosphorus precipitation as a result of the high pH induced by the algae [29].

Some reports reveal that a large part, sometimes up to 90 %, of the phosphorus removal is due to this effect [21]. In addition to tertiary treatment, microalgae may provide heterotrophs in secondary treatment with oxygen, and can also be used to absorb e.g. metals from mine waste-water. The increase in pH during photosynthesis also has a disinfecting effect on the wastewater [18]. Some species of microalgae are used in the biological treatment of the wastewater e.g., *Chlorella* sp. *Chlorella sorokiniana* and *Chlorella vulgaris* efficiently remove nitrogen than phosphorous from effluent [30] carried out extensive research to analyze the effect of starvation and co-immobilization of *C. sorokiniana*, *C. vulgaris* with microalgae growth-promoting bacterium (MGPB) *Azospirillum brasilense* for nutrient removal capability. The synthetic wastewater used mimicked the chemical composition similar to real domestic wastewater collected from the city of Mexico. Both the *C. sorokiniana* and *C. vulgaris* microalgae cultures were co-immobilized with bacteria and starved for 3 and 5 days by cultivating in sterile saline with continuous light. This study concluded that the starvation for 3days does not affect the growth of *C. vulgaris* and *C. sorokiniana*, whereas starvation for 5 days negatively affects the growth of *C. vulgaris*. In case of nutrient removal efficiency, both the cultures showed more phosphorus removal capability when co-immobilized with *Azospirillum brasilense* and starvation of these cultures further increased the phosphorus removal efficiency of *C. sorokiniana*.

Several studies were performed to analyze the capability of microalgae consortium along with symbiotic bacteria for nutrient removal capacity from wastewater. One such study was the analysis of euglenophyt, cyanobacterium, green microalgae and two types of indigenous microalgae consortium along with symbiotic bacteria for the nutrient removal capability from diluted [57] piggery wastewater. The result of this study showed that the unialgal cultures like *Euglena viridis* (*E. viridis*), *Chlorella sorokiniana* (*C.sorokiniana*) were able to grow in both types of diluted wastewater, while *Scenedesmus obliquus* (*S. obliquus*) and consortium 2 were able to grow in eight times (1:8) diluted wastewater, whereas consortium1 and *Spirulina platensis* showed no growth. In case of phosphorus and nitrogen removal capacity, *E. viridis* and *C. sorokiniana* showed more nitrogen removal in both the dilutions; on the other hand, *C. sorokiniana*, *S. obliquus* and *E. viridis* showed phosphorus (20-65%) removal in eight times diluted wastewater [19].

In a similar study, native microalgae consortium (lacking symbiotic bacteria), freshwater and marine microalgae were analyzed for the biomass production and nutrient removal capability from the treated carpet mill effluent. Totally fifteen microalgae were isolated from the wastewater using BG11 medium. Pure cultures of the isolate were mixed in equal proportion to form the native microalgae consortia. In addition to the native algal consortia, two fresh water microalgae (*Botryococcus braunii*, *Chlorella saccharophila*) and marine algae (*Dunaliella tertiolecta* and *Pleurochrysis carterae*) were selected for this study. The result of this study, indicated that the native algal consortia showed better biomass production in both treated and untreated wastewater than the unialgal cultures and the nutrient removal capability of native microalgae consortia showed more phosphate (98.8–99.1%) and nitrate removal (99.7–99.8%) in 72hours [12].

Table 6: Microalgae Cultures Examined for Piggery Wastewater Treatment [57].

S/No	Types Of Culture	Microalgae	S/No	Types Of Culture	Microalgae
1.	Consortium 1	<i>Scenedesmus Chlamydomonas</i> , <i>Microspora</i> , <i>Oocystis</i> , <i>Chlorella</i> , <i>Nitzschia</i>	4.	Cyanobacterium	<i>Spirulina platensis</i>
2.	Consortium 2	<i>Chlorella</i> strains	5.	Euglenophyt	<i>Euglena viridis</i>
3.	Green Microalgae	<i>Chlorella sorokiniana</i> <i>Scenedesmus obliquus</i>			

Biodegradation of Aromatic (Organic) Compounds: Organic pollutants in the aquatic environment are subject to biodegradation by a range of naturally occurring microorganisms, but studies have concentrated, almost exclusively, on the role of bacteria [16] and fungi [36] in the degradation processes. Algae remain the poor relations of the environmental microbiologist, in spite of their ubiquitous distribution; their central role is to turnover of carbon and other nutrient elements and recognition of their heterotrophic abilities [53]. Information on the relationship between algal heterotrophy and biodegradation of xenobiotic compounds is far less than the information accumulated concerning bacteria and fungi. Some information on the interactions between pesticides and eukaryotic algae was compiled by Kobayashi and Rittman [33].

The enzymology of the degradation of phenol in the (Figure 1) below by *Ochromonas danica* has also been investigated. Specific activities for the hydroxylation of phenol to catechol involving a putative phenol hydroxylase could not be found in cell free extracts. These activities were elucidated using whole cells with the alga having the highest specificity for phenol, then p-cresol, o-cresol and finally m-cresol. Catabolism of phenol to catechol was confirmed by the isolation of the product from incubations in which the catechol 2, 3-dioxygenase (catechol: oxygen 2, 3-oxidoreductase) was inhibited with 3-chlorocatechol [53].

Aromatic rings can be ring opened by either ortho cleavage or Meta cleavage after the formation of a 1, 2 dihydroxybenzoid in the process of (Figure 2). Enzymes of both ortho and Meta cleavage pathways which are commonly found in bacteria [16] were assayed spectrophotometrically using extracts of phenol-induced cells. Axenic cultures of *O.danica* were found to cleave catechol in the 2-3 position resulting in the formation of 2-hydroxymuconic semialdehyde. The algal extracts also oxygenated 3-methylcatechol, 4-methylcatechol, 4-bromocatechol and 4-fluorocatechol to the corresponding ring cleavage products, which showed the expected shifts in spectral peaks under acid and alkaline conditions. Inhibition studies using 4-isopropylcatechol and 3-chlorocatechol gave results corresponding to those of bacterial studies, showing irreversible and suicide inhibitions, respectively. Specific activities in these extracts for catechol 1, 2-dioxygenase (catechol: oxygen 1, 2-oxidoreductase), the enzyme initiating the ortho pathway, were found to be negligible. Metabolism of 2-hydroxymuconic semialdehyde was catalyzed predominantly by an NAD-dependent dehydrogenase (2-hydroxymuconate semialdehyde, NAD⁺ oxidoreductase). Prolonged incubation of the muconic semialdehyde products with cell-free extracts resulted in the production of pyruvate, confirmed by its reduction to L-lactate with NADH and crystalline lactate dehydrogenase and by TLC detection of its 2,4-dinitrophenylhydrazone. (Figure 2) shows the catabolic pathway for phenol characterized in *O.danica* [53]. However, the respective importance of microalgae for the catabolism of pollutants entering their ecosystem is not known. As it is extremely rare to find a single microorganism that is capable of completely degrading a pollutant or a mixture of xenobiotics under environmental conditions. The combined action of microalgae and other microorganisms might be a rather important process for the elimination of these undesired compounds from the environment. The degradation of pollutants under these conditions usually involves the combined actions of two or more microorganisms. In the case of algae, it is clear that although the complete degradation of aromatic pollutants is rare; these organisms are capable of carrying out biotransformation on aromatic pollutants.

Degradation of the Azo dyes by Microalgae: Some azo dyes present in bodies of water exhibit high toxicity to aquatic life but do not significantly reduce algal growth [1]. Furthermore, algae have been found to grow in industrial effluents [22]. Therefore microalgae are good options for the bioremediation of colored wastewater; in addition microalgae do not need added carbon sources. In contrast to bacteria and fungi, which depend on such sources [44], Algae derive energy from sunlight and carbon from the air and some scavenge atmospheric nitrogen thus, the mass cultivation of algae is less expensive [50]. The mechanisms of algae decoloration can involve enzymatic degradation, adsorption, or both. Similar to bacteria algae are capable of degrading azo dyes through an induced azoreductase to break the azo bond, resulting in the production of aromatic amines. Oxidative enzymes are also involved in the decoloration process [47]. The efficiency of adsorption is highly influenced by the structure of the dye, the species of algae [44] and pH [37]. Both living and non-viable algae have been used in the removal of color from wastewater [54], [34]. At lower pH, biomass has a net positive charge; the higher uptakes obtained at lower pH values may be due to electrostatic attraction between the negatively charged dye anions and the positively charged cell surface [56]. High adsorption capacity has been demonstrated at pH 2 using *Scenedesmus quadricauda* [24], *Chlorella vulgaris* [2] and non-viable *Spirogyra* sp [54]. Immobilised microalgae are another alternative to decoloration. For example, *C.vulgaris* and *S.quadricauda* immobilized on alginate can remove a higher percentage of color from textile dyes than suspended algae [24].

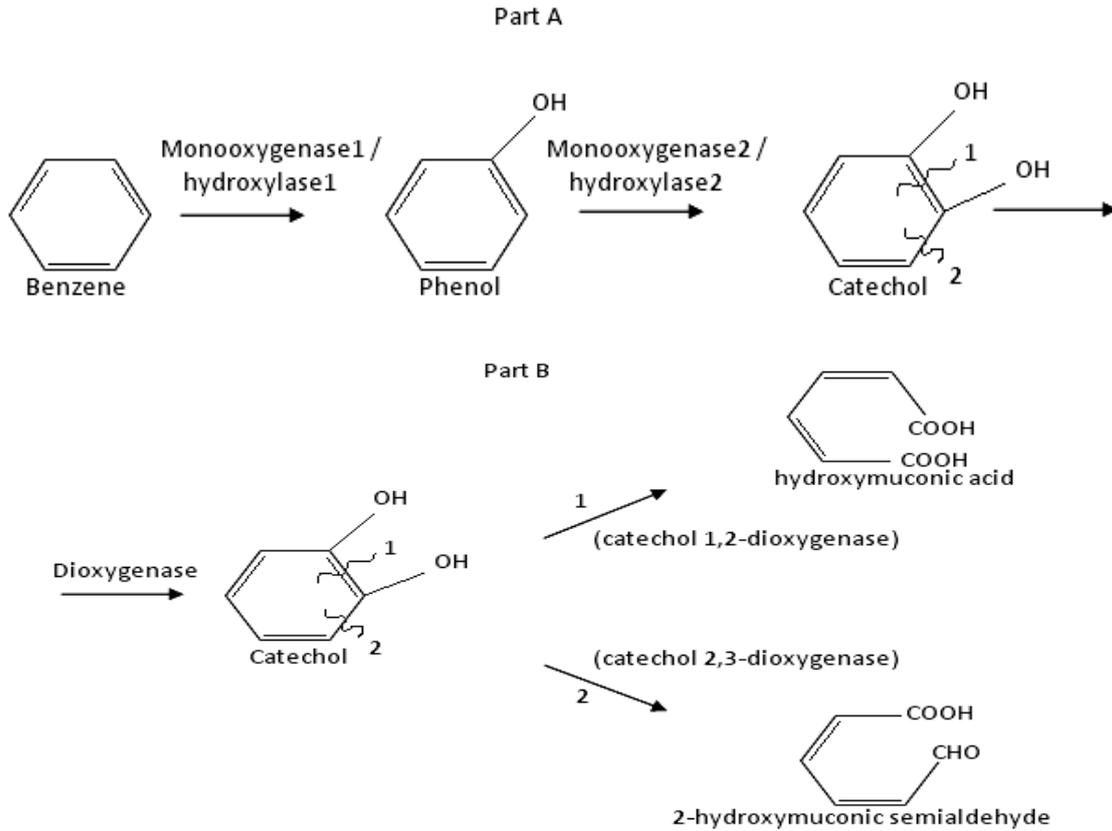


Fig 1: Biotransformation of Phenol or Benzene by Algae *Ochromonas Danica* Adapted From [16], [53].

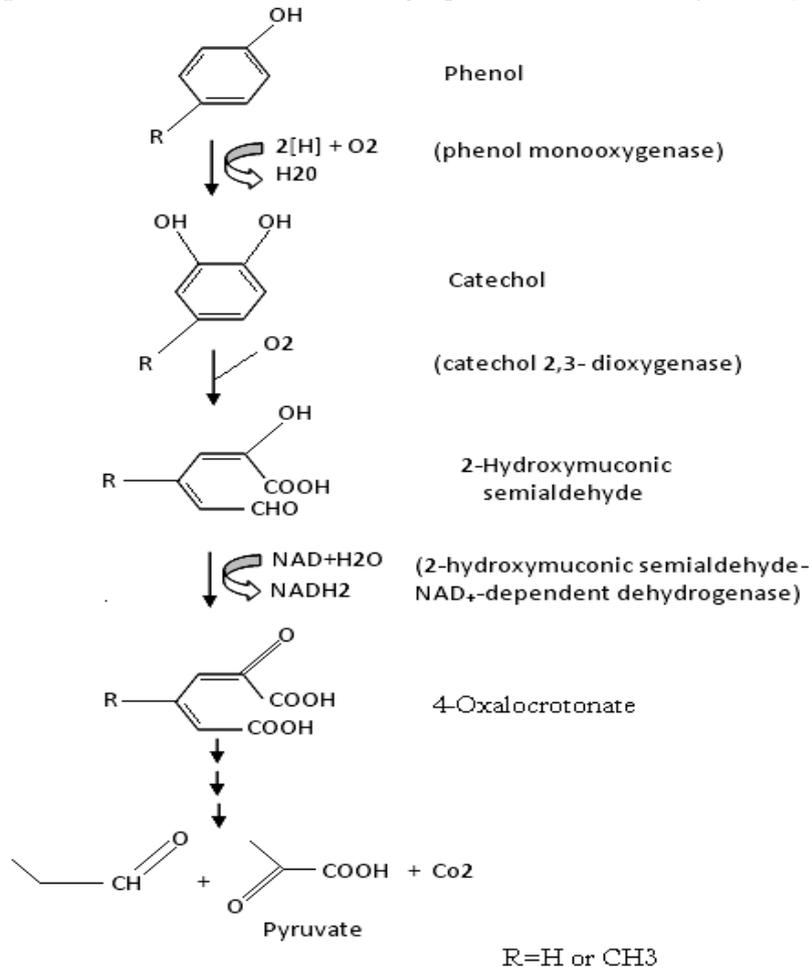


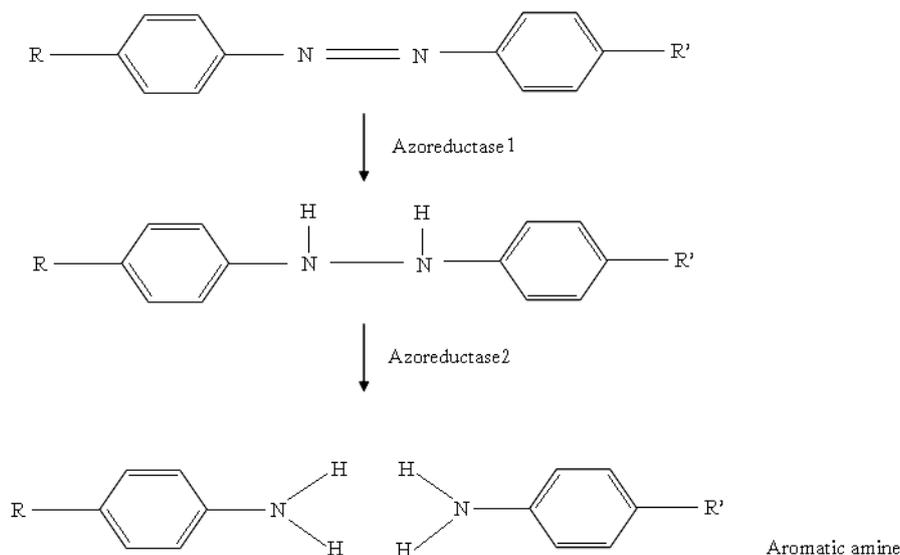
Fig. 2: Characterized Steps in the Catabolism of Phenol, Via the Meta Cleavage Pathway, by *Ochromonas Danica* Adapted From [16], [53].

Table 7: Illustrates Some Azo Dyes Decoloration Using Microalgae.

Microalgae and source	Decoloration process & conditions	(Initial concentration) Dyes, % color removal	Reference
<i>Chlorella vulgaris</i> from a collection	Adsorption; pH 7, 150 rpm, 25°C	Supranol Red 3BW, 35.62 mg dye/g biomass	[34]
<i>Scenedesmus quadricauda</i> from a lake	Adsorption; pH 2–8, 150 rpm, 30°C, 300 min	Remazol Brilliant Blue R, 48.3 mg dye/g Biomass	[24]
<i>Spirogyra</i> specie from a forestry waste	Adsorption; pH 2–10, 180 rpm, 20–50°C, 5h	15ppm Direct Brown, 70% color	[54]
<i>Chlorella vulgaris</i> from polluted water	Degradation; pH 7, 25°C, 7 days; azo reductase	20ppm each, Methyl Red, 82% color; OrangeII, 47% color; G-Red (FN-3G), 59% color	[23]
<i>Oscillatoria curviceps</i> from collection	Degradation; pH 7, 100 rpm, 4°C, 8 days; azoreductase, polyphenol oxidase	100ppm Acid Black, 84% color	[47]

Algae mediated azo bond cleavage via azoreductase enzymes is non-specific with regard to the organisms involved and the substances reduced [4]. The cleavage of the azo bond occurs in two stages in the environment, as presented in (Figure 3) where two electrons are transferred at each stage to the azo substance, which acts as a final electron acceptor [27].

The (Figure 3) illustrates how in the presence of specific oxygen-catalyzed enzymes called azoreductase, some microalgae species are able to reduce azo compounds and produce aromatic amines. The azo-bond ($-N=N-$) cleavage involves a transfer of four electrons (reducing equivalents), which proceeds through two stages at the azo-linkage. In each stage, two electrons are transferred to the azo dye, which acts as a final electron acceptor.

**Fig. 3:** Degradation of Azo Dyes by Algae *Chlorella Pyrenoidosa* [32]

We observed that eukaryotic algae are capable of biotransformation and biodegradation of aromatic pollutants commonly found in natural and wastewaters. Furthermore, these organisms are able to enhance the degradation potential of the micro biota present and therefore contribute to the elimination of pollutants from the respective ecosystem. Traditionally, plant ecology has regarded microalgae as an essential part of the aquatic food web by providing reduced C and N in aquatic ecosystems. In addition, many of these organisms were found to be useful indicators of environmental pollution and therefore applied in toxicity testing [43].

4. Conclusion

It is concluded that various environmental pollutants can be removed by different forms of algal species. Some specific micro algal species are used for the removal and degradation of specific pollutants from the environment and some are used for the biotransformation and bioaccumulation of several environmental pollutants. Enormous micro algal species

are found which are generally used as pollution indicators and pollution remover effectively. To utilize the biomass of algae, physical-chemical parameters should be maintained at optimum conditions for intense micro algal growth. Copious micro algal species are used in biosorption process for the remediation of heavy metals from effluent and soil which are very crucial from environmental point of view. Moreover, a big advantage is that microalgae cultivated in the wastewater can be used for the biodiesel production and as feed for animals. In addition, it is also observed during studying over different micro algal species, the dual process (microalgae cultivation in effluent coupled with biodiesel production) has several advantages such as less cost and less energy input for biodiesel production and less greenhouse gas emission during biodiesel production. Perhaps, microalgae removal of environmental pollutants is cost effective and efficient technique as compare to other conventional techniques.

References

- [1] Acuner, A. and F.B. Dilek. (2004). Treatment of tectilon yellow2G by *Chlorella vulgaris*. *Process Biochem.* 39: 623–31. [http://dx.doi.org/10.1016/S0032-9592\(03\)00138-9](http://dx.doi.org/10.1016/S0032-9592(03)00138-9).
- [2] Aksu, Z. and S. Tezer (2005). Biosorption of reactive dyes on the green algae *Chlorella vulgaris*. *Process Biochem.* 40: 1347–61. <http://dx.doi.org/10.1016/j.procbio.2004.06.007>.
- [3] Allen, M.M. (1968). Simple conditions for growth of unicellular blue-green algae on plates. *J Phycol.* 4: 1-4. <http://dx.doi.org/10.1111/j.1529-8817.1968.tb04667.x>.
- [4] Anjaneyulu, Y., Sreedhara Chary, N. and Raj, D. (2005). Decolorization of industrial effluents--available methods and emerging technologies--a review. *Rev Environ Sci Biotechnol.* 4: 245–273.
- [5] Ashraf, M.A., Maah, M.J., Yusoff, I. and Mehmood, K. (2010). Effects of Polluted Water Irrigation on Environment and Health of People in Jamber, District Kasur, Pakistan. *International Journal of Basic & Applied Sciences.* 10(3): 37-57.
- [6] Awasthi, M. and L.C. Rai. (2004). Adsorption of nickel, zinc and cadmium by immobilized green algae and cyanobacteria. A comparative study. *Annals Microbiol.* 54: 257-267.
- [7] Aziz, M. A. and Ng, W. J. (1992). Feasibility of wastewater treatment using the activated-algae process. *Bioresource Technology.* 40: 205–208. [http://dx.doi.org/10.1016/0960-8524\(92\)90143-L](http://dx.doi.org/10.1016/0960-8524(92)90143-L).
- [8] Becker, E. W. (1994). Microalgae. Cambridge University press. *Biotechnology and microbiology.* 293.
- [9] Cañizares-Villanueva, R.O. and Travieso, L. (1991). Inmovilización de microalgas para el tratamiento de residuales. *Informe CONACyT, Proyecto.* 8: 07-91.
- [10] Cañizares-Villanueva, R.O. and Travieso, L. (1992). Inmovilización de microalgas para el tratamiento de residuales. *Informe CONACyT, Proyecto.* 8: 18-92.
- [11] Cerniglia, C.E., van Baalen, C. and Gibson, D.T. (1980). Metabolism of naphthalene by the cyanobacterium *Oscillatoria sp.* strain JCM. *J. Gen. Microbiol.* 116: 485-494.
- [12] Chinnasamy, S., A. Bhatnagar, R. Claxton and K.C. Das. (2010). Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. *Bioresour Technol.* 101: 6751–6760. <http://dx.doi.org/10.1016/j.biortech.2010.03.094>.
- [13] Costa, A.C.A. and Leite, S.G.F. (1991). Metals biosorption by sodium alginate immobilized *Chlorella homosphaera*. *Biotechnol Lett.* 13: 559-562. <http://dx.doi.org/10.1007/BF01033409>.
- [14] Costa, A.C.A. and Leite, S.G.F. (1992). Cadmium and zinc biosorption by *Chlorella homosphaera*. *Biotechnol Lett.* 12: 941-944. <http://dx.doi.org/10.1007/BF01022595>.
- [15] Costa, Antonio Carlos, A. and Selma Gomes (1990). Cadmium and zinc biosorption by *Chlorella homosphaera*. *Biotechnol Lett.* 12: 941-944. <http://dx.doi.org/10.1007/BF01022595>.
- [16] Dagley, S. (1978). Microbial catabolism, the carbon cycle and environmental pollution. *Naturwissenschaften.* 65: 85-95. <http://dx.doi.org/10.1007/BF00440546>.
- [17] Davis, Thomas, A., Bohumil Volesky and Alfonso Mucci. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Res.* 37: 4311-4330. [http://dx.doi.org/10.1016/S0043-1354\(03\)00293-8](http://dx.doi.org/10.1016/S0043-1354(03)00293-8).
- [18] De la Noüe, J., Laliberté, G., and Proulx, D. (1992). Algae and waste water. *J. Appl. Phycol.* 4: 247–254. <http://dx.doi.org/10.1007/BF02161210>.
- [19] De-Godos, I., V.A. Vargas, S. Blanco, M.C. GarciaGonzalez, R. Soto, P.A. Garcia-Encina, E. Becares and R. Muioz. (2010). A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. *Bioresour Technol.* 101: 5150–5158. <http://dx.doi.org/10.1016/j.biortech.2010.02.010>.
- [20] Dilna Damodaran, Gummadi Suresh, and Raj Mohan, B. (2011). 2nd International Conference on Environmental Science and Technology. *Ipcbee.* 6: 9-18.
- [21] Doran, M.D. and Boyle, W.C. (1979). Phosphorus removal by activated algae. *Water Res.* 13: 805–812. [http://dx.doi.org/10.1016/0043-1354\(79\)90246-X](http://dx.doi.org/10.1016/0043-1354(79)90246-X).
- [22] Dubey, S.K., Dubey, J., Viswas, A.J. and Tiwari, P. (2011). Studies on Cyanobacterial biodiversity in paper mill and pharmaceutical industrial effluents. *Br Biotechnol.* 1: 61–67. <http://dx.doi.org/10.9734/BBJ/2011/395>.
- [23] El-Sheekh, M.M., Gharieb, M.M. and Abou-El-Souod, G.W. (2009). Biodegradation of dyes by some green algae and cyanobacteria. *Int Biodeter Biodegr.* 63: 699–704. <http://dx.doi.org/10.1016/j.ibiod.2009.04.010>.
- [24] Ergene, A., Ada, K., Tan, S. and Katircio'u, H. (2009). Removal of Remazol Brilliant Blue R dye from aqueous solutions by adsorption onto immobilized *Scenedesmus quadricauda*. Equilibrium and kinetic modeling studies. *Desalination.* 249: 1308–1314. <http://dx.doi.org/10.1016/j.desal.2009.06.027>.
- [25] European Public Health Alliance, (2009). Air, Water Pollution and Health Effects.
- [26] Fukami, M. (1988). Effects of zinc on metal metabolism on the zinc tolerant chlorotic mutants of *Euglena gracilis*. *Agric Biol Chem.* 52: 2343-2344. <http://dx.doi.org/10.1271/bbb1961.52.2343>.
- [27] Guo, J., Kang, L., Wang, X. and Yang, J. (2010). Decolorization and degradation of azo dyes by redox mediator system with bacteria. Biodegradation of azo dyes. *Handbook of Environmental Chemistry.* Springer-Verlag. 9: 85–100.
- [28] Hall, K.R., L.C. Eagleton, A. Acrivos and T. Vermeulen. (1996). Pore and solid diffusion kinetics in fixed-bed adsorption under constant-pattern conditions. *Ind. Eng. Chem. Fund.* 5: 212-223.
- [29] Hammouda, O., Gaber, A. and Abdel-Raouf, N. (1994). Microalgae and wastewater treatment. *Ecotoxicol. Environ. Saf.* 31: 205–210. <http://dx.doi.org/10.1006/eesa.1995.1064>.

- [30] Hernandez, J.P., L.E. de-Bashan and Y. Badhan. (2006). Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* sp. co-immobilized with *Azospirillum brasilense*. *Enzyme Microb. Technol.* 38: 190–198. <http://dx.doi.org/10.1016/j.enzmictec.2005.06.005>.
- [31] Ilangoan, K. (1992). Interaction of cadmium, cooper and zinc in *Chlorella pyrenoidosa*. *Chick Environ Technol.* 13: 195-199. <http://dx.doi.org/10.1080/09593339209385144>.
- [32] Jinqi, I. and Houtian, O. (1992). Degradation of azo dyes by algae. *Environ.Pollut.* 75: 273-278. [http://dx.doi.org/10.1016/0269-7491\(92\)90127-V](http://dx.doi.org/10.1016/0269-7491(92)90127-V).
- [33] Kobayashi, H. and Rittman, B.E. (1982). Microbial removal of hazardous organic compounds. *Environ. Sci. Technol.* 16: 170-183. <http://dx.doi.org/10.1021/es00097a002>.
- [34] Lim, S.L., Chu, W.L. and Phang, S.M. (2010). Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresour Technol.* 101: 7314–22. <http://dx.doi.org/10.1016/j.biortech.2010.04.092>.
- [35] Mallick, N. (2002). Biotechnological potential of immobilized algae for wastewater N, P and metal removal. A review. *BioMetals.* 15: 377–390. <http://dx.doi.org/10.1023/A:1020238520948>.
- [36] Middlehoven, W.J. (1993). Catabolism of benzene compounds by *ascomycetous* and *basidiomycetous* and yeast-like fungi. *Antonie van Leeuwenhoek.* 63: 125-144. <http://dx.doi.org/10.1007/BF00872388>.
- [37] Mohan, S.V. Ramanaiyah, S.V. And Sarma, P.N. (2008). Biosorption of direct azo dye from aqueous phase onto *Spirogyra* sp evaluation of kinetics and mechanistic aspects. *Biochem Eng J.* 38: 61–69. <http://dx.doi.org/10.1016/j.bej.2007.06.014>.
- [38] Mostafa, M. El-Sheekh, Wagieh, A. El-Shouny, Mohamed E.H. Osman, Eman and W.E. El-Gammal. (2005). Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. *Environ. Toxicol. Pharmacol.* 19: 357-365. <http://dx.doi.org/10.1016/j.etap.2004.09.005>.
- [39] Mulbry, W., S. Kondrad and P. Pizarro. (2006). Biofertilizers from algal treatment of dairy and swine manure effluents. Characterization of algal biomass as slow release fertilizer. *J. Vegetable Sci.* 12: 107–125. http://dx.doi.org/10.1300/J484v12n04_08.
- [40] Mulbry, W., S. Kondrad, C. Pizarro and E. Kebede-Westhead. (2008). Treatment of dairymanure effluent using freshwater algae. Algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresour. Technol.* 99: 8137–8142. <http://dx.doi.org/10.1016/j.biortech.2008.03.073>.
- [41] Nirmal Kumar, J.I., Cini Oommen and Rita N. Kumar. (2009). Biosorption of heavy metals from aqueous solution by green marine macroalgae from Okha Port, Gulf of Kutch, India. *Am-Euras. J. Agric. Environ. Sci.* 6: 317-323.
- [42] Nirmal Kumar, J.I., George Basil, Rita N. Kumar, Sajish, P.R. and Vijol Shailendra. (2010). Biosorption of mercury and lead by dried *Aspergillus niger* Tiegh. Isolated from estuarine sediments. *Int. J. Environ. Stud.* 67: 735-746. <http://dx.doi.org/10.1080/00207233.2010.517644>.
- [43] Nyholm, N. and Ka ëllquist, T. (1989). Methods for growth inhibition toxicity tests with freshwater algae. *Environ. Toxicol. Chem.* 8: 689-703. <http://dx.doi.org/10.1002/etc.5620080807>.
- [44] Omar, H.H. (2008). Algal decolorization and degradation of monoazo and diazo dyes. *Pak J Biol Sci.* 11: 1310–16. <http://dx.doi.org/10.3923/pjbs.2008.1310.1316>.
- [45] Ozer, Ayla and Ozer Dursun. (2003). Comparative study of the biosorption of Pb (II), Ni (II) and Cr (II) ions onto *S. cerevisiae*. Determination of biosorption heats. *J. Hazard. Mat.* 100: 219-229. [http://dx.doi.org/10.1016/S0304-3894\(03\)00109-2](http://dx.doi.org/10.1016/S0304-3894(03)00109-2).
- [46] Pedroni, P., Davison, J., Beckert, H., Bergman, P. and Benemann, J. (2001). International network on biofixation of CO₂ and greenhouse gas abatement with microalgae. *Journal of energy and environmental research.* 1: 136-150.
- [47] Priya, B., Uma, L., Ahamed, A.K. Subramanian, G. and Prabakaran, D. (2011). Ability to use the diazo dye C. I. Acid Black 1 as a nitrogen source by the marine cyanobacterium *Oscillatoria curviceps* BDU92191. *Bioresour Technol.* 102: 7218–7223. <http://dx.doi.org/10.1016/j.biortech.2011.02.117>.
- [48] Rawat, I., R. Ranjith Kumar, T. Mutanda and F. Bux. (2010). Dual role of microalgae. Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *App Energy.* 10: 1010-1016.
- [49] Rosenstock, L. (2003). The Environment as a Cornerstone of Public Health, *Environmental Health Perspective.* 111(7): 376-377. <http://dx.doi.org/10.1289/ehp.111-a376>.
- [50] Saha, S.K., Swaminathan, P., Raghavan, C., Uma, L. and Subramanian, G. (2010). Lignolytic and antioxidative enzymes of a marine cyanobacterium *Oscillatoria willei* BDU 130511 during Poly R-478 decolourization. *Bioresour Technol.* 101: 3076–84. <http://dx.doi.org/10.1016/j.biortech.2009.12.075>.
- [51] Scheffler and John. (2007). Underwater Habitats. *Illumin.* 9: 4.
- [52] Semmler, H., Bailly, X. and Wanninger, A. (2008). Myogenesis in the basal bilaterian *Symsagittifera roscoffensis*. *Frontiers in zoology.* 5. <http://dx.doi.org/10.1186/1742-9994-5-14>.
- [53] Semple, K.T. and Cain, R.B. (1995) and (1996). Metabolism of phenols by *Ochromonas danica*. *FEMS Microbiol. Lett.* 133: 253-257. <http://dx.doi.org/10.1111/j.1574-6968.1995.tb07893.x>.
- [54] Sivarajasekar, N., Baskar, R. and Balakrishnan, V. (2009). Biosorption of an azo dye from aqueous solutions onto *Spirogyra*. *J Univ Chem Technol Metal.* 44: 157–164.
- [55] Solisio, C., A. Lodi, P. Torre, A. Converti and Del M. Borghi. (2006). Copper removal by dry and re-hydrated biomass of *Spirulina platensis*. *Bioresour. Technol.* 97: 1756-1760. <http://dx.doi.org/10.1016/j.biortech.2005.07.018>.
- [56] Srinivasan, A. and Viraraghavan, T. (2010). Decolorization of dye wastewaters by biosorbents. A review. *J Environ Manage.* 91: 1915–1929. <http://dx.doi.org/10.1016/j.jenvman.2010.05.003>.
- [57] Sriram and R. Seenivasan. (2012). Algal Biomass Utl. *Microalgae for nutrient removal.* 3: 9- 13.
- [58] Teresa M. Mataa, António A. Martinsa, and Nidia, S. Caetanob. (2010).
- [59] Thurman, H.V. (1997). Introductory Oceanography. New Jersey, USA. Prentice Hall College. ISBN. 13: 262072-73.
- [60] Ting, Y.P. Lawson, F. and Prince, I.G. (1989). Uptake of cadmium and zinc by the algae *Chlorella vulgaris*. Part I. Individual iron species. *Biotechnol Bioeng.* 34: 990-999. <http://dx.doi.org/10.1002/bit.260340713>.
- [61] Valiente, V. and Travieso, L. (1992). Catalogo de la colección microalgal. Algal growth potential measurement in distillery waste. *Bull. Environ. Contam. Toxicol.* 62: 483-489.
- [62] Vilchez, C., I. Garhayo, M.V. Lobato. And J.M. Vega. (1997). Microalgae-mediated chemicals production and wastes removal. *Enzyme Microb. Technol.* 20: 562-572. [http://dx.doi.org/10.1016/S0141-0229\(96\)00208-6](http://dx.doi.org/10.1016/S0141-0229(96)00208-6).
- [63] Wilde Edward, W., Benemann John, R., Weissman Joseph, C. And Tillett David, M. (1990). Bioremoval of heavy metal. *Biotechnology advances.* 11: 781-812. [http://dx.doi.org/10.1016/0734-9750\(93\)90003-6](http://dx.doi.org/10.1016/0734-9750(93)90003-6).
- [64] Yanqun, Li. (2008). Algal Biomass. South China Normal University, Guangzhou. P.R. China, *Biotechnol.* 24: 815-820.
- [65] Yuan, X., A. Kumar, A. K. Sahu and S. J. Ergas. (2011). Impact of ammonia concentration on *Spirulina platensis* growth in an airlift photobioreactor. *Bioresour. Technol.* 102: 3234–3239. <http://dx.doi.org/10.1016/j.biortech.2010.11.019>.