Review Paper:

A biological and technological approach to treat wastewater by using macroalgae and microalgae

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Abstract

Water pollution started way before the mid 19th century during the industrial revolution. Water is being contaminated continuously by heavy discharge of industrial effluents, agricultural runoff, mining activities, discharge of household products, burning of fossil fuels etc. which impose a potential risk to human health. The treatment of wastewater involves transforming the reject water that can be directly or indirectly reused or recycled for different purposes. Chemical and physical wastewater treatment plants adopt an expensive and energy-consuming reactions while the physical processes for screening, primary and tertiary treatment of wastewater produces toxic by products.

Hence biological treatment process is a feasible alternative to chemical and physical wastewater treatment. In the biological treatment process, microbes mostly mixed consortia of bacteria are used for the treatment of contaminated water. Several researches has proved the inherent potential of both macroalgae and microalgae for removing organic carbon, nitrogen, phosphorous, sulfates, heavy metals from wastewater. The most promising advantage in use of macro and micro algae is the conversion of rich chemical energy (in the form of pollutatns) in wastewater to various products (biofuel, nutraceuticals and value added products) which is not otherwise possible through currently practiced activated sludge process. There are however changes in algae based approach to wastewater treatment such as removal of microbes from treated water and optimizing the conditions favorable of algal growth and contaminant removal. In spite of such challenges, wastewater treatment by algae has been shown at lab and pilot scale studies to be economical, eco-friendly and a promising alternative to effective wastewater treatment.

Keywords: Macroalgae, microalgae, wastewater, phycoremediation, photo bioreactors, textile wastewater.

Introduction

Every country in the world is facing several problems due to water pollution. Right now in India, water pollution as well as its treatment is a pressing issue. The central pollution control board (CPCB) has reported that the number of polluted rivers in India has more than doubled in the last five years. World Health Organization (WHO) in a recent report found that Delhi, the capital city of India, was the most polluted city on the planet, with an annual production of 153 µg of the most dangerous small particulates known as PM 2.5 per cubic meter in a survey during the year 2019.

Wastewater is generally a kind of used water that is a byproduct of domestic, industrial, agricultural and commercial activities or any sewer infiltration that leads to organic and inorganic pollution. “Contaminants/pollutants” are generally any kind of physical, chemical, biological and radiological substances present in water which renders the water unfit or unsafe for human/animal consumption or use. Chemical contaminants are pesticides, metals, nitrogen, bleach, salts and organic matter either suspended or dissolved in water etc. while the biological contaminants are usually infectious microbes which include bacteria, viruses, protozoans and several parasites and pathogens.

Stages of wastewater treatment: Wastewater treatment can be categorized into three stages according to the types of pollutants removed or treatment undergone, these are known as primary, secondary and tertiary treatments. Depending upon the inlet water quality (also called as influent), different treatment stages are used either used independently or in combination. In part per million to part per billion levels of contamination, a more advanced treatment process is adopted called quaternary water treatment which involves oxidation or fine filtration processes to make the water amenable for human consumption.

Primary wastewater treatment: For settling solids with densities higher that water, a settling tank or quiescent basin is generally used in primary wastewater treatment. During this process, heavy solids including suspended particles get clarified from water. These also includes grits and sediments which escape from screening chambers and grit removal tanks. Particles such as oils and grease which are lighter in densities than water float to the surface and are skimmed from the sedimentation basin. After settling the heavier material to the bottom, now known as primary sludge, the remaining liquid gets discharged for further treatment.

Secondary wastewater treatment: The organic and dissolved content of wastewater is typically degraded either (or by combined) aerobically or anaerobically in secondary wastewater treatment processes. It uses the biological process containing mixed microbial consortium with billions of cells per gram of biomass sludge. The dissolved and suspended organic pollutants gets oxidized under aerobic condition and reduced under anaerobic conditions to covert into a settable biomass. It is usually done in three ways. The first one is the **"**bio-filtration" process where different filters like sand filters, contact filters and trickling filters containing microbes are used to remove the dissolved pollutants from the water. The second one is "aeration" where wastewater is supplied with air (containing oxygen). The third one is the **"**waste stabilization ponds" which are artificially built to remove pathogens and organic pollutants from the wastewater. Influents or wastewater enters on one side and effluents come out from another side of the pond after retaining for two to three weeks.

Tertiary wastewater treatment: Tertiary treatment of wastewater aims to remove the remaining organic or inorganic contaminants untouched by the microbes due to their toxic or recalcitrant nature. Chemicals such as nitrogen, ammonia, phosphorous are also removed from the wastewater during this stage and this could be separately called as BNR (Biological Nutrient Removal) process. The BNR process is cost-effective as well as eco-friendly as compared to the other physical and chemical treatment process. Treatment of wastewater using both microalgae and macroalgae cultures offer a tremendous solution to tertiary and quaternary treatment due to its ability to tolerate inorganic and organic pollutants and toxic heavy metals in wastewater.

Algae and Wastewater Treatment

Algae are the having photosynthetic eukaryotes having chlorophyll pigment but lacking distinctive tissue types such as roots, shoots, flowers etc. unlike their common counterparts as water plants. Algae belong to the Kingdom "Monera" and are grouped with bacteria in the modern classification system. But in the 5-kingdom classification system, the algae belong to Kingdom Protista. The algae are divided into various phyla like green algae (Chlorophyta), red algae (Rhodophyta), brown algae (Phaeophyta), diatoms (Chrysophyta), euglenoids (Euglenophyta) and dinoflagellates (Pyrophyta). The commercial production of different algal strains such as *Dunaliella* and *chlorella* species with application to wastewater treatment is since over 75 years. Remarkable interest has been developed in some advanced world nations such as the USA, Mexico, Thailand, Japan, Australia and Taiwan[44,59] towards the use of algal methods for wastewater treatment.

Currently, algae are the most expedient alternative organisms for biological decontamination of wastewater due to specific features such as accumulation of organic and inorganic substances, toxic heavy metals and radioactive matters in their cells.[23,24,61] Heterotrophic bacteria use the oxygen produced by algae to convert the wastewater nutrient into useful biomass.[15] Biodegradation is the result of oxygen produced by algae from pollutants present in wastewater in natural water treatment systems. Whereas, in activated sludge systems, oxygen needs to be supplied to aeration tanks to meet the demands of bacterial oxidation. This energy consumption by bacteria can be greatly reduced in algal technologies since algae are able to generate oxygen during light reaction. Algae has also been shown to degrade pollutants during dark reactions (absence of light). Apart from efficient degradation rates, algal biomass leads to the production of pharmaceuticals and genetically engineered products such as antitumor/ anticancer, antibacterial, antihistamine, antiviral and many more high-value products.[45]

Macroalgae and wastewater treatment

Nutrient, BOD and COD removal: Neveux et al[47] demonstrated decrease in COD, Nitrogen, phosphorus by 57%, 62% and 75% respectively along with the reduction in microbes by 99% in the treated water after cultivation of *Oedogonium* species. He et al[18] observed that when *Porphyra yezoensis (seaweed)* were cultivated in the open sea, nitrite, nitrate, ammonia and phosphate were decreased by 42–91%, 21–38%, 50–94% and 42–67% respectively as compared with the control area.

Sode et al[63] tested the wastewater from anaerobically digested sewage sludge by cultivating *Ulva Lactuca*, green macroalgae as a nutrient source and resulted in the highest removal of phosphorous and nitrogen in sewage. Marinho-Soriano et al[19] studied the biofiltration capacity of *G. Birdiae* by culturing it in aquaculture wastewater for 4 weeks. A significant reduction in PO_4, NH_4 and NO_3 were confirmed i.e. NO_3 decreased by 100%, PO_4 by 93.5%, NH_4 by 34%. Mithra et al[43] experimented on nutrient absorption by seaweed *C. taxifolia*. under different pH (4-10) with 6, 12, 18 and 24 hrs duration were maintained and observed the utmost removal of zinc and other tested nutrients at pH 7 within 24 hours. Ge et al[16] tested the nutrient removal efficiency of *Chaetomorpha linum*, marine macroalgae in municipal wastewater where 86.8 ± 1.1% reduction in nitrogen and 92.6 ± 0.2% reduction in phosphorous were achieved.

Wu et al[72] experimented with growth and nutrient bio extraction of four different macroalgae i.e. *G. vermiculophylla, U. compressa, Gracilaria chorda and Ulva prolifera* under hypo- and hyper-osmotic conditions and *U. compressa, G. vermiculophylla and U. prolifera* proved to be the best in high nutrient uptake, rapid growth, nitrogen accumulation, removal capacities and high tissue carbon. Cultivation of seaweed has been suggested as an efficient tool in urbanized estuaries because of the high nutrient removal efficiency of seaweed.[29,72]

Heavy metal removal: Strongly acidic sulfonic groups and weakly acidic carboxylic groups are present on the surface of brown macroalgae[58] which have high affinities towards metallic groups. Three different species of dead red algal biomass such as *Hypnea sp*, *Laurancia obtusa and Geldiella acerosa*, were used to prepare three types of a fixed-bed column for the removal of toxic heavy metal ions such as Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} from industrial effluent and reported high removal efficiencies of metal ion bioremoval in an algal column of *L. obtusa* was 94%, in *G. acerosa* was 85% and the lowest one in *Hypnea* sp. was 71%.[22]

Four different species of red seaweeds *Galaxaura oblongata, Pterocladia capillacea, Corallina Mediterranea and Jania rubens* were used to remove Co, Cr, Pb and Cd ions from aqueous solution. *Galaxaura oblongata* showed optimum biosorption efficiency i.e. 84% with a contact time of one hour.[21] The efficiency of removal of eutrophication factors and toxic heavy metals of macroalgae *Gracilaria* sp was observed in a closed cultivation system. A decrease in the concentration of Cr, Al and Zn by 52.5%–83.4%, 10.1%–72.6% and 36.5%–91.7% respectively was observed[25].

Matheickal et al[40] used *Ecklonia radiate*, brown marine algae to develop a biosorbent material, having the potential to remove Cu^{2+} ions from wastewater. Within 15 minutes of the initial time of contact, almost 90% of adsorption was observed. Many studies has proved the efficiency of using macroalgal species for the removal of heavy metal from contaminated water by freshwater algae[51], marine algae and most of these studies concluded on using *Ascophyllum* and *Sargassums* species[13] for metal removal. Biosorption capacity of marine brown macroalgae *Sargassum wightii*, red algae *Gracilaria corticata* and green algae *Ulva fasciata* were tested from an aqueous solution of heavy metal arsenic(As) and found to concentrate the metals on to algal cell walls. Utmost removal of arsenic i.e. 90.2% was noted in *G. corticata* and *S. wightii* at a contact time of 90 minutes.[8]

Textile wastewater treatment: Mahajan et al reported 68%, 78%, 82%, 86% decrease in TDS (total dissolved solids), COD, BOD and EC (electrical conductivity) respectively after culturing *Chara Vulgaris* in 10%, 25%, 50%, 75% diluted textile wastewater for 120 h. Omar et al[48] found the highest adsorption i.e. 95.6% -98.3% of the dye using *Sargassum crassifolium* at the optimal conditions and lowest adsorption i.e. 69%– 77.1% of the dye at a high dye concentration (35 mg L–1). Dry biomass of *Ulva Lactuca* and *Cladophora vagabunda Hoek* was treated with textile wastewater and it was concluded that *Ulva Lactuca* is more suitable than cladophora species for textile wastewater treatment.

Neveux et al[47] observed that when Oedogonium species were cultured in textile wastewater, COD, phosphorus and nitrogen decreased by 57%, 75% and 62% respectively in the decontaminated water within 42 days of algal culture.

Khataee et al[28] investigated decolorization of malachite green dye by using Chara species and the efficiency of decolorization was governed by optimized parameters such as optimum dye concentration, temperature and pH. Deokar and Sabale[10] performed the adsorption of methylene blue (M.B.) and malachite green (M.G.) onto dried biomass of *Ulva Lactuca*. Optimum adsorption of both dyes was reported in 100 ppm dye solution at pH 6. Thus *Ulva Lactuca* was proved to be efficient for the elimination of both dyes from a binary mixture, this study was however with synthetic wastewater, while the degradation process becomes complex with mixture of pollutants.

Microalgae and wastewater treatment: Microalgae species grow well in wastewater as it absorbs organic nutrients and converts them into useful biomass. Being a photosynthetic organism, algae uses solar radiation to convert inorganic carbon into useful biomass and accumulate various nutrients like phosphorus and nitrogen which help to prevent eutrophication.[9] The earliest research about these sailent features of algae was performed more than 60 years before by Oswald and Gotaas in 1957.[50]

Palmer[52] proposed a pollution index based on the algal genus and species. Based on pollution tolerance, the top five species were found to be *Scenedesmus quadricauda, Euglena viridis, Oscillatoria limosa, Oscillatoria tennis* and *Nitzschia palea* and the top eight genera were found to be *Chlorella, Euglena, Scenedesmus, Chlamydomonas stigeoclonium, Nitzschia, Oscillatoria* and *Navicula.* This genus and species indices of Palmer are mostly used in the rating of highly polluted water containing heavy organic loads. Another important aspect of Macroalgae is it is dominance and hence cannot be superseded by other microbes this is an important factor to maintain species uniformity while cultivating algal cells.

Sewage treatment: Sewage is a liquid containing wastes primarily from domestic activities of a locality discharged into the water containing mixtures of toxic chemicals (emerging contaminants, persistent organic pollutants etc.) as well as disease-causing organisms. Currently, sewage is the largest source of water pollution of domestic activities. Center for Science and Environment (CSE) reported that everyday Indian cities are producing over 40,000 million liters of sewage. Agal systems have shown to possess the potential to treat wastewater of domestic origin.[20]

Ahmad et al[1] investigated the capability of *Chlorella vulgaris* to treat municipal wastewater and they observed 100% removal of BOD, 99.9% removal of COD, nitrate, phosphate and total coliform. A six-day study of municipal wastewater by a mixed culture of microalgae was done by Ahmad et al[2] and they observed the fresh weight and dry weight of mixed culture to be 3.34g/day and 3g/day respectively. A notable reduction of total phosphate, sulfate, ammonia, chloride and Kjeldahl nitrogen was marked by mixed algae culture from wastewater.

Tam and Wong[66] made a comparison between *Chlorella pyrenoidosa* and *Scenedesmus* by culturing both the species in suspended and settled sewage, they concluded that *Chlorella* cells performed better than *Scenedesmus*. Significant research is being carried out using microalgal culture system for the treatment of agricultural wastes[57], agro-industrial wastes[56], food processing and other industrial wastes.[26]

Treatment of textile effluent: Recently microalgae have been offering an elegant solution for textile wastewater treatment by removing BOD, COD, azo dye and also inorganic pollutants like nitrate, phosphate, sulfate, ammonia etc. A 28 days observation by Subashini and Rajiv[65] was done using *Chlorella Vulgaris* in textile wastewater and they confirmed that *C.vulgaris* has reduced BOD, COD as well as azo compound present in textile effluent.

Anandhan et al[4] concluded that green algae *chlorella* species have the potential to remove the indigo textile dye and, COD by 46% and 89% respectively within five days duration. Argaw and Asmare[5] reported that 82.6% decolorization, 91.50% reduction in COD, 91.90% reduction in BOD and 89.10% reduction in TDS was achieved in 20 days with mixutres of *Synedra* sp., *Scenedesmus* sp, *Achnanthidium* sp. and *Chlorella* sp., when grown in a photobioreactor in optimum condition. *Chlorella pyrenoidosa*, a microalga was cultivated in different concentrations of textile wastewater and reduction in BOD, phosphate, nitrate were 63%, 87% and 82% respectively along with the reduction in methylene blue dye.[69]

Heavy metals removal: Microalgal cells are capable of removing metals present in the aqueous environment by both intracellular absorption and extracellular adsorption which are metabolic dependent and nonmetabolic dependent processes respectively.[38,42] Due to this remarkable ability, either nonliving or living cell biomass of microalgae have been used for removing heavy metals from contaminated water.[42,55] Biosorptions of cobalt (Co), zinc (Zn), copper (Cu), cadmium (Cd), lead (Pb) and nickel (Ni) in algae-treated bark and pine bark were compared.

Pseudokirchneriella subcapitata sp. and *Chlorella* sp. showed the maximum potential of metal sorption[36]. Terry and Stone[67] monitored the biosorption efficiency of water contaminated with heavy metal copper (Cu) and cadmium (Cd) by investigating both living and nonliving *Scenedesmus abundans* and concluded that living spp. resulting in maximum absorption of metal.

Travieso et al studied the effect of three heavy metals zinc (Zn), chromium (Cr) and cadmium (Cd) on the growth of two different species of microalgae *Chlorella vulgaris and Scenedesmus acutus* and marked that the efficiency of tolerance as well as up-take of these three heavy metals is higher in *Scenedesmus acutus* as compared to the microalgae *Chlorella Vulgaris*.

Shanab et al[62] tested three freshwater microalgae *Scenedesmus quadricauda, Pseudochlorococcum typicum and the cyanobacterium Phormidium ambiguu* to determine the bio removal potential of lead (Pb^{2+}), mercury (Hg^{2+}) and cadmium (Cd^{2+}) in aqueous solutions where *P. typicum* showed the highest percentage of metal bio removal i.e. 70% of lead, 86% of cadmium and 97% of mercury ion in the first 30 minutes of exposure while *S. quadricauda* and *P. typicum* were proved to be more efficient to eliminate the heavy metal contamination from wastewater.

Cameron et al[7] used *Tetraselmis marina* AC16-MESO to study the bio removal efficiency and the efficiency of tolerance to heavy metal ions where it was observed a complete removal of iron, 40-90% removal of copper and 20-50% removal of manganese ion within 72 hours. In various other researches, microalgae based sequestration of chromium, copper, nickel, lead, cadmium like heavy metals have been documented.[33,70]

Algae used as biological indicator: Biological indicators are the organisms or populations whose existence indicates the environmental condition.[32] Due to colonization in almost all habitats, diatoms sustain in a wide range of ecological conditions and hence are extensively used in water quality assessment and multiple indicators of environmental change.[60,64] (Round, 1991; Stevenson and Bahls, 1999). Algae are the most relevant organisms for the estimation of water quality due to the following reasons:

- Very short life cycle
- Rapid reproduction rate
- Sensitive to pollutants
- Wider distribution among the ecosystem
- Bioaccumulation of organic and inorganic pollutants
- Primary producer in aquatic habitat
- Directly affected by chemical and physical factors.

Future Perspectives

Advanced wastewater treatment uses “photocatalyst” such as titanium dioxide, vanadium dioxide, magnesium dioxide, iron oxide which convert phototonic solar energy to chemical energy by artificial photosynthesis process and thus remove pollutants like POP (persistent organic pollutant) effectively from wastewater. With the help of chlorophyll A pigment, photosynthetic algae capture sunlight and transform simple inorganic substances into value added bio products.

Due to the catalytic function, genetic implantation of specific enzymes to photosynthetic algal strain shall stimulate chemical reactions which would induce the degradation of several emerging pollutants. And likewise, we would be able to replace metal-based photocatalyst with algal-based “biocatalyst” which will be environmentally safe and economically beneficial for the purification of wastewater.

Conclusion

The use of algae also called “the green technique” for wastewater treatment is widely accepted because it acts as a “biofilter” for the minimization of high concentrations of several nutrients like nitrogen, phosphorous, sulfate, heavy metals, inhibition of pathogens, removal of biological oxygen demand (BOD) and chemical oxygen demand (COD). Algae are suitable alternative organisms that can utilize the CO_2 produced from the degradation of organic matter while generating oxygen to increasing the DO conent of water.

Along with the treatment of wastewater, algae can produce commercially important compounds from wastewater such as biofuels, feedstocks, nutrient supplements and secondary metabolites with the medicinal properties. When it comes to wastewater treatment, the challenge however, is in the clarification of algal cells from treated water. Since microalgal cells lack the capacity to form flocs or mats (while bacterial cells could be easily be clarified from treated water), algal cells do not aggregate, thus increasing the cost of clarification. Macro algal cells on the other hand could be easily separated (filtered) from treated water, they however lack the metabolic diversity unlike the microalgal cells.

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