***Review Article***

# Sewage Effluent: A Potential Nutrient Source for Microalgae

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Enrichment of water bodies with nutrients from sewage effluents contribute to the process of eutrophication which has developed into serious water management problems throughout the world. The excessive growth of algae or higher plants that occur as a consequence of eutrophication leads to many water quality problems including oxygen depletion, increased water purification cost, decline in the conservation value of water, loss of livestock and possible sub lethal effects of algal toxins on humans who use eutrophic water. There are concerns about the impact of the quality of recycled water being used. Water quality issues may include nutrient and sodium concentrations, heavy metals, and the presence of contaminants such as human and animal pathogens, pharmaceuticals and endocrine disruptors. In recent years, the importance of biological waste treatment systems has attracted the attention of workers all over the world and has helped in developing relatively efficient, low cost waste treatment systems. Photosynthetic organisms, which have high growth rates, produce significant biomass with efficient nutrient utilization potentials and which could be mass cultured in waste waters, play a dual role in cleaning up of polluted water and serving as a source of feed or fertilizer.

**Key Words: Sewage, Effluent, Nutrient, Source, Scavenging, Microalgae**

## Introduction

The rapid development and industrialization coupled with an increased awareness about the need for a clean environment have forced industrialists, environmentalists and governments to look for cheap, efficient and lasting solutions to waste treatment and recycling. In recent years, the importance of biological waste treatment systems has attracted the attention of workers all over the world and has helped in developing relatively efficient low cost waste treatment systems. For a number of years, there has been a series of investigations indicating that photosynthetic autotrophs could be used as organisms indicative of quality of water and for the reduction of pollution load or as scavengers for nitrogen and phosphorus [1].

A great deal of interest has been centered on microalgae as potential candidates for bioremediation of polluted water bodies [2]. These organisms hold an important position in ‘green clean’ technology and have a great capacity not only in treating the wastes but also produce a variety of useful byproducts from their biomass [3]. The ease with which, various microalgae can be cultured and the variety of biomass rendered by them as living, dead or immobilized have made them one of the most potent bioremediators. Despite the algal diversity and relatively inexpensive algal biomass, there has been little commercial exploitation of these organisms for nutrient scavenging, recovery and further prospecting. Sometimes waters suitable for reuse is produced in large volumes, which if not used would be merely discharged into the environments. It is well known that discharge of effluents, treated or non-treated, into the environment, particularly natural water bodies such as lakes, rivers and the coastal bodies can cause degradation of these water ways. This is often related to the presence of organic and inorganic nutrients, which can cause problems such as eutrophication and algal blooms. Reusing these discharged effluents can have a significant impact on reducing or completely removing the impact of these effluents from receiving environments [4].

This review deals with waste water generation, pollution load and the role of microalgae in the bioremediation and their nutrient scavenging potential from the polluted effluents.

## Waste Water Generation in India

A survey carried out by Central Pollution Control Board [5] on the status of generation, collection, treatment and disposal of waste water in 212 class I cities covering 65 percent of Indian population, indicates a total generation of 12,145 million liters of waste water per day (MLD). Out of this, only 2633 MLD (22%) are collected through sewage system and rest are directly discharged to land or water without treatment. From the total collection of 2633 MLD through sewage system, 895 MLD gets primary treatment alone and 1626 MLD gets both primary and secondary treatment.

In India, some 5000 large and medium industrial units are responsible for pollution of water bodies. But

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their contribution to the total pollution load is less than 10 percent; the rest is of domestic origin. For example, the industries of Bombay account for only 13 percent of the total waste dumped into water bodies and in Calcutta 11 per cent waste is of industrial origin. In Delhi, the river Yamuna takes in 200 million liters of untreated human wastes everyday against 20 million liters of industrial waste water. However, most of the time the industrial waste water pose a greater threat due to the presence of toxic chemicals and non biodegradable organic matter [6]. A calculation on average nutrient content of Indian sewage collected from 322 class I cities and towns [5] reveals that the total quantity of plant nutrients generated per day from sewage comes to about 400 tones of nitrogen, 136 tones of $P_2O_5$ and 480 tones of $K_2O$. This calculation is based on the average nitrogen, $P_2O_5$ and $K_2O$ content of 50, 17 and 60 mg respectively, per liter of sewage [7].

**Pollution Load in Waste Waters**

Population enhancement in the metropolitan cities has rapidly expanded the discharge of partially treated and/ or untreated sewage to the enclosed water courses and thus, has magnified the nutrient load and the eutrophication problem [8,9]. Daily $NO_3^-$ and $PO_4^-$ inputs from the new towns have been reported to be 14291 and 6280 kg respectively [10]. High amount of phosphorus content in sewage water has been recorded due to increasing use of domestic synthetic detergents [11]. A survey of rivers across Europe found that out of the 1000 monitoring stations analyzed, 90% had levels of total phosphate in excess of 50 μg P/liter [12], whereas a survey by the former U.K. National Rivers Authority found that 23% of lakes in Britain were severely affected by eutrophication [13]. The Urban Wastewater Treatment Directive 91/271 has been imposed in Europe, which specifies compliance limits for P removal from wastewater and in particular for those treatment works which discharge into areas designated as eutrophication sensitive [14].

**Growth Response and Nutrient Scavenging Potential of Microalgae**

The luxuriant growth of algal forms in eutrophic waters is a common phenomenon and the role of algae in the removal of various kinds of inorganic and related substances by their metabolic activities have been studied by several workers [15, 16, 17, 18, 19]. Cultural studies have been carried out in sewage with *Euglena gracilis* and *Chlorella pyrenoidosa*, which are frequently encountered in waste stabilization ponds [20, 21]. Owing to the ability of microalgae to utilize nitrogen and phosphorus for the growth, they have been extensively used in stabilization ponds [22, 23] in lagoons [24] and in tertiary treatment of sewage [25] for the removal of pollutants. Two different genera of photosynthetic microalgae (*Chlorella pyrenoidosa* and *Scenedesmus*) were grown in settled and activated sewage filtrates with two different inoculum sizes. High levels of nitrogenous compounds in waste waters can be effectively removed only by algae and higher growth rates were recorded in cultures with higher inoculum size and algal cells usually grew better in settled sewage than in activated sewage [26]. Hyper concentrated algal cultures are more effective in removing nutrients from the waste waters, thus having higher potential for waste water treatment [27].

Ecological study carried out in the effluent affected area of Orissa's sponge iron limited near Keonjhar reported the occurrence of five strains of blue green algae, two strains of green alga and *Euglena* in the polluted waters. The liquid waste of the factory contains excessive amounts of sodium, iron, sulphate, carbonate and traces of nitrogen and phosphorus [28]. The amount of algal material that can be produced from one milligram of nitrogen has been estimated from 10.5 milligram to 20 milligram for *Microcystis aeruginosa* [29, 30]. Attempts have been made to utilize blue green algae for low cost biological treatment of waste waters through nutrient manipulations [31]. The role of cyanobacterium, *Oscillatoria pseudogeminata* var. unigranulata has been studied in reducing the pollution load from sewage water. It was found that the maximum reduction of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) was around 80 percent and the initial dissolved oxygen increased considerably [32]. This correlation between initial increase in dissolved oxygen and removal of BOD and COD observed in the study agreed with the observations made by Kankal et al. [33]. Oswald et al. [34] observed the extent of decrease in BOD through algal growth and bacterial activity ranged from 58 to 73.8% but Sengar et al. [35] showed the range of BOD from 57.2 to 76.7%. Jayangoudar et al. [36] studied the reduction of organic pollutants and BOD load from slaughter house effluents using *Spirulina* and *Chlorella*. Others have also shown the reduction in the nutrients/ pollutants from waste water treated with blue green algae [32, 37, 38]. These hazards can be further minimized through the reduction of number of pathogenic microorganisms as well as oxidation of organic materials [30]. The phosphorus reduction in the polluted waters decreased the N fixing cyanobacteria significantly in the central parts of Finland. Therefore, the purification efficiency in the sewage treatment plants would provide an opportunity to control the intensity of toxic blooms of nitrogen fixing cyanobacteria [40].

Various dilutions of sugar factory effluent (20, 40, 60, 80 and 100%) over the control showed significant increase in the growth and pigmentation of laboratory cultured blue green alga, *Westiellopsis prolifica*. The growth stimulating effect of the effluent increased with increase in concentration without any morphological abnormalities [31]. Under laboratory conditions, enhancement in the growth of *Westiellopsis prolifica* has been recorded over control, with the increase in concentration of paper mill waste water up to 100 per cent and maximum growth was observed during 14-16 days of incubation [6]. It was also observed that there was a decrease in biomass and pigments in absence of specific nutrients from the medium and with the addition of these, there was an increase in these parameters [7].

Increase in the microalgal biomass proportionally enhanced the removal of eutrophication causing nutrients from the sewage effluents which are known to supply necessary inorganic compounds especially nitrogen and phosphorus [41, 42, 43]. On the basis of experiments with monoculture of *Scenedesmus*, it was concluded that the N: P ratio of 30:1 by moles is the optimal ratio for growth and the growth is limited at higher and lower ratio values. The lower content of total nitrogen in activated sewage than in settled sewage might decrease the maximum rate of 'P' uptake by the cells [44]. The optimal N: P molar ratio for the cyanobacterium *Anacystis nidulans* was also reported to be 30 [45]. When the productivity of two species of green algae, *Scenedesmus quadricauda* and *Stigeoclonium tenue* were compared, the critical N:P supply ratios by weight turned out to be 22:1 for the former and 17:1 for the latter [46]. A number of experiments conducted with natural fresh water phytoplankton in the laboratory conditions at N: P ratio of 5, 15 and 45 indicated that at lower N: P supply ratio, the cultures were dominated by *Diatoms* and green algae. However, under P deficiency, *Synechococcus* suppressed the growth of competing green alga *Scenedesmus quadricauda* [47]. N: P ratio values higher than 25 by mass were unfavourable for the growth of cyanobacteria [48]. Kinetics between nitrogen and phosphorous removal due to N: P ratio (mg N/mgP) was 6.6 [26], which was also close to the optimal value of 7.0 [49]. Multiple linear regression analysis of the data summarized from twenty-two lakes worldwide suggested that total nitrogen, total phosphorus as well as light interact to determine the relative biomass of blue green algae [50]. Cyanobacteria tend to become rare at total nitrogen to total phosphorous ratio of 30 [51].

Nitrogen can be removed from waste waters by a number of physicochemical methods and efforts have been made to develop techniques for removing nitrogen and phosphorus from sewage effluents destined for ocean disposal [52]. However, none of these methods is fully efficient if the waste waters carry several forms of nitrogen and concentration in excess of several hundred milligram nitrogen per liter. Two biological methods, namely nitrification and denitrification [53, 54, 55] and intensive algal cultures [56] have been proposed for the removal of nutrients from such waste waters. The two major faults of intensive algal culture are the relatively small resistance of algal cultures to ammonium [57] and the preferential utilization of the reduced forms of nitrogen (urea and ammonium) as a result of which nitrates and nitrites remain in the waste waters [56]. This form of nitrogen is removed from waste water only after the concentration of ammonical nitrogen falls below a certain level and depletes from the media and a modified system, which resulted in 94 to 99% removal of nitrogen from the waste waters has been proposed [58,59]. The specific use of cyanobacteria, both free and immobilized forms have been reported in the efficient removal of different forms of combined nitrogen and phosphorus [60].

To meet these increasingly stringent targets, which generally require P removal efficiencies from sewage of over 80%, two main processes are employed, either separately or in combination (1) chemical precipitation and (2) enhanced biological phosphate removal. Chemical precipitation is the traditional and is still the most common method of P removal from waste water streams. This method involves the use of ferric, ferrous, aluminium, or calcium salts [61].

Our awareness of the phenomenon of enhanced biological phosphorus removal dates back to 1955 when Greenberg et al. [62] proposed that under certain circumstances, activated sludge had the ability to accumulate phosphate in excess of that required for balanced microbial growth. While studying the feasibility of growing rice plants on the surface of an activated sludge plant, Srinath et al. [63] found that the plants suffered from the characteristic symptoms of phosphate deficiency exemplified by excessive vegetative growth and diminished grain formation. There was 100 per cent removal of nitrate and ammonia and 50-100 per cent removal of nitrate and a total or near total removal of all types of phosphates from sewage by *Oscillatoria* alone or in combination with natural microbial population [64]. The capacity of blue green algae to remove large amount of phosphorus from waste water has been demonstrated by numbers of other workers [26, 65]. It has been known that low carbon or unfavorably low C: P ratio could reduce cellular phosphorus uptake and may even cause the release of phosphorus from microbial flocs of the activated sludge [66].

The ammonium nitrogen reduction from 19 mg to 3 mg $NH_4^+/dm^3$ was possible within few days and the concentration of phosphorus got reduced from 24 mg to 1.1 mg $PO_4^-/dm^3$ only after 12 days using sewage treated by active sludge with the periphyton complex consisting of *Ulothrix, Oscillatoria, Phormidium* and other micro-algae and diatoms [67]. Survey of the microflora of several municipal activated sludge plants revealed that

34% of isolates were capable of enhanced phosphate uptake under acid conditions and that levels of phosphate removal could be increased by 55-124% through adjustment of culture pH to 5.5. The pH optimum for phosphate uptake varied between isolates obtained from these plants but in all cases ranged from pH 5.0 to 6.5 [68]. Phosphate deprived *Euglena* and *Chlorella* acquired the ability to rapidly incorporate added phosphate via luxury uptake with synthesis and accumulation of polyphosphates. This suggested that more efficient nutrient uptake could be obtained by regulating the physiological conditions of the cells [69].

When the aerated swine waste was diluted 50% with distilled water, 75% of ammonia, 53% of total phosphorus and 98% of orthophosphates were removed by suspended *Spirulina* which exhibited optimal growth [70]. *Chlorella* and *Scenedesmus* were the most efficient algal strains to eliminate phosphate from mixtures of municipal and refinery wastes [71]. The daily removal rates of $PO_4^{3-}$-P and $NH_4^+$-N were 0.96 and 2.0 mg/l respectively, in a batch culture of *Scenedesmus* in pig manure [72]. Most algae are unable to utilize the complex phosphates used in detergent formulations and depend upon the phosphatase activity of bacteria to hydrolyze them to the available orthophosphate forms [73]. Finstein & Hunter [74] have reported that conversion of complex phosphates to the orthophosphate form is not normally completed during biological waste treatment. Therefore, the considerable proportion of phosphorus may not be immediately available for algal assimilation.

*Oscillatoria* removed over 4-20 per cent of calcium in unsterilized and sterilized sewage respectively, and magnesium reduction in unsterilized sewage was 76 per cent [64]. In addition, other studies revealed that nutrient removal efficiency is related to the content of nutrients in waste, the degree of nutrients utilized by algal growth and the nutrient concentration in algal tissues [56, 58].

**Biotechnological Aspects**

Lot of attention has been paid to the bio-technological aspects of algal growth on sewage which not only plays a central role in the photosynthetic oxygenation of waste waters but also promotes the production of algal biomass rich in proteins having nutritional value [75, 76, 77]. The high rate oxidation pond also named accelerated photosynthetic pond or high rate waste stabilization pond has got increased attention as a means of treatment of waste water as well as for the production of algal biomass [23, 42].

Dor [78] inoculated *Scenedesmus obliquus* on tap water grown in dialysis tubing, which was suspended in raw sewage. Osmotic contact with the surrounding bacterial medium had a powerful stimulatory effect on algal growth and the algal biomass proliferated on the nutrients extracted from the waste water without being mixed with it. Characteristic feature of dialysis culture to extract growth materials from the great volume of nutritional medium and the dense biomass yield makes this method particularly suitable for the production of single cell algal proteins from waste waters [79].

A satisfactory method for biological recycling of pig manure with aerobic fermentation followed by *Scenedesmus* culture has been reported. The results indicated that the hyperconcentrated algal cell suspension grown on slightly diluted aerated manure is a promising way of simultaneously treating pig waste and producing good quality biomass that can be used in animal feeding [72]. Such a scheme has also been worked out in Singapore where microalgae grown on swine manure are returned into the animal diet [80]. Many other authors have also recommended aerobic fermentation prior to or during cultivation of algae as this operation leads to good depollution performance and high biomass yield [76]. Oswald et al. [23] and Schroeder & Hepher [81] showed that undesirable pig waste could be converted into proteins, which can be utilized for animal feeding.

Limited information is available in use of algae for removing nitrogen and phosphorus from sewage effluents with high salinity [82, 83]. This can be accomplished by the use of unicellular green alga *Chlorella salina* before discharging the effluent in coastal marine water [65]. Uptake of nitrate was reported to occur in their study after the ammonia level was reduced to level below 0.5 ppm. Such cultures with high protein content can be used to serve the dual function of waste water purification and waste recycling through the production of algal protein. Although low concentration of ammonia upto 2 mM were found to be beneficial to various algae in field condition [84], unionized ammonia at higher concentration is known to be toxic to wide range of organisms such as *Prymnesium parbum* [85, 86], marine diatoms [87] and, prawns and fish [88]. According to Golueke [89], high rate oxidation ponds failed to operate when supplemented with over doses of ammonia.

Active uptake of nitrate and soluble inorganic carbon during algal photosynthesis results in rise in the pH value of the culturing media [75, 90]. It is evident from the studies undertaken by Hemens & Mason [25] that lagoons of more than 10 cm depth would be unnecessary for exploiting photosynthetic activity in contrast to oxidation ponds treating raw sewage in which bacterial stabilization is the important process. During active photosynthesis in algal culture, the bicarbonate-carbonate equilibrium is disturbed due to algal uptake of $CO_2$ and high pH values may result that may cause precipitation of phosphates in pond systems [91, 92]. Kaneshige [93] found it necessary to add $CO_2$ to algal cultures to prevent this as the growth was severely affected. An increase in pH value above 10 in sewage algal system may cause coagulation and absorption of inorganic phosphate [25, 90] and evolution of $NH_3$ into the atmosphere due to its decreased ionization [94, 95].

In sewage plants, with enhanced biological phosphorus removal, the activated sludge must pass alternately through anaerobic and aerobic stages. During the anaerobic phase, the phosphates that had been previously taken up aerobically by the biomass and stored as polyphosphate are partially released. The higher anaerobic release, the more effective is the subsequent aerobic phosphorus removal and a deficiency in dissolved oxygen was proposed as a triggering factor for the phosphate release [25]. However, others found a correlation between phosphate release and low oxidation-reduction potential (ORP) [96, 97]. Earlier investigators showed that nitrate can also influence phosphate release in activated sludge differently. These depend upon the composition of bacterial flora that in turn can change with alternations in the processes used for phosphorus removal, the sewage composition and other environmental conditions [98]. For the application of enhanced biological phosphate removal, several types of activated sludge plants have been designed mainly based upon empirical data and undefined sewage as influents [99]. A simple, one reactor vessel system called a fill and draw system was developed for the study of enhanced biological phosphate removal under defined conditions [100]. Schon et al. [101] worked on influence of dissolved oxygen and oxidation-reduction potential on phosphate release and uptake by activated sludge from sewage plants with enhanced biological phosphorus removal.

Addition of 2, 4-dinitrophenol to the biomass inhibited phosphate uptake thus indicating that the excess phosphate removal was biologically mediated [102]. The exploitation of waste water treatment phenomenon of luxury phosphate uptake suggested a potential biotechnological approach that ultimately led to the development of the Enhanced Biological Phosphorus Removal (EBPR) process. The exact physiological reason why EBPR microorganisms should replenish poly phosphate reserves at the expenses of PHB/PHV, instead of utilizing these biopolymers solely for growth and biomass production, is at present unknown [103]. The unicellular alga *Dunaliella salina* and *Saccharomyces cerevisiae* utilize their intracellular poly-phosphate reserves to provide a pH-stat mechanism to counterbalance alkaline stress [104, 105, 106].

## Potential Risks from Using Recycled Water and Public Concern

Some risk factors are short term and vary in severity depending upon the potential for human, animal or environmental contact (eg. microbial pathogens) while others have long term impacts which might increase with the continued use of recycled water. Enteric pathogens (viruses, bacteria, protozoa and helminthes) can enter water either directly through defecation into water through sewage effluent or from run-off from soil and other land surfaces [107]. The risk of water-borne infection from these pathogens would depend upon pathogen numbers and dispersion in the water, infective dose required and susceptibility of an exposed population, the chance of faecal contamination of the water and the amount of treatment undertaken [108]. Bacteria are the most common of the microbial pathogens found in the recycled waters [109]. There are several protozoan pathogens which have been isolated from waste waters and recycled in water sources [110]. Public and commercial concern does exist regarding pathogens through the use of recycled water [111]. Heavy metals are easily and efficiently removed during common treatment processes and the majority of heavy metal concentrations in raw sewage end up in the biosolid fraction of the treatment process with very low heavy metal concentrations present in the treated effluents [112]. It is well known that the algae grown for cattle fodder using sewage as the source of nutrients can easily transfer toxic metals present in the sewage to the feeding animals.

It has also been noted that the organic carbon present in the sewage effluent can stimulate the activity of soil microorganisms [113]. Chakrabarti [114] observed that the rice crops gave a higher yield when irrigated with raw or partially diluted sewage effluent compared to unamended groundwater. Effluents commonly used for reuse purposes, for example treated sewage effluent can have electrical conductivity less than 1dS/m [115, 116] which may not cause any problem regarding utilization of such waters.

Most people tend to become less supportive toward use of secondary treated sewage effluent when the chances of personnel contact increases [117]. While the actual physical risk from the treated sewage effluent can be similar or less than that of untreated stormwater, the public perception can lead to a belief within the community of a greater risk from the effluent [118]. Consumers and exporters are still concerned about the potential for negative health and environmental impacts [111]. More scientific research and effective communication as well as effective interaction with government agencies is needed to provide information on the merits and potential risks of using treated sewage effluents.

## Algal Hazards

Though blue green algae are of a great value to us, yet the negative aspect of these aquatic plants cannot be overlooked. These may produce coloration, off flavours and toxins making the water unfit for drinking and even washing. These are also implicated as causative agents for several instances of dermatitis and other itching problems. The respiratory disease, tamadare fever is related with blooms of *Trichodesmium erythraeum*. Algal blooms due to luxuriant growth of blue green algae can cause severe economic losses to aquaculture, fisheries and tourism operations, and have major environmental and human health impact [119].

## Conclusions

Regions with high and constant solar radiations which offer appropriate conditions for the operation of conventional algal ponds are considered suitable for the implementation of algal technology for bioremediation of sewage effluent [120]. These can be applied for waste water stabilization systems in the country on the whole.

Strategies that exploit alternative triggers for the induction of N and P accumulation by microalgae are potentially of great biotechnological benefit. The imposition of any condition under which these mechanisms are necessary for the survival of micro-organisms should result in enhanced nutrient removal from waste waters. Knowledge of such conditions might be exploited to provide alternative and possibly superior treatment options for biological removal of N and P from municipal effluents.

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