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RESEARCH ARTICLE

SUSTAINABLE METHOD OF WASTE WATER TREATMENT USING MICRO-ALGAE

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Abstract

The present study illustrates the efficiency of microalgae based treatment system. Microalgae can treat the wastewater very efficiently (that contain organic components in abundance). The paper emphasizes on the practical aspect of biological removal process that is natural in which Microalgae removes substances (both organic and inorganic), BOD, COD and other impurities present in the wastewater in the presence of sunlight and CO₂. During the study it was found that variability in algae play a different role in treatment process. It has been observed that nutrients removal efficiency of microalgae is very high and effective as it removes 78-99% of Nitrogen and Phosphorus. The treatment system also succeeds to remove 40-65% of COD, BOD and other impurities present in wastewater. This paper reveals that the bioremediation capacity of microalgae to treat wastewater would be a better alternative to the classic wastewater treatment process. This study focused on the sorption capacity of two algal species Chlorella vulgaris and Hydrodictyon reticulatum in four different places (1. Civil line, Chaupati, B.T. Ganj and Sonali Bridge of Roorkee).

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Introduction:-

Water is the most essential need for the survival in the earth. Water is present around 60% (of body weight) in adult human and 75% (of body weight) in children. Due to industrialization and development there is an increase in population which is the most critical environmental problem in developing countries like India. Due to this rapid industrialization and urbanization the amount of wastewater generated every day is very huge. Due to this, water pollution is one of the most critical environmental problems. For the treatment of waste water various conventional methods are used in India but they are not so economical and have a negative impact on the surrounding environment. Due to rapid advancement in technology, some green technical methods are introduced for the treatment of waste water to solve the problems of domestic waste water treatment.

There are some unconventional biological methods that are very helpful in treating water pollution just like the bioremediation technology that has been used for treating toxic and hazardous waste. It is ecologically reliable and economic as compared to other technology that uses some chemicals, many countries successfully use these ancient technologies in the current scenario (Enrica, 1994).

In bioremediation technology or biological treatments different types of microorganisms are capable of utilizing organic waste and reducing the pollutant content of waste water. These microorganisms utilize organic content as a

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food source and takes energy from them by releasing soil nutrients as a remaining substance with lots of oxygen content (Metcalf & Eddy, 2003)

In last 55 years the treatment of Biological wastewater through Microalgae have gained importance and now it is accepted widely that microalgae based wastewater treatment system are as effective as conventional wastewater treatment system.

In early 1970s the first cultivation of Microalgae was started but in the initial stage it was grown in ponds containing wastewater, in order to prevent Eutrophication, it was first tested on secondary effluent. Treatment through Microalgae is very efficient as it removes nutrients very effectively from wastewater.

Algae are small photosynthetic plants that perform photosynthesis process for own needs and release back O₂ after utilizing CO₂ in this way microalgae based treatment plants require less energy resources and deduct the use of electromechanical blowers that consumed large amount of energy (Green et al. 1995). These microalgae do not require anaerobic digestion (McCarty et al. 2011) and hydrothermal liquefaction (Chakraborty et al. 2012).

Due to this reason Tam & Wong (1989) used Microalgae in secondary treatment rather than in tertiary treatment. Recent studies shows that some algal species like Chlamydomonas (Elshorbagy and Chowdhury 2013) Chlorella (Sekaran et al., 2013) Botryococcus (Travieso 1999) Haematococcus (Dalrymple, et al., 2013), Scenedesmus (Campbell, 2008), Spirulina (Shen, Y. 2014) etc can effectively remove the nitrogen, phosphorus and other organic (biochemical oxygen demand, BOD) and inorganic impurities (COD) from raw sewage or wastewater. Now a day's Chlorella algae has gained more importance as it is widely used to treat wastewater by removing nitrogen, phosphorus, BOD and COD very efficiently with different retention period ranging from 10hrs to 42 days. During the cultivation process of these algae, a large amount of biomass is produced, which is a great source of food and energy in the form of Biodiesel.

There are number of benefits of growing algae in wastewater as it absorbs nutrient thus reducing the treatment cost of wastewater. Secondary it digests large amount of organic carbon to produce its biomass which can further processed to the production of biodiesel. The growing Environmental problem such as Global warming, the increase of Ozone hole and climate changed can be solved by using Microalgae treatment system as it has ability to consume high amount of carbon dioxide in Photosynthesis process to produce oxygen and glucose. By using sunlight, CO₂ and various nutrients Microalgae has ability to remove nutrients, heavy metals, organic and inorganic toxics substance and other impurities which is present in the wastewater. Algal system is very unique; they have ability to absorb solar radiation in the form of energy in its chloroplast cell and it absorbs CO₂ along with nutrients from the wastewater to synthesis their biomass and produce oxygen. The produce oxygen from microalgae is enough to fulfill most of the aerobic bacterial requirements simultaneously it metabolizing the residual organics in the treated wastewater. In algal system large amount of simpler organic compound formed which can be digested in the aqueous system. These bacteria are good source of CO₂ which is required for algal growth, stimulate the release of organic growth factor & vitamin and change the PH of the supporting medium for algal growth.

Factor Affecting for the Growth of Algae:

Microalgae not only used in secondary or biological treatment but also these are helpful in tertiary treatment as removing the phosphorus and nitrogen that causes the eutrophic conditions in any stagnant water body. De-Bashan et al., (2010).

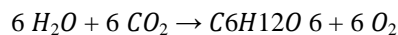
Advantages of using algae for this purpose include: low operational cost, possibility of recycling assimilated nitrogen and phosphorus within the algae biomass as a fertilizer, avoidance of the sludge handling problem, and direct discharge of oxygenated effluent water into the water bodies. In addition, this process is not associated with carbon, as usually required for nitrogen and phosphorus removal, which is an additional advantage for the treatment of secondary effluents.

Sunlight:

Algae are unicellular and photosynthetic microorganisms and they use sunlight for photosynthesis process and converted into organic molecules as carbohydrates. Sunlight is the most important factor for the growth of microalgae and without sunlight microalgae growth would suppressed or reduced.

Carbon Dioxide (CO₂):

Global warming is concern as a major world-wide problem. The increase level of CO₂ causes global warming. All the algae in any ecosystems utilize the Carbon dioxide and sunlight for photosynthesis activity and release Oxygen in to the environment. Algae are the best option for carbon sequencing.



It can fix CO₂ from three different sources viz. atmospheric CO₂, discharge gases and soluble carbonates. According to the studies microalgae cells contain approximately 50% carbon, in which 1.8 kg CO₂ are fixed by producing 1 kg of microalgal biomass (Shen 2014). For the fixing of CO₂ microalgae are considered as more efficient than terrestrial plants.

Nutrients (Nitrogen, Phosphorus and other minerals):

The use of municipal and industrial wastewater effluent as a nutrient feedstock for the production of algal has environmental and economic benefits (Sturm and Lamer 2011). Wastewater nutrients are fed microalgae by different nutrients like nitrogen, phosphor, ammonia, sulphur, iron, toxins and all the metals in wastewater to production of microalgae biomass (Darmaki et al., 2012). Phosphorus and Nitrogen are the primary nutrient for the growth of microalgae while carbon and nitrogen are the secondary nutrient to microalgae growth. Phosphorus is another macro-nutrient essential for growth, which is taken up by algae as inorganic orthophosphate (Larsdotter 2006)

pH:

PH of the waste water is also consider one of the important factor for the growth rate of microalgal and treatment of wastewater. Availability of inorganic carbon also affected by pH, even if pH is high for other reasons than photosynthetic CO₂-exhaustion, the pH regulates what species of inorganic carbon that is available. The increase in amount of oxygen concentration and pH in the wastewater is the cause of phosphorous sedimentation and also ammonia and hydrogen sulphur removal. High pH in algal ponds also leads to pathogen disinfection. Fontes et al (1987) observed that optimal productivity of the cyanobacterium *Anabaena variabilis* were obtained at pH 8.2–8.4, being slightly lower at 7.4–7.8, decreasing significantly above pH 9, and at pH 9.7–9.9 the cells were unable to grow well (Larsdotter 2006).

Temperature:

Temperature is proportional to the availability of sunlight and has little effect when light is limiting. When light availability is not limiting increase in temperature can increase the rate of photosynthesis, growth and doubling rates are consequently (Kendrick, M. 2011). However, light is the important factor for the growth of microalgae, but too much light may also cause the reduction in photosynthesis. With the increment of temperature, the growth rate of algal enhance until an optimum temperature is reached. Further increase in temperature leads to a rapid reduction in algal growth rate. Temperature ranges generally within 20 to 30 °C for the maximum growth of microalgae.

Depending upon the nutritional present in the wastewater, nutrition could be consumed partially or totally. In case of anaerobic digestion process the effluent discharge is rich in nutrition due to which it is recycled to the head of the wastewater treatment plant and can increase the cost destabilized the overall process due to accumulation of large amount of phosphorus. The microalgae system can treat various types of wastewater like, domestic sewage, industrial waste water etc. and reduce the nutrients (Nitrogen, phosphate and other minerals) from the waste water. The effluent of wastewater is rich in nutrients which causes the problem of Eutrophication, due this problem it cannot be discharged directly into water bodies.

There are lots of unique benefits of the Microalgae based treatment systems. Since microalgae are an aquatic species, so they do not require arable land for their cultivation. It means the cultivation of microalgae does not need to compete with agricultural commodities for growing space. The cultivation of algae uses fresh water or saline wastewater and salt concentrations up to twice that of seawater can be used effectively.

By choosing algae for the waste water treatment have large advantages over the conventional treatment methods due to its low cost, production of biomass (assimilation of nitrogen and phosphorus) as a fertilizer, very low or no sludge generation and after the treatment water can be directly dumped in to any water body. Therefore, the aim of this study was to investigate the remediation capability of two fungal species viz. *Chlorella vulgaris* and *Hydrodictyon reticulatum* in four different waste waters.

Material and Methods:-

For this study raw wastewater was collected from the four different sites of Roorkee viz. Civil line (near kathi roll) Fast food cuisine, Chaupati (civil line), B.T. Ganj and Sonali Bridge (near CCD, Café, Coffee Day). Algae were (*Chlorella vulgaris* and *Hydrodictyon reticulatum*) collected from the Roorkee and Haridwar region near the Ganga Canal. Both the cultures were incubated at 25°C at constant temperature and continuous light exposure of 5,000 lux for 14 days in Environmental Engineering Laboratory, COER. All the Physicochemical parameters (pH, Turbidity, BOD, Total hardness, Alkalinity and Acidity) of waste water were analyzed in the laboratory on the same day (Trivedi, and Goel 1986). Whereas with algal samples (50g/l) waste water were analyzed after 14 days of biosorption process The study was conducted using raw wastewater for 14 days at raw waste water pH, constant temperature $25 \pm 1^\circ\text{C}$, and at constant light intensity ($100 \mu\text{E}/\text{m}^2/\text{sec}$).



Fig. 1:- Collection of wastewater near Roorkee city.

Algal Characterization:

Chlorella vulgaris *Chlorella vulgaris* is a eukaryotic, unicellular green alga. It is a genus of single-cell green algae belonging to the family Chlorophyte. It contains green photosynthetic pigments chlorophyll a and b in its chloroplast. Throughout photosynthesis, it multiplies swiftly, requiring only CO_2 , water, sunlight, and a little amount of minerals to reproduce. It is a possible food source since it is high in protein and other essential nutrients; when dried, whereas 45% of protein, 20% of fat, 20% of carbohydrate, 5% of fibre, and 10% of minerals and vitamins.



Fig 2:- Collection of algae near Haridwar.



Fig 3:- *Hydrodictyon reticulatum*.

Hydrodictyon:

Hydrodictyon, also known as “Water Net”, has large colonies composed of elongate cells linked in a reticulated, net-like pattern. Each cell is connected at its end walls to two other cells forming, meshes of five or six cells.

Experimental Design for algal biosorption:

For biosorption of organic impurities wastewater samples were sterilized by autoclaving for 30 min. At the beginning of experiment 1000 mL of wastewater was inoculated in the flasks with pre-weighted (50g/l) *Chlorella vulgaris* and *Hydrodictyon reticulatum* for evaluate the efficiency of nutrients removal by both the algae. The experiments were conducted using municipal wastewater for 14 days at waste water pH and constant temperature $25 \pm 1^\circ\text{C}$ with continuous light intensity ($100 \mu\text{E}/\text{m}^2/\text{sec}$).

The biosorption capability was evaluated after 14 days in terms of values reduction of each parameter was calculated at the interval of 14 days. At the time of analysis samples were withdrawn from flasks, and were centrifuged at 5,000–6,000 rpm, to separate both the algae. After selection of optimum days and pH of raw water (as it was at the time of sampling) the wastewater was treated with *Chlorella vulgaris* and *Hydrodictyon reticulatum* algae separately of 50 g/l. Aeration for all the treatment was provided at 9 l/min. The samples were analysed after treatment. The same has been repeated with the combination of algal species. All the physico-chemical parameters were done according to the standard methods (Trivedi, and Goel 1986) in the Environmental Engineering laboratory, COER. *Chlorella* sp. was selected for treatment of the process water samples due to its ability to quickly use the nutrients found in the hydrothermal process liquid (Chen et. al., 2003).

Result and Discussion:-

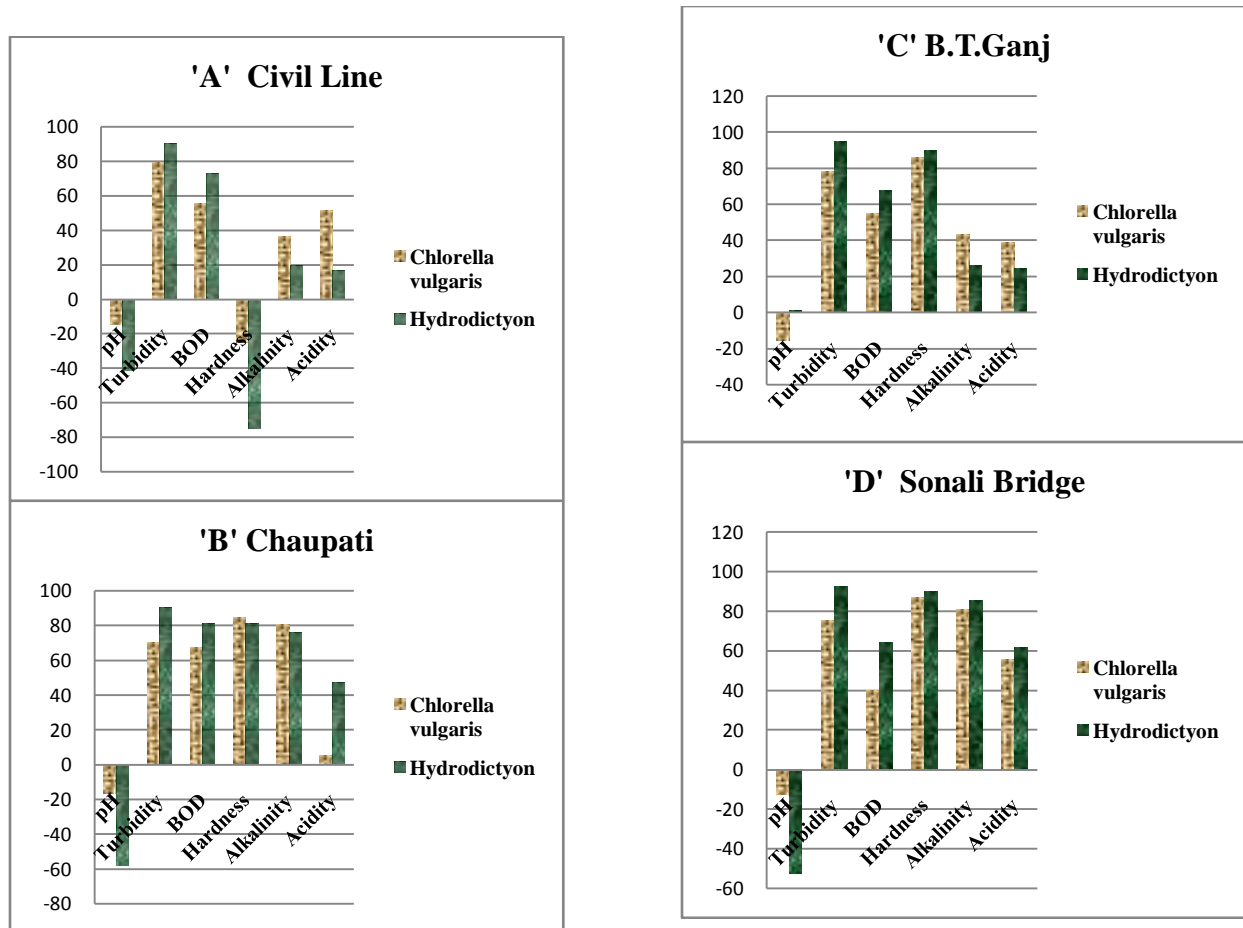
The treatment raw wastewater of Roorkee region with algae (*Chlorella vulgaris* and *Hydrodictyon* algae) was studied earlier with varying conditions (Days) and the optimum amount of algae (weight wise in gm/l) has been finalized. In this study it was 14 days and amount of algae was 50gm (based on earlier study, performed in COER environment laboratory) without adjusting pH. Performance of for the treatment of wastewater was first carried out. The physico-chemical parameters (pH, Turbidity, BOD, Total hardness, Alkalinity and Acidity) of 4 different sites are listed in the Table 1.

S.no.	Locations	Physico-chemical Parameters. (control)	Treatment with <i>Chlorella vulgaris</i> 5g/l	Treatment with <i>Hydrodictyon reticulatum</i> 5g/l
1.	Civil line (near kathi roll)	pH 8.36 Turbidity-61 NTU BOD- 200mg/l Total Hardness-160mg/l Alkalinity-104 mg/l Acidity-96 mg/l	pH-8.49 Turbidity-12.5 NTU BOD- 89mg/l Total Hardness-200mg/l Alkalinity-66 mg/l Acidity-46.8 mg/l	pH -11.83 Turbidity-5.8 NTU BOD- 54 mg/l Total Hardness-280 mg/l Alkalinity-124 mg/l Acidity-80
2.	Chaupati (civil line)	pH-7.10 Turbidity-64 NTU BOD-107mg/l Total hardness-158 mg/l Alkalinity-220 mg/l Acidity-148 mg/l	pH -8.26 Turbidity-19 NTU BOD-35 mg/l Total hardness-24 mg/l Alkalinity-42.8 mg/l Acidity-140 mg/l	pH -11.21 Turbidity-6 NTU BOD- 20mg/l Total hardness-30 mg/l Alkalinity-52.6 mg/l Acidity-78 mg/l
3	B.T. Ganj	pH -8.61 Turbidity-60 NTU BOD-129mg/l Total hardness-144 mg/l Alkalinity-68 mg/l Acidity-96 mg/l	pH -9.94 Turbidity-13 NTU BOD-58mg/l Total hardness-20 mg/l Alkalinity- 38.5 mg/l Acidity-58.6	pH -8.52 Turbidity-3 NTU BOD- 42 mg/l Total hardness-15 mg/l Alkalinity-50.2 mg/l Acidity-72.4 mg/l
4	Sonali Bridge (near CCD)	pH -8.64 Turbidity-69 NTU BOD-107mg/l Total hardness-154 mg/l Alkalinity-104 mg/l Acidity-104 mg/l	pH -9.72 Turbidity-17 NTU BOD-64mg/l Total hardness-20 mg/l Alkalinity- 46.5mg/l Acidity-53.6 mg/l	pH -13.18 Turbidity-5 NTU BOD-38mg/l Total hardness-15mg/l Alkalinity-53.4 mg/l Acidity-39.6 mg/l

Table 1:- Characteristics of wastewater at various location of Roorkee with control sample and algal treatment.

Chlorella vulgaris and *Hydrodictyon* sp. showed maximal growth in municipal waste water at normal room temperature at $24 \pm 1^\circ\text{C}$, and without adjusting pH. Results presented in table 1 indicate that the cells of these algae (50gm/l) without any extra nutrients (for algal growth) showed a remarkable reduction in all the parameters about more than 50 percent reduction on an average. In the present study the reduction or enhancement in all the physiochemical parameters was variable in all the four sites as calculated percentage wise. Percentage reduction or enhancement are well demonstrated in the graphs (A, B, C and D).

The percentage decrement or enhancement in physico-chemical parameters (pH, Turbidity, BOD, Total hardness, Alkalinity and Acidity) of 4 different sites are shown in the graphs 'A', 'B', 'C' and 'D'



pH Enhancement:

In the present study both the algae induced the pH levels in all the four sites differently viz. 15%, 16%, 15% and 13% by the *Chlorella vulgaris* in sites A, B, C and D where as *Hydrodictyon reticulatum* showed maximum enhancement of 58% in site B (Chaupati), 53% (D Sonali Bridge) 42% (A Civil Line) respectively with minimum 1% in site C (B.T.Ganj). The increment of pH in both the culture was due to photoautotrophic growth of *Chlorella vulgaris* and *Hydrodictyon reticulatum* algae they consume consumption of CO₂ in waste water produced by the some bacteria during the natural decomposition and increase the pH level in water (Borowitzka, 1998). In all the seven days there was no pH adjustment in the experiment. Increase in pH was due to the phosphorus precipitation by the formation of hydroxyapatite. According to Diamadopoulos and Benedek (1984) increased pH helps the flocculation process of sludge in biological treatment (Borowitzka, 1998).

Turbidity removal:

Turbidity of four samples was decreased drastically by both algae. It can be seen from the table that reduction in turbidity was more by *Hydrodictyon reticulatum* in four samples 'A' 90%, B 91%, C 95% and D 93% respectively. Whereas *Chlorella vulgaris* showed 75% decrement in turbidity by showing maximum in sample A 79%, C 78%, D 75% to minimum 70% in sample B. Reduction in turbidity was due to the reduction in total coliform bacteria (Riaz et.al 2018).

BOD removal:

Unfiltered BOD in the raw wastewater in all four sites ranged from 100 to 200 mg/l. The decrease of BOD level for all sites was 40% D, 55% C, 56% A and 67% B respectively in *Chlorella vulgaris* treated samples. Whereas *Hydrodictyon reticulatum* showed slightly more reduction in four sites viz. 73% A, 81% B, 67% C and 64% D.

According to the Faleschini et al. (2012), large amount of BOD decreased by sedimentation in the first stage and remaining BOD removed may be due to the oxygenation of sample during the aeration process by which aerobic Bacteria use more oxygen produced by the photosynthetic activities of the algae (Mahapatra et.al. 2013).

Hardness removal:

In the present study hardness of all the four samples was significantly reduced in three sites B 885%, C 86%, D 87% respectively. In the site A, *Chlorella vulgaris* show increment of 25% in hardness, it may be due to the presence of some heavy metals so that *Chlorella vulgaris* do not utilize the CO₂ and precipitation process. Whereas for *Hydrodictyon reticulam* the percentage of reduction was maximum for site 'C' and 'D' with 90% to minimum 75% for site 'A'.

According to the Lu et al. (2017) the calcium ions present in the raw waste water form precipitation with CO₂ as Calcium carbonate which are the contributing components to water hardness. Algal photosynthetic reaction consume CO₂ enabling the calcium ions to precipitate as calcium carbonate (Usui & Azuma 1992) lowering the hardness.

Alkalinity and Acidity removal: It is evident from the figure that both the parameters alkalinity and acidity reduced significantly with both algal treatments in all four sites. The decrement was highest 86% 'D' and lowest 19% 'A' with *Hydrodictyon reticulam* algae. *Chlorella vulgaris* showed height reduction (81%) for site B. In case of acidity there was more variability in the decrement process with 5.4% 'B' (min.) to 56% 'D' for *Chlorella vulgaris* and for *Hydrodictyon reticulam* minimum reduction was observed in site A with 17% to maximum 62% 'D'. The amount of alkalinity depends on the inorganic and organic compounds dissolved in the water with suspended amount of organic matter. For the good growth of beneficial bacteria's there should be required amount of alkalinity so that all the biological species optimize the decomposition process. Decrement in alkalinity means proper utilization of organic compounds by the algae by neutralization with acid present in water sample (Neo Performance Materials 2019). Gross (2000) stated the majority part of the acidic environment reduced by some acidophilic or acid tolerant algae as they utilize them by metabolic process. In this study results showed that both algal species reacted differently according to the amount of acidity.

Conclusion:-

Algal treatment of wastewater is a traditional technique that has been used for over five decades due to environment friendly alternative for removing inorganic and organic nutrients and some heavy metals. Several algal species have been identified and utilized for such studies. *Hydrodictyon reticulam* and *Chlorella vulgaris* were capable of reducing Turbidity, BOD, Hardness, Alkalinity and Acidity to good extend. It was observed in the present study that the reducing capacity of *Hydrodictyon reticulam* was more as compared to the *Chlorella vulgaris*. This technology allows algae to be used as economic with low maintenance for small contaminated systems. In the climate of Haridwar District *Hydrodictyon reticulam* and *Chlorella vulgaris* are abundantly available and easily harness for waste water treatment.

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