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Research Article

Removal of Coliform Bacteria from Municipal Wastewater by Algae

Farooq Ahmad[1]*, Aqsa Iftikhar[1], Adnan Skhawat Ali[1], Syed Ahtisham Shabbir[1], Abdul Wahid[2], Nazish Mohy-u-Din[1] and Abdul Rauf[3]

[1]Sustainable Development Study Centre, GC University, Lahore, Pakistan
[2]Department of Environmental Science, Bahauddin Zakariya University, Multan, Pakistan
[3]Department of Zoology, University of Agriculture, Faisalabad, Pakistan

Abstract: The present work was conducted in Sustainable Development Study Centre, Government College University, Lahore. The focus of the study was the removal of coliform bacteria from municipal wastewater by *Rhizoclonium implexum,* a freshwater algal species. Experiments were conducted on the basis of pond size, algal and water quantity and optimum conditions were determined i.e. Pond size (P2), algal quantity (150 g) and wastewater quantity (7 liters). In this study maximum percent reduction for Chemical oxygen demand (99.4%), Biochemical oxygen demand (99.5%), Total solids (95.1%), Total dissolved solids (97.2%), Total suspended solids (96.1%), Total Kjeldahl nitrogen (98.2%), Total phosphorus (96.8%), Total coliforms (100%) and Faecal coliforms (100%) were obtained. Increase in accumulation of nitrogen (10.75 to 34.9mg/g), phosphorus (257.40 to 268.1ppm) and organic matter (290 to 315.3mg/g) in algal biomass were also observed besides other operating parameters. Biochemical oxygen demand (BOD) and chemical oxygen demand of treated sewage were found in NEQS (2000) permissible limits while total coliform and faecal coliform were totally removed from sewage by *Rhizoclonium implexum* therefore, it could be an efficient technique for sewage effluent treatment.

Keywords: Coliform bacteria, municipal wastewater, sewage, faecal coliforms, algae

1. INTRODUCTION

Human activities are continuously affecting the quality and quantity of water. Water after adversely affected in its quality by various anthropogenic activities is termed as wastewater. It includes an extensive range of waste in the form of pathogens, metals and other contaminants from domestic, agricultural, industrial and other activities. Studies revealed that the major cause of water pollution is the discharge of sewage into rivers and irrigation canals. Release of wastewater without any treatment directly into water bodies is a destructive act because it badly pollutes the water resources. After treating wastewater, it can safely be released into water bodies because treated wastewater is not as much harmful as wastewater without treatment but safe for water bodies [1].

Mainly the municipal wastewater contains various categories of pathogenic agents including bacteria, helminthes, viruses and several protozoan and fungi. Sewage containing these pathogens causes many health problems including water borne diseases especially in the developing nations. There are a number of harmful diseases that can caused by these categories of pathogens which are commonly exist in municipal wastewater. Pathogens of bacteria are very injurious for health and they are source of deaths especially in those regions where sanitation is deprived or improper. In municipal wastewater pathogenic bacteria are specifically in large quantity [2].

Pakistan was already struck with a similar kind of outbreak in 2006 reported by ADB [3]. There is wide range of pathogenic contamination

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*Corresponding author: Farooq Ahmad; Email address: fagondal82@yahoo.com

includes in municipal wastewater. There is group of bacteria known as coliform bacteria which are used as indicators for various disease producing pathogens. This group of indicator bacteria consists of *Escherichia coli* along with other type of bacteria that are found in human faeces or originated from other sources. Water quality can checked for disease causing pathogen of human by the presence of faecal coliform bacteria which are mostly used as an indicator to assess water contamination [4].

Although, there are large number of wastewater techniques and facilities such as adsorption methods, chemical techniques, precipitation, constructed wetlands and other processes are present but the most suitable is biological method because of its least expensiveness for developing countries particularly [5]. Algal-based wastewater treatment method is a better option for areas where expensive conventional methods are not affordable [6].

As algae plays a significant role in removing nutrients, pathogens, heavy metals and other pollutants from the sewage and this type of treatment process is easy to handle, more effective and environment friendly. Another benefit of algal use is that nutrient extraction from sewage by means of algae provides useful biomass that can be used to biofuels. Open ponds and closed photobioreactors of various designs are used for algal growth but microalgae grow efficiently in ponds containing wastewater [7].

Wastewater treatment ponds are considerably used for sewage treatment in most of the developing countries where wastewater treatment plants are not available. In such settings biological treatment systems using algae are useful. It has many advantages because they are inexpensive, easy to assemble, manage and no skilled labor is required to run it. Particularly, the removal of pathogens must be stressed in developing areas because of vulnerability towards various diseases in such areas due to improper ways of sanitation. In this perspective, wastewater treatment ponds are considered to be more efficient and effective as compared to other conventional treatment systems [2]. Major objectives of the study were to determine the presence of total coliform and faecal coliform in municipal wastewater and their removal by algae based treatment methods.

2. MATERIALS AND METHODS

2.1. Experimental Layout

Aquatic cultures were conducted in synthetic ponds with dimensions of 0.15 x 0.15 x 0.3 m^3 having maximum capacity of 6.75 liters; the dimensions of second pond were 0.3 x 0.3 x 0.15 m^3 with maximum capacity of 13.5 liters, and 0.9 x 0.15 x 0.45 m^3 were the dimensions of third pond with maximum capacity of 60.75 liters.

2.2. Wastewater Sampling

Water samples were collected in plastic cans from sewage drain passing through Shadman, Lahore by grab sampling methods then these (samples collected at morning, noon, afternoon and evening) were mixed with each other to form composit sample. Tests were performed immediately or the sample was refrigerated (at 12 ^{0}C) for further analysis.

2.3. Algal Sampling

Algal samples were collected from Botanic Garden of GC University Lahore and forms of Department of Fisheries near Manawa police station Lahore. These samples were grown in wastewater ponds for experiments to optimize the conditions for wastewater treatment.

2.4. Identification and Inoculation of Algae

Collected algae were first identified and then kept at room temperature as local outdoor cultures. Inocula of algae were transferred in previously mentioned ponds for sewage treatment. The rate of growth of these algal species was measured by the estimation of fresh weight. These samples were dried in an oven at 60 °C for 48 hours to calculate its dry weight.

2.5. Wastewater Analysis

2.5.1. *Physical Analysis of Wastewater*

Temperature and pH were determined immediately after sample collection with digital meter as mentioned in APHA [8] standard methods.

2.5.2. *Total Coliform*

Sample was analyzed for biological parameter i.e.

total coliform. It was determined throughout the experiment by MPN/100 method. Culture media was prepared by adding 17.5 g LT-broth into 500 mL of distilled water and 5 mL from this culture was put into the required number of pre sterilized test tubes. All the test tubes were cotton plugged. Test tubes were autoclaved at 120 ºC for 20 minutes after covering them with aluminium foil. After removing from autoclave these were cooled at room temperature and filled with wastewater sample i.e. 0.1 mL, 1 mL and 10 mL in separate test tubes except one which served as a blank. Put them in an oven at 35 ºC for 48 hours. After the incubation period these were compared with blank to count the total coliform MPN/100 [8].

2.5.3. *Faecal Coliform*

Samples were analyzed for faecal coliform bacteria following the standard procedures [8] and reported as MPN/100 mL.

2.6. Algal Analysis

Algal samples were dried in an oven at 60-80 ^{0}C for 48-72 hours. This dried algae was weighed and then crushed by grinder and after grinding samples were weighed again. These powdered algal samples were utilized for further study.

2.7. Total Nitrogen Determination

Nitrogen is determined by Kjeldahl method [8].

Calculations

%N = (V-B) ×N×R×E×100/ Wt

V = Volume of 0.01 N H_2SO_4 titrated for the sample (mL)

B = Blank titration volume (mL)

R = Ratio between total volume of the extract and extract volume used for titration.

N = Normality of H_2SO_4 solution.

Wt = Weight of air dry algae (g)

E = Atomic weight of Nitrogen

2.8. Phosphorus Test

Available phosphorous was determined by Olsen method [8] and ammonium molybdate-developed color intensity was measured colorimetrically using spectrometer 0-D.

2.9. Organic Matter

Organic matter was determined by Walkley-Black method [8].

2.10. Experimental Setup on the Basis of Variation in Pond Size

Experimental setup was conducted on the basis of variation in pond size. Where the algal and water quantity were taken constant i.e. 150 g and 7 L, respectively. Pond size was varied from P1 to P3.

2.11. Experimental Setup on the Basis of Variation in Algal Quantity

Second experimental setup was conducted on the basis of variation in algal quantity. In this setup, the water quantity and pond size were constant i.e. 7 L and P2 respectively. P2 was selected on the basis of its efficiency in comparison to P1 and P3. Three replicates of each experiment were conducted to minimize error. Different Algal quantities used in this setup were; 50 g, 100 g, 150 g and 200 g.

2.12. Experimental Setup on the Basis of Variation in Water Quantity

Third experimental setup was performed on the basis of water quantity, where pond size and algal quantity were remained constant i.e. P2 and 150 g respectively. This algal quantity showed maximum removal of pollutants from municipal wastewater therefore it was selected for further experiments. In this setup, different water quantities were used in four ponds of similar dimensions (3 L, 5 L, 7 L and 9 L).

2.13. Experimental Setup on Monthly Basis

This setup was conducted to observe the effect of seasonal variation on percent reduction of various parameters. In this regard, 100 g of Algae was taken with 5 liters of water in P2. Chemical oxygen demand (COD), Biochemical oxygen demand (BOD), Faecal coliforms (FC), Total coliforms (TC), Total Kjeldhal nitrogen (TKN), Total phosphorus (TP), Total solids (TS), Total suspended solids (TSS) and Total dissolved solids (TDS) were observed after each month from September to July.

3. RESULTS AND DISCUSSION

3.1. Pollutant Load in the Samples

Sample collected from the sewage drain was found to be rich in pollutants. The results shown in Table 1 indicated that the concentrations of the parameters were above National Environmental Quality Standards (2000). Table 1 also compared the concentrations of pollutants in untreated sample immediately after collection with concentration of pollutants after treatment with algae and control setup conducted without inoculation of algae. The alga used in the current study was identified as *Rhizoclonium implexum.*

3.2. Mechanism for the Removal of Coliforms from Wastewater by Algae

In wastewater ponds of algae, sunlight give rise to those processes which are related with dissolved oxygen, pH and sedimentation. It can play a key role in pathogen removal. If there are high amounts of protozoa present, it can be significant to overcome the bacterial population. There are some mechanisms of pathogen removal from wastewater by algae as under:

- Nutrient competition of algae with coliform bacteria.
- Increase in pH due to CO_2 consumption which is lethal for pathogens.
- Algae produce toxins of long chain fatty acids which kill pathogens.
- Aeration (addition of O_2 by algae) enhances faecal coliform die-off rates.
- Adhesion/Attachment to the algal cells [2].

3.3. Effect of Treatment on Total Coliforms Reduction

In this experiment, the measurement of total coliform (TC) reduction was done to observe the effect of pond size with varying treatment duration but fixed algal and water quantity (150 g and 7 L, respectively). Reduction in TC was detected to be maximum in pond P2 (100%) low percentage

Table 1. Wastewater analysis before and after treatment and its comparison with National Environmental Quality Standards (2000).

Parameter	Unit	Sewage analysis, before treatment	Reduction after treatment (%)	Without treatment reduction (%)	NEQS 2000
Colour	---	grayish	---	---	---
Odour	---	Irritating	---	---	---
pH	---	6.7	0.1±0.08	0.4±0.2	6-9
Temperature	^{0}C	25±3	---	---	≥3 ^{0}C
Chemical oxygen demand	mg/L	580±20	99.4±0.02	13±1.2	150
Biological oxygen demand	mg/L	321±15	99.5±0.01	15±2.4	80
Total solids	mg/L	5720±20	95.1±0.05	10±0.6	3700
Total dissolved solids	mg/L	4500±20	97.2±0.07	4±2.5	3500
Total suspended solids	mg/L	1220±15	96.1±0.11	3±1.6	200
Total Coliforms	MPN	1.6×10^3	100±0	8±1.9	---
Fecal Coliforms	MPN	1.6×10^3	100±o.06	11±1.5	---
Total Kjeldahl nitrogen	mg/L	30.2±2.1	98.2±0.12	4±0.8	---
Total phosphorus	mg/L	13±1.6	96.8±0.24	5±1.3	---

reduction was observed in pond P3 (89.5%) whereas the percentage reduction was decreased to 78.3 % in pond P1, as a result pond P2 was selected for further study (Fig. 1).

In this experiment, the effect of variation in algal quantity was observed for total coliform (TC) reduction with alteration in treatment time at constant water quantity and pond size (7 L and P2 respectively). Maximum TC reduction was indicated with 150 g and 200 g algal quantity i.e. 100% and 100% respectively whereas lower TC reduction was found with 50 g and 100 g algal quantity i.e. 79.6% and 86.3% respectively. Since the percentage reduction was similarly observed with 150 g and 200 g of algae thus, for 7 liters of water quantity, 150 g of algae were found to be most favorable (Fig. 2).

Percent reduction in total coliform (TC) was also observed with variation in treatment time at constant pond size and algal quantity (P2 and 150 g respectively). Maximum percent reduction was found with 3L, 5L and 7 L of wastewater i.e. 100%, 100% and 100% respectively however the reduction percentage was decreased with 9liters of wastewater (89.4%). Therefore, for further study 7 liters of wastewater was selected (Fig. 3).

3.4. Effect of Treatment on Faecal Coliforms Reduction

In this experiment, the measurement of fecal coliform (FC) reduction was done to observe the effect of pond size with varying treatment duration but fixed algal and water quantity (150 g and 7 L, respectively). Reduction in FC was detected to be maximum in pond P2 (97.8%) low percentage reduction was observed in pond P3 (84.6%) whereas the percentage reduction was decreased to 77% in pond P1, as a result pond P2 was selected for further study (Fig. 4). 100% reduction of all fecal indicator bacteria was observed by [9] during the 6.6-day system residence time. In the presence of sunlight, fecal coliforms removal increased because sunlight promoted their inactivation.

In this experiment, the effect of variation in algal quantity was observed for fecal coliform (FC) reduction with alteration in treatment time at constant water quantity and pond size (7 L and P2 respectively). Maximum FC reduction was indicated with 150 g and 200 g algal quantity i.e. 100% and 100% respectively whereas lower FC reduction was found with 50 g and 100 g algal quantity, i.e., 78.3% and 86.1% , respectively. Since the percentage reduction was similarly observed with 150 g and 200 g of algae thus, for 7 liters of water quantity, 150 g of algae were found to be most favorable (Fig. 5). 100% reduction of all fecal indicator bacteria was observed by [9] during the 6.6-day system residence time. In the presence of sunlight, fecal coliforms removal increased because sunlight promoted their inactivation. Attachment of fecal coliforms along with other bacteria on the surface significantly enhanced the fecal coliform die-offs [2].

Percent reduction in fecal coliform (FC) was also observed with variation in treatment time at constant pond size and algal quantity (P2 and 150 g respectively). Maximum percent reduction was found with 3L, 5L and 7 L of wastewater i.e. 100%, 99.9% and 100% respectively however the reduction percentage was decreased with 9liters of wastewater (87.7%). Therefore, for further study 7 liters of wastewater was selected (Fig. 6). [10] reported phosphorus reduction almost 96.2%.

100% removal of all fecal indicator bacteria was observed by [9] during the 6.6-day system residence time. In the presence of sunlight, fecal coliforms removal increased because sunlight promoted their inactivation. Attachment of fecal coliforms along with other bacteria on the surface significantly enhanced the fecal coliform die-offs [2].

3.5. Effect of Treatment on Reduction of Pollutants

The effect of seasonal variation on percent reduction was observed in wastewater parameters with 150 g of algal quantity and pond P2. In November, maximum percent reduction of parameters was found. Nearly, same results were observed in the month of March whereas results obtained in other months were not satisfactory. Therefore, month of November is found to be favorable for percent reduction in wastewater parameters (Fig. 7). Kadam [11] reported percent reduction of biochemical oxygen demand (BOD), total kjeldahl nitrogen

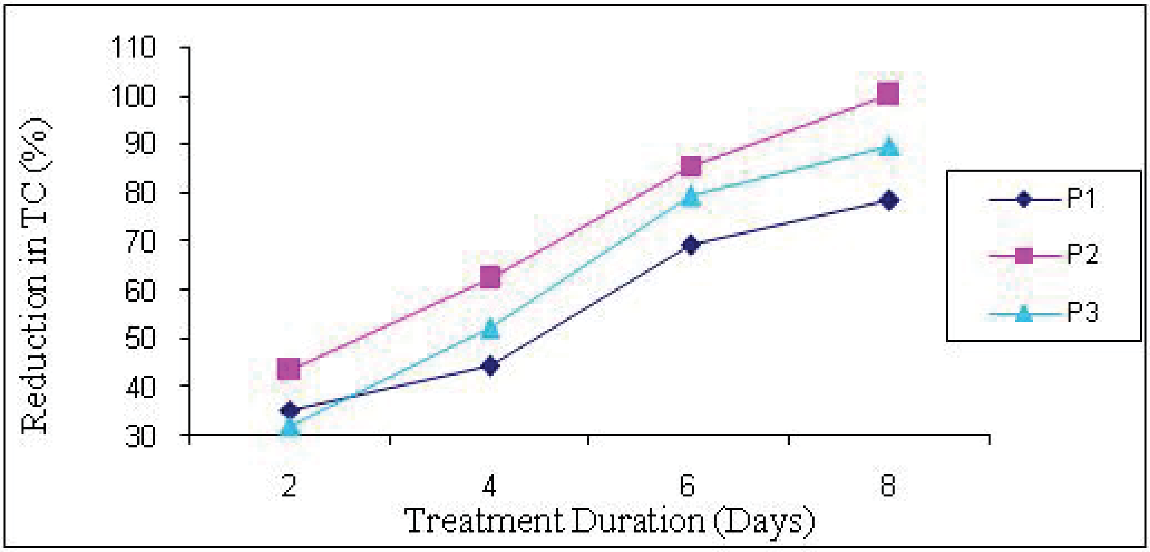

Fig. 1. Effect of pond size and treatment duration on percent reduction in total Coliforms (TC) with 7 liters of water and 150 g of algae.

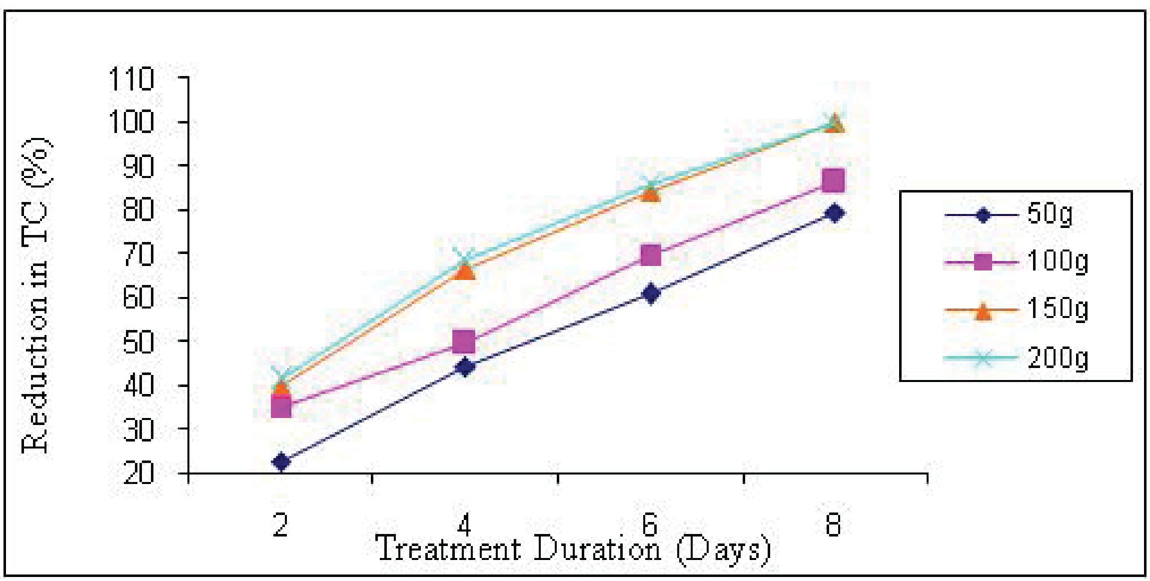

Fig. 2. Effect of algal quantity and treatment duration on percent reduction in total Coliforms (TC) with 7 liters of water in P2.

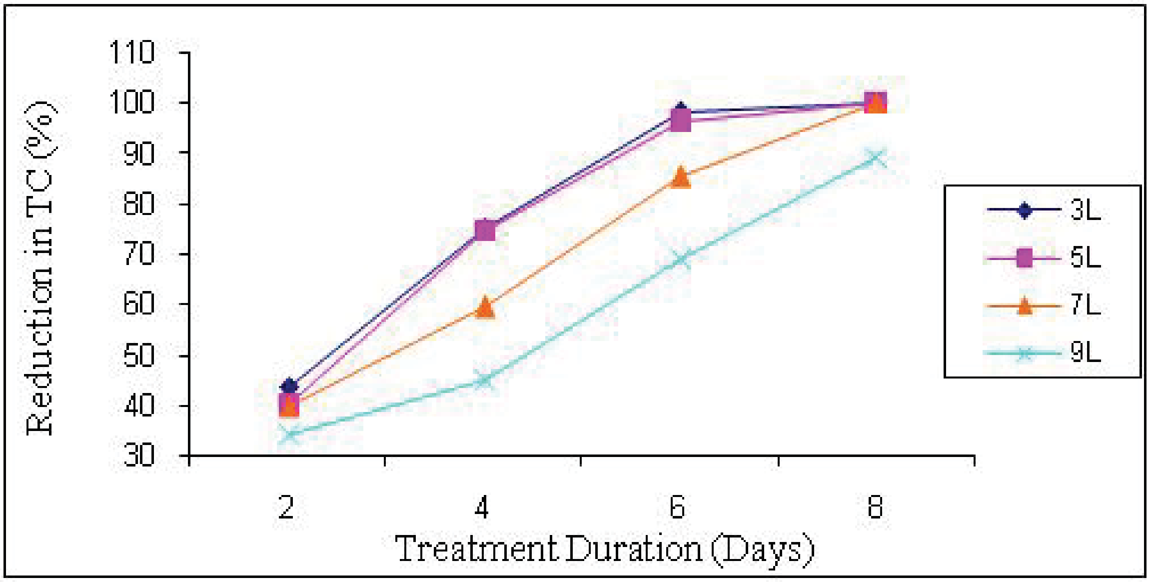

Fig. 3. Effect of water quantity and treatment duration on percent reduction in total Coliforms (TC) with 150 g of algae in P2.

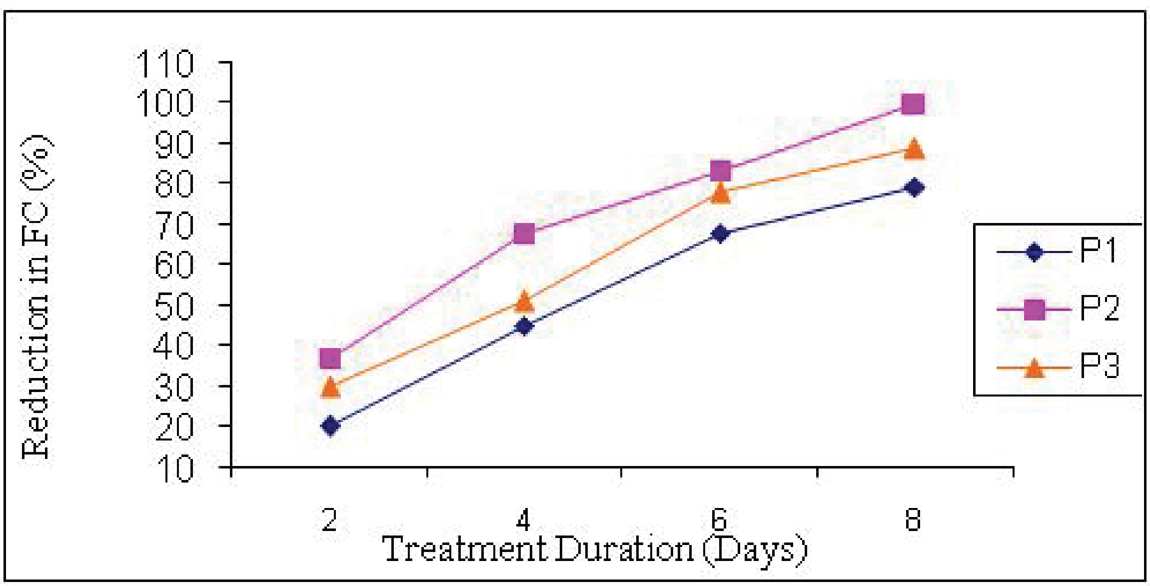

Fig. 4. Effect of pond size in percent reduction of Faecal Coliforms in 7 litres of water and 150 g of algae.

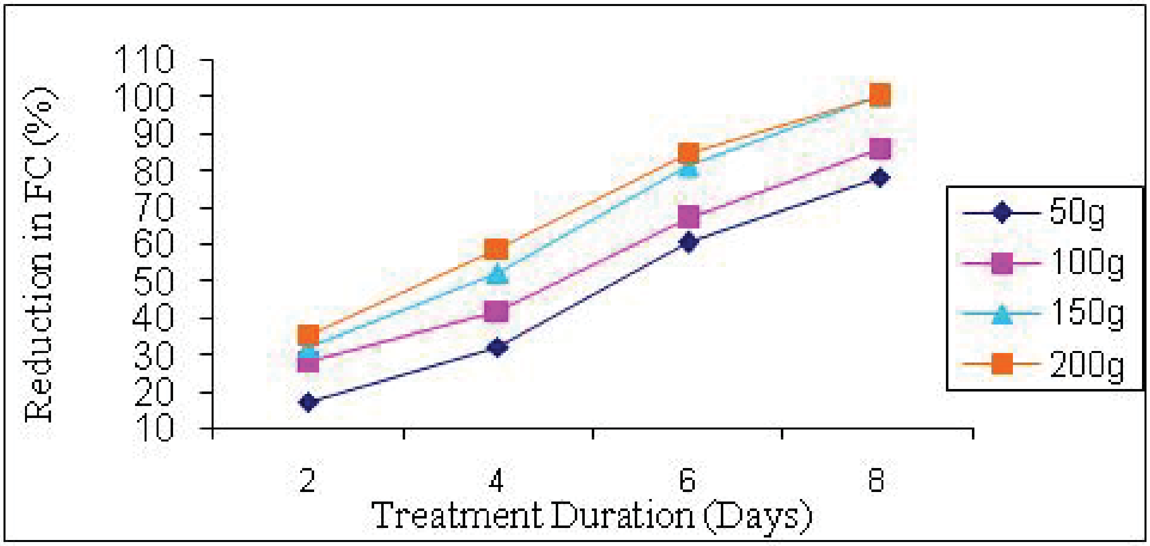

Fig. 5. Effect of algal quantity and treatment duration in percent reduction of Faecal Coliforms with 7 liters of water in P2.

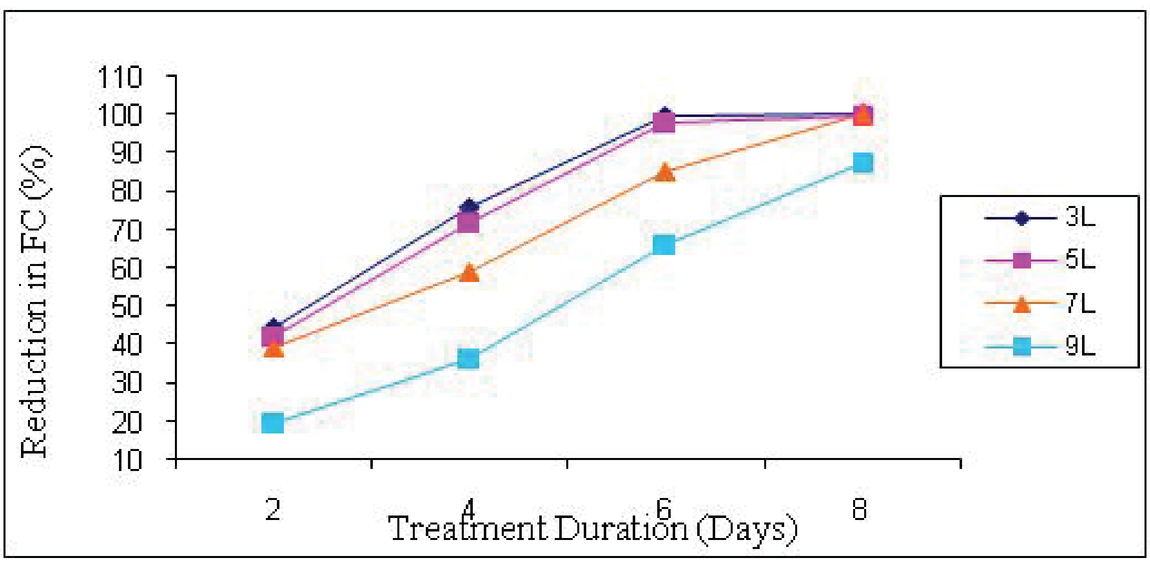

Fig. 6. Effect of water quantity and treatment duration in percent reduction of Fecal Coliforms with 150 g of algae in P2.

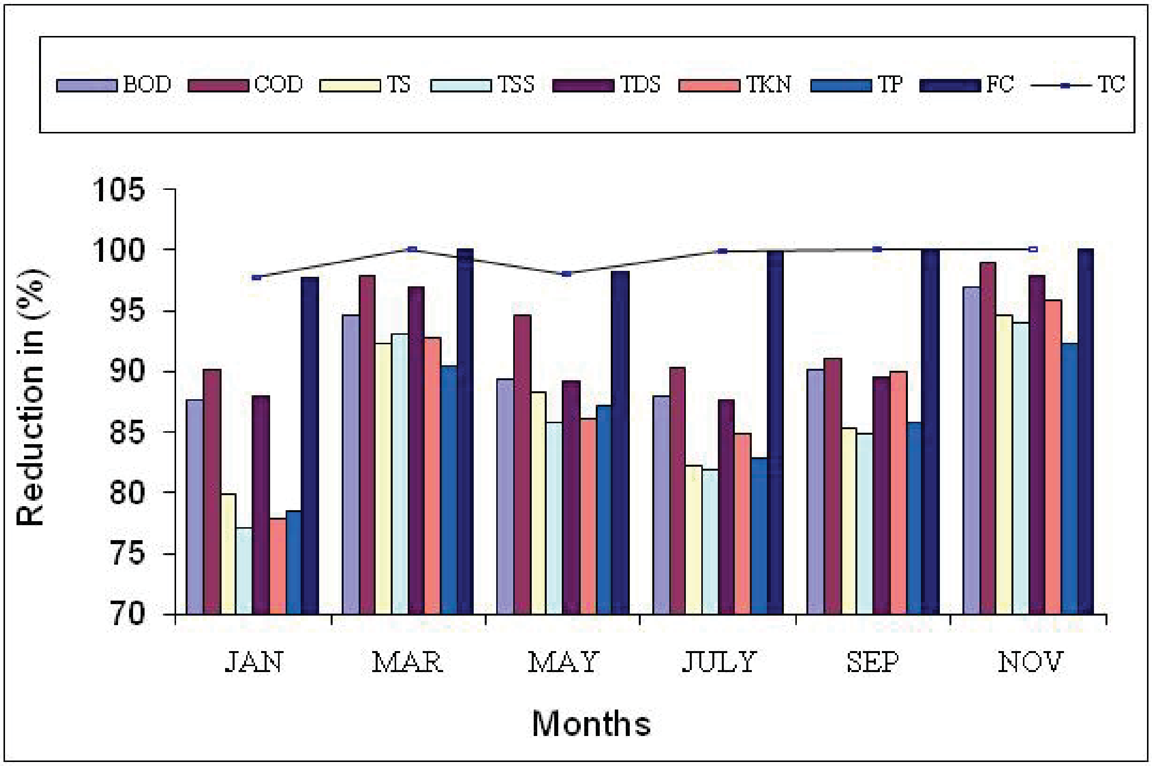

Fig. 7. Percent reduction of wastewater parameters in alternative months of the year under optimum conditions.

Table 2. Mean accumulation of nitrogen and phosphorous in algae with change in treatment duration under optimum conditions.

Parameters	Treatment duration (Days)				
	0	2	4	6	8
Nitrogen (mg/g)	10.75±1	13.5±1.7	23.3±0.8	32.2±0.6	34.9±2
Phosphorus (μg/g)	257.40±2.9	259.4±3.2	264.4±2.2	267.6±2.6	268.1±1.7
Organic matter (mg/g)	290±2	293.2±3.1	299±4	314.5±1.5	315.3±2.1

(TKN) and chemical oxygen demand (COD) as 83% 21% and 68%, respectively.

3.6. Accumulation of Nitrogen and Phosphorous in Algal Biomass

In this experiment, total nitrogen, total phosphorus and organic matter was calculated in algae with the change in treatment duration and it was observed the accumulation of both these nutrient increase with increase in treatment time. Maximum nitrogen, phosphorous and organic matter accumulation was found on the final day of treatment i.e. 34.9mg/g, 268.1ppm, 315.3mg/g respectively (Table 2).

4. CONCLUSIONS

Present study revealed that algae for wastewater treatment are very effective for reduction of coliform bacteria along with other various parameters from municipal wastewater. Algal based wastewater treatment system was proved to be a good alternative to the conventional wastewater treatment systems in many ways, due to its efficiency in pollutant removal, cost-effectiveness and other advantages. It not only gives 100% removal efficiency but also bring other pollutants into their permissible limits. Consequently, this wastewater treatment process can become very cheaper and efficient.

Based on the results of this study, it is recommended that algae-based wastewater treatment system is a cost-effective and environment friendly way to remove pathogens. It is more efficient system as compared to other conventional treatment systems. The optimum conditions obtained in the current study can become very helpful in launching this technique at pilot scale. The natural biomass produced can also be utilized for production of biofuels. The process consume CO_2 a major green house gas for pollutant removals. Therefore, it is recommended to take these aspects for future studies in developing countries like Pakistan. There is need to identify the algal species with high oil content and wastewater treatment efficiency so that dual benefits can be obtained from this natural resource.

5. ACKNOWLEDGEMENTS

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