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Research Article

Comparative Phycoremediation of Sewage Water by Various Species of Algae

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Abstract: In this study sewage water treatment efficiency of *Chlorella vulgaris, Rhizoclonium hieroglyphicum* and mixed algae culture (*Microspora* sp., *Navicula* sp., *Lyngbya* sp., *Cladophora* sp., *Spirogyra* sp. and *Rhizoclonium* sp.) was compared. Sampled wastewater was analyzed for various parameters (i.e., COD, BOD, TS, TSS, TDS, TC, FC, TKN, TP, NO_3-N, PO_4, SO_4 and Cl^-) and concentrations of all these parameters in the untreated water were above the permissible limits of National Environmental Quality Standards of Pakistan (2000). Various algal species were used to treat sewage water by varying pond size, treatment duration, seasonal variation and growth rate of algae to arrive at the optimum outcome. Maximum percent reductions of various parameters, attained with *C. vulgaris,* were: chemical oxygen demand (98.3%), biochemical oxygen demand (98.7%), total Kjeldahl nitrogen (93.1%), total phosphorus (98.0%), nitrate (98.3%), phosphate (98.6%), chloride (94.2%), total coliforms (99.0%), faecal coliforms (99.0%) and total dissolved solids (98.2%) while maximum reduction in total suspended solids (92.0%) was obtained with a mixed algae culture and maximum increase in biomass by *R. hieroglyphicum* (0.75 g L^{-1} day^{-1}). Reduction in the concentration of pollutants in sewage water was to such a low level that it can be thrown in waterbodies without any further treatment.

Keywords: Algae, Chlorella, phycoremediation, pollution, pond, rhizoclonium, wastewater

1. INTRODUCTION

Phycoremediation is the use of micro- or macro-algae for the removal or biotransformation of pollutants, including nutrients and toxic chemicals from wastewater [1, 2]. The term phycoremediation was introduced by John [3] to refer to the remediation carried out by algae. Phycoremediation is comprised of several applications: (i) nutrient removal from municipal wastewater and effluents rich in organic matter; (ii) nutrient and xenobiotic compounds removal with the aid of algae-based biosorbents; (iii) treatment of acidic and metal wastewaters; (iv) CO_2 sequestration; (v) transformation and degradation of xenobiotics; and (vi) detection of toxic compounds with the aid of algae-based biosensors. A distinct comparison can be made between microalgae and other conventional technologies on the basis of nutrient removal [4].

The prevalent sewage treatment strategies are costly and not affordable in the developing countries of the world due to high consumption of energy. Sewage irrigation is another big problem which affects human health directly and indirectly. Pakistan is also facing problems related to contamination of water, where excessive and untreated sewage is discharged directly into water bodies as a result this water can not even be used for irrigation purposes. Sewage is also loaded with diseased pathogens, where they get their direct entry into the drinking water by leakage and cause serious intestinal infections especially in children. Prolong exposure to these hazards can cause several diseases including cancer and birth defects in young ones [5].

Microalgae can be used to treat municipal wastewater, industrial effluents and solid wastes aerobically as well as anaerobically. The use of algae to treat wastewater has been in practice for over 40

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years and first descriptions of this application was reported by Oswald [6]. So the use of microalgae for the treatment of municipal wastewater has been a subject of research and development for several decades. Extensive work has been conducted to explore the feasibility of using microalgae for wastewater treatment, especially for the removal of nitrogen and phosphorus from effluents [7, 8] which would otherwise result in eutrophication if dumped into water bodies.

Biological treatment enhances the removal of nutrients, heavy metals, pathogens and provides O_2 to heterotrophic aerobic bacteria for mineralization of organic pollutants, as a result CO_2 is released from bacterial respiration [9]. Photosynthetic aeration is important to reduce operation costs and limit the risks for pollutant volatilization under mechanical aeration. Recent studies have shown that microalgae can indeed support the aerobic degradation of various hazardous contaminants [10]. The hyper concentrated algal cultures, called 'activated algae' can be used to decrease the land and space requirements for wastewater treatment. This process removed nitrogen and phosphorus in a very short period of time, i.e., less than 1 hour [4]. Microalgae can be efficiently used to remove significant amount of nutrients because they require high amounts of nitrogen and phosphorus for the sysntheisis of proteins (45–60% of microalgae dry weight), nucleic acids and phospholipids. Nutrient removal can further be increased by NH_3 stripping or NH_3 precipitation due to the raise in pH [11]. This method is not appropriate for large scale wastewater treatment, therefore there is need to improve the technology.

The advantages of using algae for sewage treatment include low cost of the operation, possibility for assimilation of nitrogen and phosphorus into algal biomass, which consequently can be used as fertilizer thus avoiding sludge handling problem and the discharge of oxygenated effluent into the water body. In addition, the process has no carbon requirement for nitrogen and phosphorus removal, which is sustainable for the treatment of sewage effluents [2].

The current study was conducted to assess the pollution load carried by municipal drains into the water bodies and its treatment with environment friendly and naturally available fresh water algae. A comparison was made to find out the algal species with best reduction efficiency. Treatment efficiency was also measured in different months of the year to assess the effect of seasonal variations.

2. MATERIALS AND METHODS

2.1. Experimental Location and Layout

The experimental outdoor work was carried out in Botanic Garden and roof of Sustainable Development Study Centre, GC University, Lahore. The locations were selected in consideration of availability of enough light, space and ponds facility. The study was conducted to test the feasibility of growing algae as a sewage nutrients reduction agent. These experiments were carried out in batch mode and cultivation time for each condition was 8 days with three replications. The effect was studied in terms of algal growth and wastewater nutrients reduction. Aquatic cultures were conducted in synthetic ponds with different sized and having dimensions of 0.15 x 0.15 x 0.3 m (P1), 0.3 x 0.3 x 0.15 m (P2) and 0.9 x 0.15 x 0.45 m (P3) with final capacity 6.75 L, 13.5 L and 60.75 L respectively. In addition a pilot scale large pond with dimensions of 8 x 2 x 1 m (P4) and final capacity of 16000 L was used for sewage treatment [12]. Initially the algal biomass was kept constant in each pond with equal quantity of wastewater except in pond P4 where biomass was added on per litre bases deduced from other ponds.

2.2. Wastewater Sampling and Analysis

Waste water samples were collected from various sewage drains of Lahore city by grab sampling techniques; then these samples were mixed to form composit sample [13]. The composite sample was filtered to remove coarse particles and divided into three replicates. The analysis for various physicochemical parameters like biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solid (TDS), total suspended solids (TSS), total phosphorus (TP), total Kjeldhal nitrogen (TKN), Nitrate-nitorgen (NO_3-N), phosphate (PO_4) and chloride (Cl^-) was carried out by using standards methods of American Public Health Association [14]. The sampled untreated wastewater was stored at 4^{o}C until used for algae cultivation.

2.3. Algal Sampling, Identification and Incubation

R. hieroglyphicum was collected from the fish forms of the Department of Fisheries near Manawa police station, Lahore. *C. vulgaris* sample was obtainted from Mr. Tariq Rashid, working in the same department on utilization of *Chlorella species* as fish feed. Mixed algae culture was collected from Baradari in River Ravi, near Lahore and Botanic Garden of GC University, Lahore. These algal species were identified by adopting a standard methods described by Zarina et al [15, 16].

Collected algae were kept under optimum conditions as local outdoor cultures and inocula of algae were transferred in previously mentioned ponds for sewage treatment. All the experimental ponds with same quantity of selected algal species were kept under similar light intensity and photoperiod to get comparative results. Before analysis samples were filtered to remove algal biomass present in the sample after treatment, it was done to avoid the interference caused by algal biomass in final results. The rate of growth of these algal species was measured by the estimation of fresh weight. These algal species were harvested every second day to measure its fresh weight.

3. RESULTS AND DISCUSSION

3.1. Sewage Treatment with Algae

The collected sample was characterized for various parameters showing the results which were above National Environmental Quality Standards (NEQS) [17] values. It was observed from the study that in comparison to *R. hieroglyphicum* and mixed algae culture, the *C. vulgaris* was more efficient in treating wastewater (Table 1). Przytocka-Jusiak et al [18] reported that the nutrient reduction depends on the quantity of nutrients present in wastewater and extent of these nutrients absorbed by the algae for incorporation into algal tissues. *Chlorella* has the ability to uptake maximum phosphorous for the synthesis and accumulation of polyphosphates in their bodies.

3.2. Effect of Algal Species on Pollution Reduction

Various parameters of wastewater were analyzed after treatment with *R. hieroglyphicum*, mixed algae culture and *C. vulgaris*. Before analysis samples were filterd to remove algal biomass which can interfer with the results. The reduction percentage of COD (98.27%), BOD (98.69%), TKN (93.14%), TP (98%), NO_3-N (98.33%), PO_4 (98.63%), Cl^- (94.16%), TC (99%), FC (99%) and TDS (98.21%) was noted to be maximum with *C. vulgaris*. The treatemnt efficiency of *R. hieroglyphicum* was

Table 1. Characterization of untreated wastewater and quantity of pollutants reduced by *C. vulgaris*, *R. hieroglyphicum* and mixed algae culture.

Parameters	Untreated	Reduction with *C. vulgaris*	Reduction with *R. hieroglyphicum*	Reduction with Mixed algae culture	NEQS 2000
COD (mg/L)	721	708.5	705.8	683.6	150
BOD (mg/L)	407	401.7	396.4	389.8	80
TSS (mg/L)	970	836.1	869.6	891.9	200
TDS (mg/L)	4650	4567.2	4546.2	4546.2	3500
TC (MPN)	$1.6x 10^3$	$1.58x 10^3$	$1.58x 10^3$	$1.57x 10^3$	---
FC (MPN)	$1.6x 10^3$	$1.58x 10^3$	$1.58x 10^3$	$1.57x 10^3$	---
TKN (mg/L)	35	32.6	31.8	30.5	---
TP (mg/L)	22	21.6	21.1	20.9	---
NO_3-N (mg/L)	12	11.8	11.8	11.7	---
PO_4 (mg/L)	10	9.86	9.86	9.4	---
SO_4 (mg/L)	55	53.5	50.2	53.5	600
Cl^- (mg/L)	60	56.5	55.5	51.5	1000

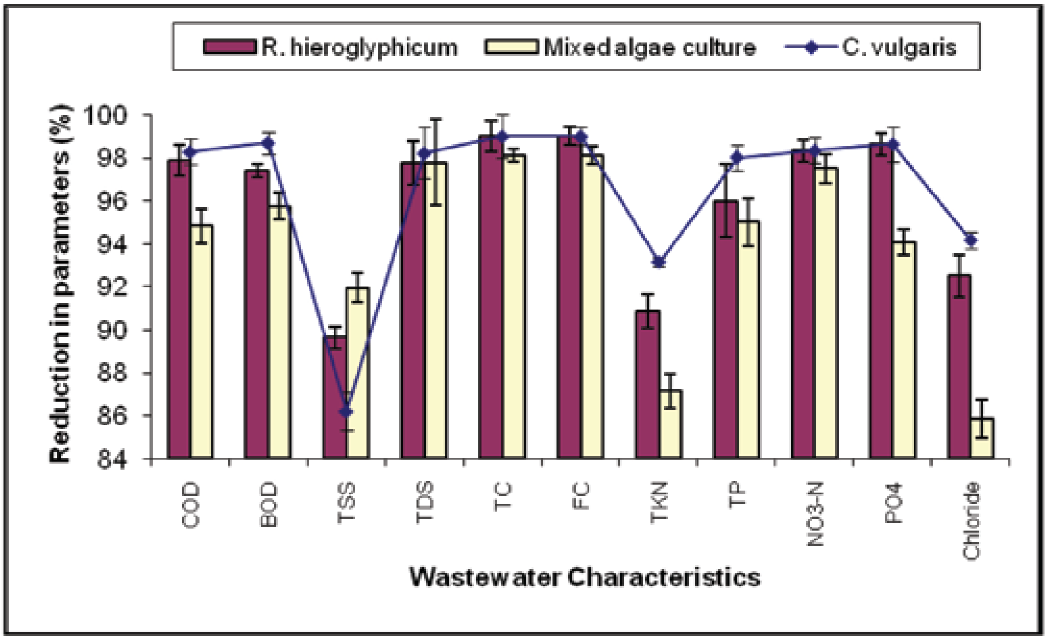

Fig. 1. Comparative efficiency of algal species in removing wastewater pollutants.

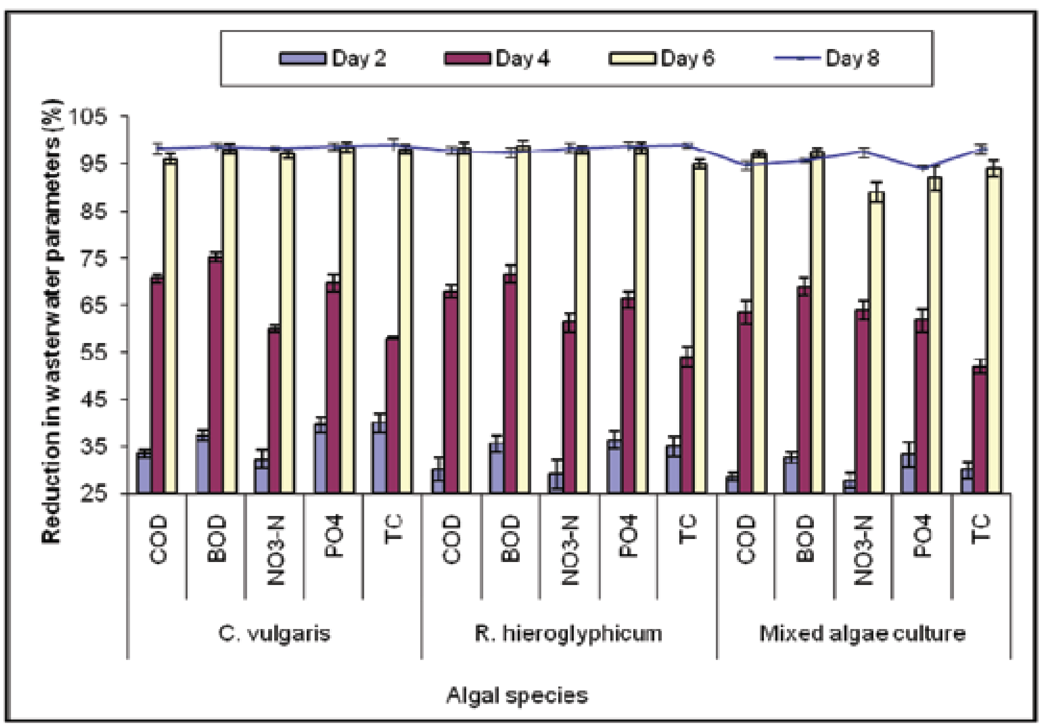

Fig. 2. Comparative percentage reduction of various parameters with different species of algae in time.

less than *C. vulgaris* but it was found to be higher than mixed algae culture. However the reduction percentage of TSS (91.3%) with mixed algae culture was higher than both *R. hieroglyphicum* and *C. vulgaris* (Figure 1). Microalgae showed efficient results in removing N and P from sewage-based wastewater either applied in free-swimming suspension or in an immobilized form. Increase in pH to 8 or above causes precipitation of phosphorous from the wastewater so it will not be wrong to say that nutrient reduction in wastewater occurs due to algal uptake as well as other reasons. Algal biomass contain phosphorous concentration ranging from 0.5-3.3 % of its dry mass which means 3-23 % of phosphorous was reduced due to algal uptake. In secondary treated wastewater more than 80% reduction of nitrate and total phosphorous was shown by various species of *Chlorella* and *Scenedesmus* [19, 20, 21].

3.3. Effect of Treatment Time on Pollution Reduction

Treatment duration has profound effect on treatment efficiency as the treatment time increases, algal biomass increases and it absorbs more nutrients from wastewater which results in the reduction of those nutrients. In the current study treatment time was taken in days (0-8) and wastewater was analyzed after every two days. It was observed that reduction became constant on 8th day with all the three types of algae however minimum reduction was achieved with mixed algae culture (Figure 2). Algae species *Chlorella* was widely applied for wastewater treatment and had proven abilities of removing nitrogen, phosphorus, and chemical oxygen demand (COD) with different retention times ranging from 10 hrs to 42 days [22].

3.4. Increase in Biomass of Algae in Wastewater

Increase in biomass of algae with the passage of time was measured by taking into account its fresh weight. Increase in biomass was observed from day 2 to 8 in all three types of algae. As five litre of wastewater was used to grow algal species for measurement of growth so increase in biomass on 8th day was divided by five for per litre and 8 for per day calculation. Maximum growth rate (0.75g.L^{-1}.day^{-1}) was shown by *R. hieroglyphicum*

Table 2. Increase in fresh weight of algal species with the passage of time in five litre of wastewater.

Algal species	Algal biomass (g)	Days					Increase in weight *(g. L^{-1} .day^{-1})
		0	2	4	6	8	
C. vulgaris	Fresh weight	100	102.7±2	115±0.6	121.8±2.3	122±1.5	0.55
R. hieroglyphicum	Fresh weight	100	109±1.1	120±2.1	128.5±1.6	130.01±1.8	0.75
Mixed algae culture	Fresh weight	100	105.5±0.8	118.4±0.9	122.3±1.5	124.0±1	0.6

* 8th day increase in biomass was divided by 5 (for L^{-1}) and 8 (for day^{-1}) measurement

Table 3. Percent reduction in various parameters with algal species in different sized ponds.

Parameters	*C. vulgaris*				*R. hieroglyphicum*				Mixed algae culture			
	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4
COD	89.4	98.6	88.1	98.7	86.6	98.8	89.9	98.5	85.9	94	87.2	98.7
BOD	93.7	98.8	92.9	98.9	89.5	98.9	93.7	98.8	90	93.3	92.2	98.9
TS	86.2	95.3	93.1	95.1	84.2	97.6	87.5	92.4	83.5	96.2	89.4	97.6
TC	94.1	99	95.2	99	92.4	99	93.8	98.2	93.2	98.8	96.1	99
TKN	89.4	97.7	87.7	97.2	86.3	97.7	90.9	95.8	85.4	95.3	90.2	97.7
TP	89.7	93.8	92.6	94	86.9	98.4	91	93.5	86.1	97	92.9	98.4
NO_3-N	91.2	98.1	94.7	98.5	89.1	98.9	92.8	95.9	89	94.5	92.1	98.9
PO_4	88.4	94.3	88.9	95	86	98.7	89.9	92.4	85.7	97.1	89.4	98.7

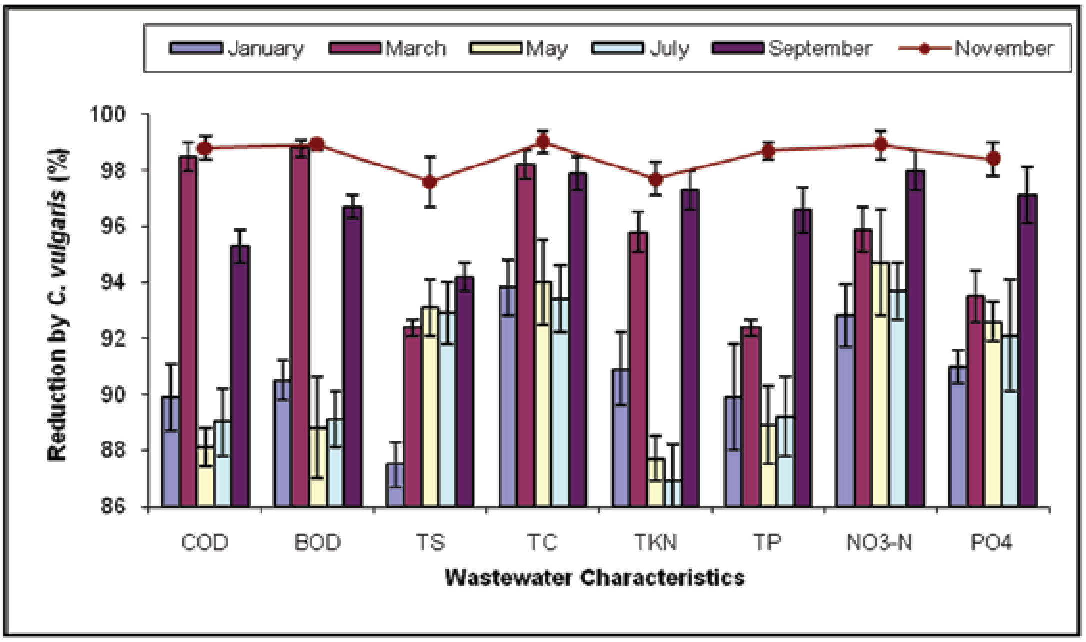

Fig. 3a. Percent reduction in wastewater characteristics in different months of the year with *C. vulgaris* species.

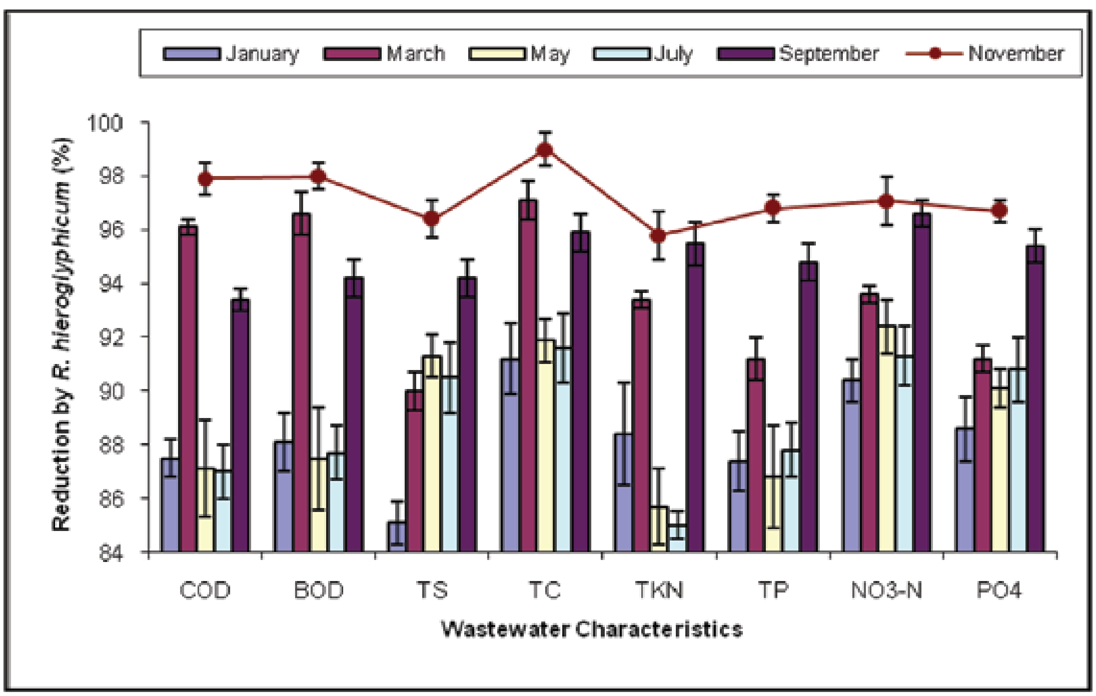

Fig. 3b. Percent reduction in wastewater characteristics in different months of the year with *R. hieroglyphicum*.

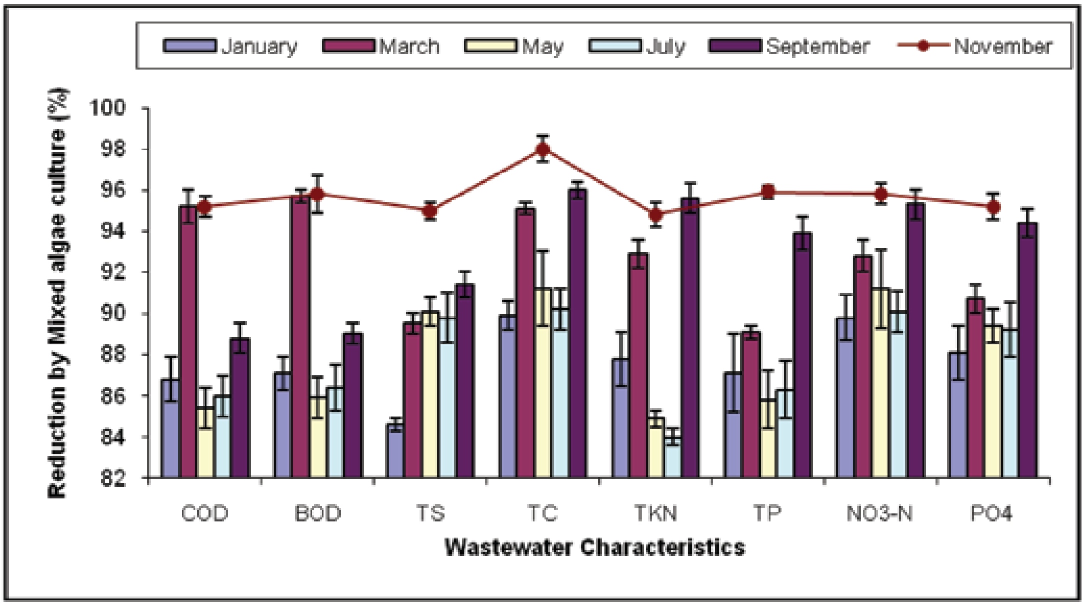

Fig. 3c. Percent reduction in wastewater characteristics in different months of the year with mixed algae culture.

while the increase in fresh weight resulted by *C. vulgaris* and mixed algae culture was observed to be 0.55 g L^{-1} day^{-1} and 0.6 g L^{-1} day^{-1}, respectively. It was also obseved that increase in growth became almost constant after 6^{th} day due to deficiency in the availablity of the nutrients in the medium (Table 2). Ruiz-Marin et al [20] performed many experiment under batch culture condition in which microalgae showed high growth rates in initial days but growth and chlorophyll contents were decreased after four cycles of culture indicating collapse of the culture due to nutrient deficiency.

3.5. Effect of Pond Size in Pollution Reduction

Ponds of different shapes and sizes (P1, P2, P3 & P4) were selected to find out the relationship of exposed surface to pollution reduction. Results showed that maximum reduction was carried out in P2 and P4 with all three types of algae. It was because in these pond there was more surface area exposed for algae to be incontact with the wastewater and more light was available for the growth of the algal species. *C. vulgaris* also showed more reduction in P1 and P3 (Table 3) as compared to *R. hieroglyphicum* and mixed algae culture because microalgae remained suspended in the wastewater while macroalge come at the surface mostly therefore the wastewater at the depth was not incontact with the biomass which can be the reason of least reduction so in order to get maximum reduction a pond should be designed in such a way that it should have more surface area exposed to light and shallow water for maximum penetration of light.

3.6. Effect of Seasonal Variations on Pollution Reduction

In order to evaluate the seasonal variation on pollution reduction all three types of algal cultures were grown under optimum conditions in alternative months of the year. Experiments were conducted for 6 alternative months by renewal of sewage and algae but the initial concentration of pollutants in experimental wastewater was kept similar in all the selected months with same initial quantity of biomass of three selected algae. It was concluded from these experiments that maximum pollution reduction was observed in the month of november and march with all three types of algal cultures (Figure 3a,3b & 3c). It was due to moderate temperature found in these months which promote algal growth while minimum reduction was found in January, May and July due to low temperature in January and very high temperatur in May and July. It was observed that the reduction percentage of pollutants in sewage in all the months was enough to be used for irrigation purposes. Green algae demands more nitrogen and phosphorous than do many other species, and they can take up generous nitrogen when the phosphorous content is relatively high [23].

4. CONCLUSIONS

The study revealed that algae are an effective organism for the reduction of biological and chemical pollutants from sewage water. Growth rate indicated that algae can luxuriously grow in wastewater medium due to availability of all necessary nutrients. Wastewater treated by algae can be used for irrigation purpose or released into water bodies as all the measuring parameters were in the permissible limits of National Environmental Quality Standards (2000) with selected species of algae. The study showed that phycoremediation process can be inversely affected by high temperature (May and July) and low temperature (January) but the reduction of pollutants in these months was enough to bring the remaining concentration into permissible limits. Consequently, this algal based treatment can be a sustainable technique for wastewater treatment.

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