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Bioremediation of sewage wastewater through microalgae (*Chlorella minutissima*)

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ABSTRACT

Phycoremediation is a cost effective, environmentally sustainable, safe and alternative technology for remediation of wastewater. The present work was aimed to evaluate the role of inoculated microalgae (*Chlorella minutissima)* in phycoremediation of sewage wastewater. The selected microalgae removed 94.4% TDS, 88.9% of NO_3^-N, 66.3% potassium, 67.4% phosphorus, 48.2% NH_4^+, 93% (Biological Oxygen Demand) BOD_5 and 80.5% (Chemical Oxygen Demand) COD. The wastewater obtained after phycoremediation have safe limit of nutrients to be used as irrigation water in agricultural fields. The results of this study suggested that growing algae in nutrient-rich sewage wastewater offers a new opportunity to bio-remediate pollution load of wastewater and use it for irrigation purposes.

Key words: *Chlorella minutissima,* Phycoremediation, Pollution, Sewage wastewater

Water is a source of life and energy for all the living organism on the planet Earth, although millions of people worldwide are woeful with the shortage of fresh and clean drinking water. Rapid pace of industrialization, population expansion and unplanned urbanization have largely contributed to the severe water pollution. In India it was estimated that more than 80% of wastewater is discharged without any treatment. The total sewage wastewater generated in India is 61754 million litre per day (MLD), out of which 22963 MLD get treated (MoEF&CC, GOI, 2018). The discharge of untreated domestic and industrial wastewater contaminates both surface and ground water with erratic pollutants. With the scarcity and increasing pollution of the groundwater, it has become crucial to make the water reusable by removing the pollutants; therefore, wastewater treatment has become both an ecological as well as an inevitable demand. The present wastewater treatment processes are really expensive and energy-consuming. The alternative to the current treatment methods, microalgae-based remediation also called as phycoremediation could be employed in the treatment process (Sharma *et al.* 2014). Phycoremediation is the process of using algae for removing an excess nutrient load from wastewater and consequently reduce the pollutants content in wastewater. This technology can be coupled with conventional treatment process in an economical and sustainable way. Algae plays an effective role in removing nitrate - nitrogen (NO_3 -N), ammonical - nitrogen (NH_4 -N), phosphorus (P), potassium (K), biological oxygen demand (BOD_5) and chemical oxygen demand (COD) (Khan *et al.* 2019). In contrast to physicochemical treatment technologies for wastewater, phycoremediation techniques are safer and more efficient specifically during the secondary treatment process (Malla *et al.* 2015; Sharma and Khan 2013). Hence, the present study is an effective, sustainable and economical approach of reusing the sewage wastewater for irrigation after phycoremediation by microalga (*Chlorella minutissima).*

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MATERIALS AND METHODS

Study area

The study was carried out at the Indian Council of Agricultural Research-Indian Agricultural Research Institute, New Delhi, India (28°38`23” N , 77°09`27”E and 228.16 m above mean sea level). The region lies between humid subtropical and semi-arid climate (Koppen climate classification). The average annual temperature is around 29°C. May and June are the hottest months and experience extreme heat waves. While, in January month of winter season sometimes the minimum daily temperature goes below 5°C.

The monsoon starts from June and lasts till mid-September, with mean annual rainfall of about 700 mm. The driest months (April-May) have average relative humidity of 20% to 30% while, maximum RH (63-75%) is received in the morning hours during monsoon season (July-August). The sewage wastewater were collected from IARI campus having discharge amounts of about 20 MLD.

Microalgae and growth condition

Microalgal strains *C. minutissima* were obtained and cultured at National Centre for Cultivation and Utilization of Blue Green Algae, IARI, New Delhi, India. The obtained culture of alga *C. minutissima* had multiplied for mass multiplication in culture room. *C. minutissima* was grown on Bold's Basal Media (BBM) whose composition is given in Table 1 (Stein, 1973)

Analysis of physico-chemical parameters

The parameters like pH, electrical conductivity (ECw), total dissolved solids (TDS), nitrate-nitrogen, ammonical-nitrogen, phosphorus, potassium, biological oxygen demand (BOD), and chemical oxygen demand (COD) were analysed initially and after harvesting of algae biomass (i.e. after 25 days) to investigate its suitability for irrigational use. The difference between the initial and final value gave the estimate of phycoremediation by microalgae. The methods and techniques being employed for the physico-chemical analysis is given in Table 2.

Estimation of NPK content in algal biomass

The harvested algal biomass was harvested through muslin cloth. The algal biomass was air dried followed by oven dry. The dry algal biomass was acid digested for estimation of NPK.The NPK content in dried algal biomass was estimated through Kjeldahl, calorimetric and flame photometer methods respectively.

Table 1 Composition of Bold's Basal Media

Chemical	Concentration (g/L)	Amount in culture medium (ml/L)
$NaNO_3$	25	100
$MgSO_4.7H_2O$	7.50	10.0
NaCl	2.50	10.0
K_2HPO_4	7.50	10.0
KH_2PO_4	17.50	10.0
$CaCl_2.2H_2O$	2.50	10.0
Trace element	8.82	1.0
a) $ZNSO_4.7H_2O$	1.44	-
b) $MnCl_2.4H_2O$	0.71	-
c) MoO_3	1.57	-
d) $CuSO_4.7H_2O$	0.94	-
e) $Cu(NO)_3.6H_2O$		-
H_3BO_3	11.40	1.0
EDTA-KOH Solution	50	1.0
a) EDTA Na_2	31	-
b) KOH		-
$FeSO_4.7H_2O$	4.98	-
Conc. H_2SO_4	1.0 ml/L	1.0

Table 2 The standard methods of estimation for physico-chemical parameters

Parameter	Units	Method
Physical parameters		
Electrical conductivity (EC_w)	dS/m	Conductivity bridge
Total dissolved salts(TDS)	mg/L	Gravimetric method
Chemical parameters		
Acidity/basicity		pH meter
Total ammonical nitrogen	mg/L	Colorimetric method
Nitrate nitrogen	mg/L	Colorimetric method
Total phosphate phosphorus	mg/L	Colorimetric method
Potassium	mg/L	Flame photometer
Biological oxygen demand (BOD)	mg/L	Incubation method (5 days at 20^{o}C) (Winker method)
Chemical oxygen demand (COD)	mg/L	Titrimetric method

Reduction efficiency

The following equation calculated the reduction/removal efficiencies (%) of various parameters (Li *et al.* 2012).

$$R = \frac{Ci\text{-}Ce}{Ci} \times 100$$

where, R = the removal/reduction percentage at each measurement time, Ci = the initial concentration of a given parameter, Ce = the remainder concentration on a given sampling day.

Statistical analysis

To observe the variation in selected parameters of algae the initial value (sewage wastewater before remediation) and final value (sewage wastewater after 25 days of remediation) the P test was done at 95% confidence level through IBM-Statistical Package of Social Sciences (SPSS 21).

RESULTS AND DISCUSSION

Phycoremediation potential of Chlorella minutissima for sewage wastewater

Chlorella minutissima was found to be very effective in the removal of electrical conductivity (EC), total dissolved solids, phosphorous (P), potassium (K), ammonium, nitrate, biological oxygen demand and chemical oxygen demand of sewage wastewater.

Temporal dynamics in sewage wastewater quality

There was significant decrease in the electrical conductivity, TDS, P, K, NH_4^+, NO_3^-, BOD and COD content while, increase in pH (insignificant) and DO (significant) of sewage wastewater after the inoculation of algae (Table 3). The EC, TDS, P, K, NH_4^+ and NO_3^- value decreases continuously during 25 days of inoculation however, rate of decrease was higher during 0-15 days of inoculation (Fig 1). It states that *C. minutissima* can effectively reduce the EC, TDS, P, K, NH_4^+, NO_3^-, BOD and COD within 15 days of inoculation. In contrast, BOD and COD initially increased up to 5 days of algal inoculation followed by continuous decrease while, DO initially decreased up to five days of algal inoculation followed by continuous increase.

Changes in electrical conductivity (EC) and total dissolved solids (TDS)

Electrical conductivity and TDS are the prime parameters for determining quality of water for irrigation. Total dissolved solids (TDS) is total amount of any metals, cations, anions, minerals and salt dissolved in water. Irrigation water with high EC and TDS lead to accumulation of salt and higher soil water osmotic potential thereby reduces water availability to the crop and their yield (Zaman *et al*. 2018). According to FAO, irrigation water quality parameters; the electrical conductivity and TDS of irrigation water should be in the range of 0.7-3 dS/m and 450- 2000 mg/L respectively, to avoid secondary soil salinization. The initial EC and TDS of sewage wastewater in this study was 3.52±0.07 dS/m and 2416±202 mg/L (Table 3). After 25 days of phycoremediation it significantly decreased to 0.25±0.03 dS/m and 136±12.58 mg/l respectively. The decrease in EC and TDS was about 93% and 94.3% respectively (Fig 2). The EC and TDS level in sewage wastewater after phycoremediation was within the prescribed maximum limit for irrigation. Khan *et al*. (2019) has reported the 96%

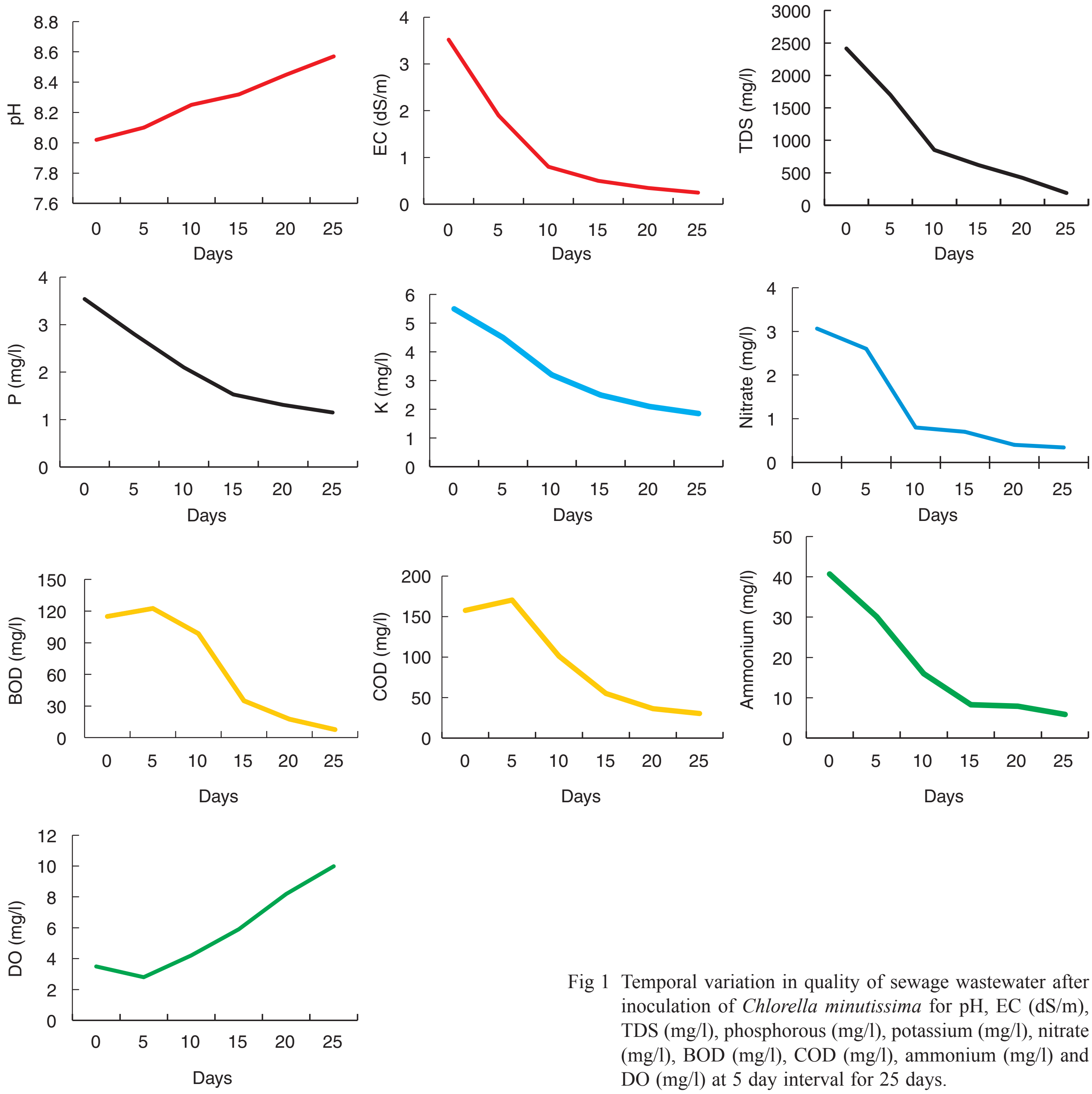

Fig 1 Temporal variation in quality of sewage wastewater after inoculation of *Chlorella minutissima* for pH, EC (dS/m), TDS (mg/l), phosphorous (mg/l), potassium (mg/l), nitrate (mg/l), BOD (mg/l), COD (mg/l), ammonium (mg/l) and DO (mg/l) at 5 day interval for 25 days.

Table 3 Phycoremediation of sewage wastewater by *Chlorella minutissima*

Parameter	Initial value	Final value	P value (t test)	Water quality for irrigation*
	Average ± Stand. dev			
pH	8.02 ± 0.05	8.57 ± 0.27	0.06	6.0 – 8.5
EC (dS/m)	3.52 ± 0.07	0.25 ± 0.03	<0.001	0.7 – 3.0 dS/m
TDS (mg/L)	2416 ± 202	136 ± 12.58	<0.001	450–2000 mg/L
P (mg/L)	3.54 ± 0.05	1.15 ± 0.01	<0.001	0-2 mg/L
K (mg/L)	5.5 ± 0.09	1.85 ± 0.03	<0.001	0-2 mg/L
NH_4^+ (mg/L)	5.6 ± 0.18	2.9 ± 0.15	<0.001	0-5 mg/ L
NO_3 (mg/L)	3.06 ± 0.01	0.34 ± 0.05	<0.001	5 – 30 mg/ L
DO (mg/L)	3.5 ± 1.04	8.1 ± 0.40	<0.001	0-7 mg/ L
BOD_5 (mg/L)	114 ± 7.08	7.75 ± 1.12	<0.001	100 mg/ L
COD (mg/L)	157 ± 0.68	30.58 ± 1.39	<0.001	NA

*FAO Soils Bulletin 10 (Dewis and Freitas 1970)

reduction of TDS by *C. minutissima* in sewage wastewater. The reduction in TDS was due to consumption of dissolved solid from wastewater which is nutrient rich for the growth of microalgae. Malla *et al*. (2015) reported the reduction of EC and TDS of about 90-98% by alga *C. minutissima* from tertiary treated wastewater of CETP (Common Effluent Treatment Plant).

Changes in Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Dissolved Oxygen (DO)

BOD and COD are indicator of organic pollutants load in wastewater. Wastewater with high BOD and COD reduces dissolve oxygen availability to the aquatic organisms. Therefore, BOD and COD load of wastewater should be reduced before discharging it in to the fresh water bodies. A standard BOD_5 test conducted over a five-day period is typically used to estimate the decomposable organic compounds in a water bodies. In present study, C. *minutissima* was found very effective in reducing BOD and COD and subsequently increases DO content of the sewage wastewater. The BOD and COD was reduced from 114±7.08 mg/l and 157±0.68 to 7.75±1.12 mg/L and 30.58±1.39 mg/l respectively after 25 days of algal inoculation (Table 3). Besides, DO increased from 3.50±1.04 mg/l to 8.10±0.4 mg/l. The BOD and COD were reduced by 93% and 80.5% respectively (Fig 2), while increased in DO was more than twice to its initial value. The decrease in BOD and COD could be, due to an increase in DO through photosynthesis of algae that might have led to aerobic decomposition of organic matter in the wastewater. Sharma *et al*. (2013) had reported that microalgae *C. minutissima* reduced TDS, BOD and ammonium-N by more than 90% while, COD and Nitrate-N by more than 80% and total P by more than 70% in raw wastewater. Similarly, Govindan (1984) reported BOD and COD reduction from 75% to 95% and from 72% to 91%, respectively, from a mixture of dairy and sewage wastewater. Choudhary *et al*. (2016) have reported that algae removed COD and nitrate below permissible limit from rural sector wastewater by 87% and 70% respectively within 12 days. Malla *et al*. (2015) stated that *C. minutissima* effectively removed TDS (90–98%), ammonical-N and nitrate-N (70–80%), P (60–70%) and K (45–50%) from the wastewater within 12 days. Similarly, about 82% reduction of TDS, nitrate, ammonia, and P from dairy effluent were also reported by Ummalyma and Sukumaran (2014) within 15 days of incubation. Hena *et al*. (2015) had reported the reduction of BOD up to 82% from dairy effluent by using consortium of microalga. The reason for increased in DO might be due to absorption of oxygen by algae from atmosphere, reduction of nitrite and nitrate, and photosynthetic activity performed by algae. High rate of COD removal indicates the ability of microalgae species to tolerate and survive in the high COD levels (Wang *et al*. 2012).

Changes in NPK content

Nitrogen, phosphorus and potassium are primary plant nutrients. Nitrogen is absorbed by the algae in the form of nitrate and ammonia, while phosphorus is absorbed in the form of phosphate and potassium in the form of K^+.Therefore, inoculation of algae in the sewage wastewater significantly reduced amount of the nitrate, ammonia, phosphate and potassium. The initial content of nitrate, ammonium, phosphate and potassium in sewage wastewater were 3.06±0.01 mg/l, 5.6+0.18 mg/l, 3.54±0.05 mg/l and 5.5±0.09 mg/l respectively (Table 3). These nutrients get reduced to 0.34±0.05 mg/l, 2.9±0.15 mg/l, 1.15±0.01 mg/l, and 1.85±0.03 mg/l respectively after 25 days of phycoremediation by *C. minutissima*. The percent reduction was 89%, 48.2%, 67.4% and 66.3% respectively for nitrate, ammonium, phosphate and potassium (Fig 2). Sharma

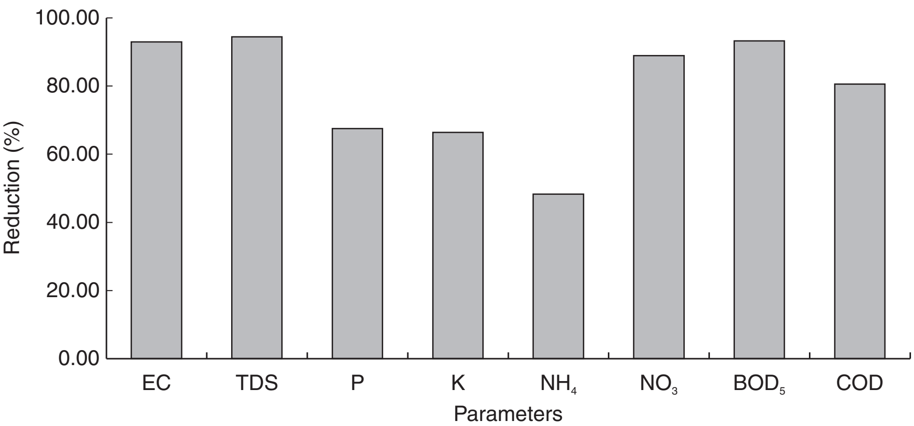

Fig 2 Phycoremediation potential of *Chlorella minutissima* (removal of pollutants) in collected sewage wastewater.

et al. (2013) had reported that *C. minutissima* reduced ammonium-N by more than 90% and Nitrate-N by more than 80% and total P by more than 70% in raw wastewater. While, (Cho *et al*. 2011) had reported a significant reduction in both nitrate and phosphate concentrations (up to 92%) remediated by *C. vulgaris* grown in municipal wastewater. Similarly 96.8–98.4% decreased in ammonical nitrogen and 94.2–97.8% reduction in nitrate nitrogen was recorded by *Chlorella sp*. cultivated in municipal wastewater (Kiran *et al*. 2014).

Algal biomass production

The algal biomass produced per unit of wastewater after inoculation was determined by filtering algal treated wastewater after 25 days of phycoremediation using muslin cloth. The obtained fresh and dry biomass was 1.26±0.07 g/L and 0.44±0.04 g/l of wastewater respectively.

NPK content in dry algal biomass

Nitrogen is one of the most important macro-nutrient for plant. The effectiveness of any organic manure depends on the percentage of nitrogen content in it. The dried algal biomass was used for the determination of NPK content. The NPK content in dry algae biomass was 6.0±0.17%, 1.0±0.10% and 0.48±0.04% respectively. Wilkie and Mulbry (2002), have reported 4.9-7.1% N content and 1.5-2.1% phosphorus content in dry algae biomass, while (Mulbry *et al*. 2007) reported 3.3-6.4% N in dry algal biomass. Adey and Loveland (1998) reported 6-9% N and 1-2% P from harvested algal biomass. Khan *et al*. (2019) quoted that algae (*C. minutissima)* biomass had 5.87% nitrogen after phycoremediation (25 days) of sewage wastewater

Conclusion

Microalgae (*Chlorella minutissima)* plays a successful role in treating sewage wastewater. The maximum pollutants was absorbed by *C. minutissima* within 10-15 days from collected sewage wastewater. The algae biomass obtained after phycoremediation (25 days) typically contained 6% N, 1% P and about 0.48% K, which is higher than most of the available organic manure. The selective algae plays an important role in reducing pollutant load from sewage wastewater andcould reuse the same water for irrigation purpose. The physico-chemical parameters range of resulted wastewater obtained after phycoremediation falls under the guideline of water used for irrigation. Hence, the present study suggest that, the IARI sewage wastewater could be safely applied for the irrigation of crops at IARI fields after the phycoremediation.

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