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ORIGINAL RESEARCH ARTICLE

Adding benefits to phytoremediation of sugar mill effluent by growing water hyacinth (*Eichhornia crassipes*): Evaluation of biomass for biogas production

Vinod Kumar, Jogendra Singh* and Pankaj Kumar

Agro-ecology and Pollution Research Laboratory, Department of Zoology and Environmental Science, Gurukula Kangri Vishwavidyalaya Haridwar-249404 (Uttarakhand), INDIA
*Corresponding author's E-mail: jogendrasinghpatil@gmail.com

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ABSTRACT

In this experiment, we assessed the phytoremediation potential of water hyacinth (*Eichhornia crassipes*) for pollutants removal from sugar mill effluent amended at different concentrations and further biogas production from its grown biomass co-digested with cow dung in a laboratory scale anaerobic digester. The results showed that the maximum values of kinetic growth rate (2.56gg^{-1}d^{-1}), total chlorophyll content (4.10±0.10mg/gfwt.) and fresh plant biomass (393.87±4.67g/Kg) of *E. crassipes* were achieved in 75% concentration of sugar mill effluent after 60 days of phytoremediation experiments. Also, the enrichment factor (E_f) and bioaccumulation factor (B_f) of heavy metals were greater than or equal to 1 in the roots and leaves of *E. crassipes* which indicated efficient elimination of these metals from the sugar mill effluent. Significant values of cumulative biogas production (5195 ml) and predicted by modified Gompertz kinetic model (5238.71 ml) were found after 15 days of anaerobic digestion at 40°C with maximum reduction of COD (83.11%) of the substrate slurry. The plot of log(COD) *vs.* t (HRT) suggested good fitness of first order kinetic equation (R^2= 0.9746) for reduction of co-substrate COD. The different kinetic parameters of COD reduction for biogas production *viz.*, a, xc and *k* were noted as 6096.12, 7.73 and 0.26, with R^2 value of 0.99, respectively. The findings of this study concluded that *E. crassipes* can be used for the phytoremediation of heavy metals and other pollutants most efficiently in 75% concentration of the sugar mill effluent. Additionally, the biomass of *E. crassipes* grown during phytoremediation can be used for enhanced biogas production.

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INTRODUCTION

Globally, the species of the aquatic macrophytes are frequently being tested for their phytoremediation potential using diverse nature of industrial effluents with varied characteristics (Kumar and Chopra, 2016). The rapid and continuous growth of industrial sector has raised the economy of the nation, but, on the other hand it has also degraded all part of the environment as air, water and soil (Mishra and Maiti, 2016). The sugar industry effluent has various pollutants which cause water pollution in the aquatic as well as soil ecosystems when released without proper treatments. The effluent produced during the manufacture of sugar contains a greater amount of pollution load mainly the suspended solids, organic matters, press-mud, bagasse and higher microbial load (Daulta *et al*., 2014). Farmers have been frequently using these effluents for the irrigation purpose in the field crops due to its higher nutrient values while continuously irrigation gradually affect the soil health and accumulate numerous toxicants. In the aquatic environment, addition of different pollutants such as chloride, sulphate, phosphate, magnesium and nitrate of the sugar mill effluent are responsible for eutrophication in the water bodies. Therefore, disposal of the sugar mill effluent in the aquatic resources severely affect the survival of living organisms (Ayyasamy *et al*., 2008). The major

shortcomings of the electric-current, physical, chemical, filtration and adsorbent based technologies for the large scale treatment of the wastewater are the higher input cost and complex manufacture, operation, and maintenance practices makes them limited (Annadurai *et al*., 2002; Mishra and Maiti, 2016).

The phytoremediation is an alternate biological method to remediate the excess nutrients and heavy metal contaminants from the wastewaters. Phytoremediation technology is continuously receiving attention as an innovative, profitable substitute for the treatment of industrial effluents (Kumar *et al*., 2016). The efficiently capable aquatic macrophytes are widely used to eliminate a wide range of micro and macro elements, metals by means of surface adsorption and/or absorption (Fernando *et al*., 2008). These plants absorb the nutrients from the wastewaters and grow more rapidly which makes the process more sustainable to reduce the load efficiently. Hence, such plants are playing prominent role in effective management of industrial wastewaters by recycling the contaminants and help in making aquatic ecosystem cleaner (Kumar *et al*., 2017a).

Water hyacinth (*E. crassipes*) is magnificent aquatic macrophytes as it has high potential to decontaminate the submerged aquatic ecosystems. The dense hairy roots of *E. crassipes* play major role in effectively absorbing a wide range of nutrients and heavy metals from their supplemented medium and further translocate them in different aerial parts (stem and leaves) by means of biological filtration system (Dhote and Dixit, 2009). The water hyacinth plants are also biochemically rich in hemicellulosic content (22–33.97% dry weight) and carbon/nitrogen ratio (20–35) which makes it good substratum for production of biofuels (Jayaweera *et al*., 2007).

Utilization of the plant-based biomass for production of biogas has become an innovative and emerging approach for fulfilling the global energy demands. The biomass of aquatic macrophytes grown during the phytoremediation process has good potential for generation of biogas and, furthermore, the left over digested substrate can be used as biofertilizer (Kumar *et al*., 2017a). Using plant biomass with diverse types of co-substrates increases the biofuel production efficiency (Mishra and Maiti, 2016; Kumar *et al*., 2017a, b).

Thus, no comprehensive report is available on the phytoremediation of the sugar mill effluent by water hyacinth and the use of grown biomass of for biogas production. Keeping in view, the present investigation was planned to assess the potential of *E. crassipes* for pollutant elimination from sugar mill effluent and further biogas production using its grown biomass.

MATERIALS AND METHODS

Experimental setup, collection and characterization of sugar mill effluent

The phytoremediation experiments were conducted in the Multipurpose Experimental Area (MEA) of the Department of Zoology and Environmental Science, Gurukula Kangri Vishwavidyalaya, Haridwar (Uttarakhand), India (29°55'13''N and 78°70'23''E. For this, sugar mill effluent samples were taken from the effluent disposal site of Uttam Sugar Mills Ltd. in Libberheri village of Roorkee, Haridwar (Uttarakhand) (29° 44'38"N and 77°51'14"E) into 25 liter capacity plastic canes. The samples were brought to the laboratory and analyzed for various physico-chemical, microbiological and heavy metals. The phytoremediation experiments were carried out in the glass aquariums (25 liters capacity) in different concentrations (25%, 50%, 75% and 100%) of the sugar mill effluent. The different concentrations *i.e*., 25% (5 liter sugar mill effluent + 15 liter bore well water), 50% (10 liter sugar mill effluent + 10 liter bore well water), 75% (15 liter sugar mill effluent + 5 liter bore well water) and 100% (absolute effluent) of the sugar mill effluent were achieved by diluting the sugar mill effluent with bore well water. A total twenty liter of treatment sample was taken in the glass aquarium and three pre-weighed healthy plants of *E. crassipes* were transplanted in the effluent. The experiment was run for a period of 60 days and replicated three times and arranged in block design. A control investigation was also undertaken to grow *E. crassipes* in bore well water. The experiments were performed with retention time of 24 hrs. and lasted for 60 days. The various physico-chemical, microbiological and heavy metals parameter of sugar mill effluent *viz*., pH, electrical conductivity (EC), total dissolved solid (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldhal nitrogen (TKN), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K); standard plate count (SPC), most probable number (MPN); cadium (Cd), copper (Cu), chromium (Cr), iron (Fe), lead (Pb), manganese (Mn) and zinc (Zn) were analyzed by following the standard methods prescribed by AOAC (2005) and APHA (2012).

Description and collection of the test plant species (*E. crassipes*)

E. crassipes is a free floating, invasive weed and perennial aquatic macrophytes which belongs to the family Pontederiaceae. It is dominantly found in the local aquatic bodies of Northern India and able to grown in a wide range of highly enriched water bodies like ponds, lakes and wetlands. Having good ability to absorb or accumulate a wide variety of contaminants in their vegetative parts also makes this species more suitable for phytoremediation. Healthy plants of *E. crassipes* were collected from the pond situated at Jamalpur Kalan (29°91´20´´N and 78° 13´08´´E) near Gurukula Kangri Vishwavidyalaya Haridwar (Uttarakhand), India. The plants of *E. crassipes* were familiarized in the MEA for one week by placing them in a common macrophytes culture pond to let adapt in the new environment. Finally, the plants of the equal size and weight were transplanted in the glass aquariums for the phytoremediation process.

Calculation of percent pollutants removal efficiency (R_e)

The percent removal efficiency of pollutants from sugar mill effluent by *E. crassipes* was calculated by using the Equation 1 (Hurst, 1997; Kumar *et al*., 2017a, b):

$$\text{Removal Efficiency } (R_e\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

Where, C_i is the initial concentration of the pollutant in the medium and C_f is the final concentration of the pollutant in the medium.

Heavy metals analysis

Both the sugar mill effluent and plants were analyzed for heavy metal (Cd, Cu, Cr, Fe, Pb, Mn and Zn) during the phytoremediation experiments. For this, 10 mL of sugar mill effluent and 1.0g of air dried root and leaves samples of *E. crassipes* were taken out in the digestion tubes separately and 3 mL of conc. HNO_3 was added. Each digestion tube was digested in an electrical heating block for a period of 1 hr. at 150°C. The mixtures were cooled and filtered using Whatman No. 42 filter paper. The final volume was made to 50 mL by addition of 1% HNO_3 and further used for heavy metals analysis using an atomic absorption spectrophotometer instrument(PerkinElmer, Analyst 800 AAS, GenTeh Scientific Inc., Arcade, NY) following the standard methods (AOAC, 2005; Chaturvedi and Sankar, 2006; APHA, 2012).

Calculation of plant growth parameters

Fresh weight and total chlorophyll content of *E. crassipes* plants were at intervals of 0, 15, 30, 45 and 60 days in each treatment of the sugar mill effluent. Fresh weight of *E. crassipes* was determined by using a digital balance. The total chlorophyll content of *E. crassipes* was analyzed using acetone extraction method and the absorbance were recorded in a spectrophotometer (Agilent 60 Cary, UV-Vis Spectroscopy); (Aron, 1949).

Calculation of kinetic growth rate

The kinetic growth rate of *E. crassipes* plants was calculated by evaluating the initial weight with the final weight. The kinetic growth rate was calculated by using the Equation 2 (Hunt, 1978):

$$\text{Kinetic growth rate} = \frac{\ln W_a - \ln W_z}{t2 - t1} \quad (2)$$

Where, $\ln W_a$ and $\ln W_z$ are the logarithms of initial fresh biomass and final fresh biomass at harvest, respectively, while (t_2-t_1) is the duration of the experiment in days. The results were expressed as increase of biomass per unit mass per day ($gg^{-1}d^{-1}$).

Calculation of enrichment (E_f), bioaccumulation (B_f) and translocation (T_f) factors of heavy metals in tissues of *E. crassipes*

The enrichment factor (E_f) of Cd, Cu, Fe, Cr, Pb, Zn and Mn in the roots and leaves *E. crassipes* was calculated using the Equation 3 (Kim and Kim, 1999):

$$\text{Enrichment factor } (E_f) = \frac{C_s}{C_r} \quad (3)$$

Where, C_s is the mean metal concentration of treated sample and C_r is the mean metal concentration of reference.

Bioaccumulation factor (B_f) is the ratio of metal concentration in the plant to the metal concentration in its medium. It describes the accumulation of pollutant within the plant tissues. For plants, the B_f is used as a measure of the efficiency of metal accumulation, whereby the value greater than 1 is the indication of plant's best potential to phytoextraction or phytoremediation (Santillan *et al.*, 2010; Dowdy and McKone, 1997). Bioaccumulation factor was calculated using Equation 4 (Eze, 2014).

$$\text{Bioaccumulation factor } (B_f) = \frac{C_p}{C_m} \quad (4)$$

Where, C_p is the mean metal concentration in plant tissue and C_m is the mean metal concentration in the wastewater medium.

Translocation factor (T_f) is the screening index of hyper accumulator plants for phytoextraction of specific heavy metals. This ratio is an indication of the ability of a plant to translocate metals from its roots to its aerial parts (Mellem *et al.*, 2012). T_f was calculated by using Equation 5.

$$\text{Translocation factor } (T_f) = \frac{C_L}{C_R} \quad (5)$$

Where, C_L and C_R is the concentration of metal in leaves and roots respectively.

Metals that are accumulated by plants and largely stored in the roots of the plants are indicated by T_f values less than 1, with values greater than 1 indicates translocation to the aerial parts of the plant (Mellem *et al.*, 2009).

Design of anaerobic bioreactor for batch mode biogas production

A laboratory scale anaerobic bioreactor set up was designed for the bio gas production by anaerobic digestion of co-substrate. A glass aquarium (30×30×30cm) was used as the chamber and the outer walls of the bioreactor were covered with poly-styrene plastic sheet in order to avoid the temperature loss. An aspirator glass jar of 2 liters capacity was used as a bioreactor for the digestion of different co-substrate which was placed inside the substrate digestion unit having 10 liter water. A digital temperature controller (thermostat unit) was fitted inside the substrate digestion unit to maintain the temperature of water inside the bioreactor (40°C). The biomass of *E. crassipes* grown in the different concentrations of the sugar mill effluent was rinsed with distilled water and dried and then grinded with mechanical mixer to convert the biomass into granular powder. Dry powder of *E. crassipes* (200g), sugar mill effluent (200mL), cow dung (200g) and 200 ml of distilled water were mixed thoroughly to prepare the substrate slurry. The slurry was further diluted in 1:5 ratios with distilled water. 1 liter of the finally prepared sample of the co-substrate slurry was filled in the aspirator glass jar. A gas collection unit and water collection unit was also fitted in the aspirator glass bottle using IV set and rubber cork. The bioreactor was run for 15 days at 40°C for the anaerobic co-digestion of the substrate and generation of biogas. The quantification of biogas was performed by water displacement method per day basis. However, the theoretical estimation of methane was performed based on the reduction in the chemical oxygen demand (COD). The cumulative biogas production was

recorded continuously till 15 days by following the standard method suggested by Goswami *et al.* (2016).

Characterization of physico-chemical parameters of co-substrate

The slurry of co-substrates was analyzed for pH, chemical oxygen demand (COD), total organic carbon (TOC), total Kjeldahl's nitrogen (TKN) and carbon/nitrogen ratio (C/N ratio) before and after co-digestion by following standard methods cited in APHA (2012). The total solids (TS) and volatile solids (VS) of the slurry were determined after drying a small portion of the slurry at 105°C for 24 hrs. (Kumar *et al.*, 2018).

Biogas Prediction analysis using modified Gompertz kinetic model

The equation of Modified Gompertz Kinetic Model was employed to predict the cumulative biogas production and verified to fit the experimental data in order to determine some important kinetic parameters necessary for digester design and optimal operation required for large scale anaerobic plants. The equation was estimated by using nonlinear curve fitting tool, obtained by using optimization tool i.e. OriginLab Pro (version 9.1) software. Gompertz model has already been used by various authors (Atlas, 2009; Lin and Shei, 2008; Li *et al.*, 2008) for successful prediction of maximized biogas production for a perfect lag time. This model can be expressed as Equation 6.

$$Y_{(t)} = P_{exp}\left\{-\exp\left[\frac{\mu_m}{p}(\lambda - t) + 1\right]\right\} \quad (6)$$

Where, $Y_{(t)}$ = Cumulative biogas production, P = Maximum biogas production potential, μ_m = Maximum specific biogas production (ml), λ = lag time (days) and t= Observation time of biogas production (days).

Statistical analysis of the data

The values reported in this study were the mean of three replicates. The means were calculated using MS Excel 2010 while the graphs were plotted using of OriginLab Version 9.1 and Microsoft Excel, 2010 packages. Data was statistically analyzed to determine the levels of significance using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Changes in the physico-chemical and microbiological parameters of sugar mill effluent during phytoremediation

The significant changes in the various physico-chemical and microbiological parameters of sugar mill effluent *E. crassipes* was recorded during 60 days of phytoremediation experiment as presented in the Table 1. The pH of the sugar mill effluent in different was reduced by 5.60%, 7.72%, 8.95%, 9.05% and 7.28% in BWW, 25, 50, 75 and 100% treatments, respectively. The most changes in the pH was found maximum if the sugar mill effluent concentration was less or equal to 75%. Similarly, reduction of other physico-chemical and microbiological parameters *viz.*, EC (2.25±0.04 dSm^{-1}, 33.55%), TDS (422.74±0.69 mgL^{-1}, 66.98%), BOD (94.28±0.16 mgL^{-1}, 71.82%), COD (137.80±1.12 mgL^{-1}, 76.09%), TKN (11.22±0.17 mgL^{-1}, 88.22%), P (17.57±0.16 mgL^{-1}, 79.22%), Ca (31.01±0.49 mgL^{-1}, 72.90%), Mg (21.07±0.82 mgL^{-1}, 76.10%), Na (23.54±0.81 mgL^{-1}, 79.24%) and K (67.17±0.13 mgL^{-1}, 66.71%) of the sugar mill effluent was observed in 75% concentration of sugar mill effluent at statistical significance of *F>prob* (*P<0.05/P<0.01/P<0.001*). For the microbiological parameters of the effluent, the most reduction of MPN (1.059×10^3±43.15 $100mL^{-1}$, 66.10%) and SPC (2.019×10^3±24.12 CFU mL^{-1}, 67.29%) was also found in the 75% treatment. Though, there was a lag phase between 0 to 15 days where the reduction was not significant (*P>0.05*), thereafter, between 15 to 45 days or log phase, the most significant removal was observed (*P<0.05/P<0.01/P<0.001*). Finally, the time between 45 to 60 days can be termed as the stationary or decline phase as there was very less or no significant reduction again in the medium. The findings of the present study are in good agreement with previous reports of Kumar and Chopra (2017) who observed higher values of TDS, BOD_5, COD and others pollutants of sewage effluent were reduced more efficiently at 50% concentration who carried out phytoremediation using aquatic macrophyte water caltrop (*Trapa natans* L.). Alade and Ojoawa (2009), Akinbile and Yusoff (2012) and Kouamé *et al.* (2016) demonstrated reduction of COD, TKN, NO^{3-}, NH_3 and PO_4^{3-} load from the wastewater using water hyacinth and water lettuce. Kumar *et al.* (2017a) reported that the water hyacinth (*E. crassipes*) has potentially treated the paper effluent and it can be used for the elimination of nitrogen, phosphorus, calcium, magnesium and parameters as MPN and SPC of the effluent. Dar *et al.* (2011) also described that water hyacinth has future prospective for the treatment of wastewater.

Reduction of heavy metals of sugar mill effluent during phytoremediation using *E. crassipes*

A significant (*P<0.05/P<0.01/P<0.001*) reduction of heavy metals *viz.*, Cd (94.99%), Pb (94.44%), Zn (79.70%), Cr (79.31%), Mn (73.13%), Cu (70.67%), Fe (66.58%), and was noted in 75% concentration of the sugar mill effluent after 60 days of phytoremediation experiments (Table 2). The less reduction in the minimal concentration of sugar mill effluent may be subjected to the bioavailability of the net metal content in the medium as already discussed in our previous study (Kumar *et al.*, 2017). Higher concentration of heavy metals in the medium increases its toxicity and tends to decrease plants ability to survive in such stressful conditions. These results are parallel to the findings of Dhir *et al.* (2009) and Kisholay and Das (2015), who found significant reduction of Cd, Cr, Cu, Fe, Pb, Mn and Zn metals present in the paper mill effluent. These findings are also in accordance with Solomon and Marcus (2016) who reported that *E. crassipes* significantly reduced the in the content of heavy metals and others pollutants.

Occurrence of heavy metals in tissues of *E. crassipes*

The contents of Cd, Cu, Fe, Cr, Pb, Zn and Mn in the roots and

Table 1. Changes in physico-chemical and microbiological characteristics of sugar mill effluent before and after phytoremediation and removal efficiency of *E. crassipes* in percent.

Parameters	Concentration	Before phytoremediation	After phytoremediation				Removal (%) at 60 days
			15 Days	30 Days	45 Days	60 Days	
pH	BWW (0%)	7.61±0.06	7.60ns±0.05	7.44ns±0.02	7.21ns±0.03	7.19ns±0.02	5.60
	25%	7.71±0.08	7.60ns±0.01	7.44ns±0.04	7.11ns±0.02	7.09ns±0.02	7.96
	50%	7.76±0.07	7.57ns±0.04	7.24ns±0.03	7.02ns±0.02	7.02ns±0.02	9.58
	75%	7.78±0.07	7.53ns±0.03	7.27ns±0.03	7.06ns±0.03	7.04ns±0.01	7.59
	100%	7.81±0.09	7.70ns±0.03	7.45ns±0.04	7.24ns±0.04	7.22ns±0.01	7.55
EC (dS m^{-1})	BWW (0%)	0.49±0.04	0.47ns±0.04	0.45ns±0.03	0.40ns±0.04	0.38ns±0.04	22.33
	25%	1.16±0.04	1.06ns±0.04	1.04ns±0.03	0.96ns±0.04	0.95ns±0.04	18.16
	50%	2.32±0.05	2.19ns±0.02	2.08ns±0.03	1.87ns±0.03	1.84ns±0.05	20.83
	75%	3.48±0.06	3.27ns±0.02	3.07ns±0.04	2.35ns±0.04	2.25*±0.04	33.55
	100%	4.64±0.07	4.51ns±0.02	4.24ns±0.03	4.05ns±0.04	4.02ns±0.01	13.37
TDS (mg L^{-1})	BWW (0%)	144.33±0.40	132.12ns±0.20	110.53ns±0.85	96.22*±0.42	94.79*±0.54	34.32
	25%	426.66±0.23	392.81ns±1.52	376.18ns±1.01	313.08ns±1.01	305.48*±0.62	28.40
	50%	853.36±1.13	728.41ns±1.48	636.42ns±1.85	563.82ns±1.20	560.06*±0.99	34.37
	75%	1280.08±2.62	933.86ns±1.73	804.45*±1.51	425.56**±1.24	422.74**±0.69	66.98
	100%	1706.79±2.72	1623.90ns±1.41	1586.14ns±1.03	1538.47ns±1.16	1528.09ns±1.02	10.47
BOD (mg L^{-1})	BWW (0%)	3.13±0.05	3.05ns±0.03	2.63ns±0.03	2.14ns±0.03	2.12ns±0.02	32.09
	25%	111.98±0.40	95.26ns±0.19	77.88ns±0.17	58.63*±0.15	55.23*±0.09	50.68
	50%	224.04±0.60	172.69ns±0.27	143.28*±0.20	111.60*±0.16	107.28**±2.66	52.11
	75%	335.72±0.62	229.35ns±0.53	211.30ns±0.46	99.44**±0.24	94.6**±0.16	71.82
	100%	447.88±4.56	428.72ns±0.63	401.78ns±0.62	397.50ns±0.54	395.96ns±0.13	11.59
COD (mg L^{-1})	BWW (0%)	8.28±0.62	7.63ns±0.54	7.10ns±0.08	6.92ns±0.06	6.90ns±0.04	16.67
	25%	192.59±1.96	171.46ns±1.61	131.93ns±1.48	103*.03±1.24	101.91*±1.20	47.08
	50%	385.72±0.62	289.35ns±0.53	221.30ns±0.46	198.72*±0.37	191.72*±0.37	50.30
	75%	576.23±0.66	421.71ns±0.26	331.53*±0.34	142.71**±0.32	137.80***±0.12	76.09
	100%	770.88±1.15	701.14ns±1.09	689.22ns±0.57	666.47ns±0.33	662.30ns±0.32	14.08
TKN (mg L^{-1})	BWW (0%)	6.44±0.48	5.02ns±0.27	4.26ns±0.10	3.88ns±0.06	3.83ns±0.02	40.50
	25%	33.78±0.94	29.36ns±1.84	22.08ns±2.95	17.50ns±1.53	15.17*±1.01	55.11
	50%	67.58±1.53	53.82ns±1.47	44.59*±1.58	32.43*±1.16	28.53**±0.95	57.78
	75%	100.37±1.52	74.75ns±1.03	42.74*±1.51	14.62**±0.53	11.22***±0.17	88.82
	100%	135.18±1.56	120.03ns±1.23	112.85ns±1.47	108.70ns±1.02	106.07*±0.51	21.53
P (mg L^{-1})	BWW (0%)	3.67±0.09	3.19ns±0.06	2.91ns±0.07	2.14ns±0.03	2.11ns±0.03	42.47
	25%	28.19±1.09	21.79ns±1.16	16.75ns±0.98	13.93ns±0.43	10.91*±0.22	61.29
	50%	56.40±1.41	44.35ns±0.55	34.44ns±0.41	26.32*±0.19	21.62**±0.26	61.66
	75%	84.57±2.55	57.15ns±2.34	41.81ns±0.61	21.77*±0.55	17.57***±0.16	79.22
	100%	112.79±1.54	103.02ns±0.76	87.02ns±0.46	83.89*±0.31	82.06*±0.06	27.25
Ca (mg L^{-1})	BWW (0%)	14.22±0.50	12.15ns±0.36	10.61ns±0.27	9.71ns±0.16	9.10*±0.01	36.01
	25%	38.15±1.23	32.05ns±0.70	28.65ns±0.52	22.48*±0.44	20.47*±0.27	46.33
	50%	76.32±1.67	57.96ns±0.62	53.27ns±0.21	43.17*±0.06	40.12*±0.06	47.44
	75%	114.45±1.98	76.52ns±1.57	61.32*±1.49	33.06**±0.86	31.01***±0.49	72.90
	100%	152.63±2.01	146.85ns±0.81	138.66ns±0.21	132.18ns±0.06	130.03ns±0.02	13.51
Mg (mg L^{-1})	BWW (0%)	12.03±0.30	10.59ns±0.18	9.62ns±0.17	9.07ns±0.05	8.06ns±0.03	33.05
	25%	29.39±0.79	22.22ns±0.88	17.66ns±0.83	14.39*±0.36	12.11*±0.33	58.80
	50%	58.80±1.01	45.22ns±0.75	36.55*±0.67	29.72*±0.45	27.09*±0.51	60.25
	75%	88.19±.03	65.77ns±0.45	37.95*±0.35	23.13**±0.94	21.07***±0.82	76.10
	100%	117.58±1.18	104.70ns±0.85	96.88ns±0.42	91.31ns±0.45	87.75*±0.35	25.37
Na (mg L^{-1})	BWW (0%)	13.69±0.47	11.56ns±0.32	10.53ns±0.14	9.56ns±0.12	9.06ns±0.04	33.81
	25%	37.80±1.04	30.02ns±1.13	24.05ns±1.03	15.63*±0.77	13.44*±0.63	64.46
	50%	75.72±1.57	52.57ns±1.09	42.80ns±1.08	30.74*±0.63	25.59*±0.56	66.07
	75%	113.39±2.09	84.42ns±1.22	63.52*±1.50	28.85*±1.00	23.54**±0.81	79.24
	100%	151.38±1.97	138.36ns±0.93	130.46ns±0.50	124.51ns±0.55	123.31ns±0.09	18.55
K (mg L^{-1})	BWW (0%)	8.73±0.37	7.46ns±0.51	6.97ns±0.07	5.54ns±0.11	5.42ns±0.09	37.90
	25%	67.17±1.19	52.93ns±0.52	41.81ns±0.61	31.54*±0.10	31.69*±0.17	52.82
	50%	134.35±1.45	110.36ns±1.03	95.16*±0.97	63.50*±0.29	62.34**±0.19	53.60
	75%	201.76±2.12	163.86ns±1.79	101.48*±1.36	71.51**±0.51	67.17***±0.13	66.71
	100%	268.74±1.85	245.08ns±1.28	235.91ns±0.77	228.81ns±0.67	227.66ns±0.23	15.29
MPN (100 ml^{-1})	BWW (0%)	-	-	-	-	-	-
	25%	1.04×10^{3}±30.57	$0.58\times10^{3^{*}}$±22.68	$0.72\times10^{3^{*}}$±19.86	$0.58\times10^{3^{*}}$±16.70	$0.56\times10^{3^{*}}$±9.85	45.52
	50%	2.085×10^{3}±61.25	$1.738\times10^{3^{*}}$±60.91	$1.457\times10^{3^{*}}$±42.52	$1.177\times10^{3^{*}}$±36.69	$1.135\times10^{3^{*}}$±27.07	45.57
	75%	3.124×10^{3}±96.10	$2.59\times10^{3^{*}}$±87.10	$2.073\times10^{3^{**}}$±66.03	$1.106\times10^{3^{**}}$±59.34	$1.059\times10^{**3}$±48.82	66.10
	100%	4.163×10^{3}±97.73	$3.830\times10^{3^{*}}$±86.07	$3.675\times10^{3^{*}}$±71.30	$3.505\times10^{3^{*}}$±40.08	$3.440\times10^{3^{*}}$±43.15	55.84
SPC (cfu ml^{-1})	BWW (0%)	-	-	-	-	-	-
	25%	2.051×10^{3}±56.36	$1.753\times10^{3^{*}}$±38.16	$1.436\times10^{3^{*}}$±26.27	$1.376\times10^{3^{*}}$±30.05	$1.26\times10^{3^{**}}$±37.77	38.30
	50%	4.119×10^{3}±108.67	$3.536\times10^{3^{*}}$±81.74	$2.925\times10^{3^{*}}$±76.96	$2.259\times10^{3^{*}}$±53.70	$2.188\times10^{3^{**}}$±37.03	46.86
	75%	6.173×10^{3}±133.35	$5.460\times10^{3^{*}}$±81.74	$3.534\times10^{3^{**}}$±71.08	$2.076\times10^{3^{**}}$±50.85	$2.019\times10^{3^{**}}$±42.12	67.29
	100%	8.327×10^{3}±151.29	$7.912\times10^{3^{*}}$±77.54	$7.477\times10^{3^{*}}$±64.73	$7.350\times10^{3^{*}}$±41.71	$7.206\times10^{3^{*}}$±10.41	13.47

Values are presented in the table are the means ± SD of three replicates; -: Non detectable; BWW (0%): - Bore well water; ns-non significant; *, **, ***- Significantly at $P<0.05$ or $P<0.01$ or $P< 0.001$ level of ANOVA, respectively.

Table 2. Changes in heavy metals characteristics of sugar mill effluent before and after phytoremediation using *E. crassipes* and removal efficiency in percent.

Parameters	Concentration	Before phytoremediation	After phytoremediation				Removal (%) at 60 days
		Zero Days	15 Days	30 Days	45 Days	60 Days	
Cd (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.005±0.006	0.004ns±0.004	0.003ns±0.002	0.003ns±0.001	0.002*±0.001	50.00
	50%	0.010±0.005	0.007ns±0.004	0.006ns±0.003	0.005ns±0.006	0.004*±0.003	62.07
	75%	0.015±0.012	0.010ns±0.005	0.007ns±0.002	0.002ns±0.002	0.002***±0.001	94.99
	100%	0.020±0.014	0.018*±0.010	0.016ns±0.008	0.014ns±0.007	0.013ns±0.006	36.07
Cu (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.008±0.005	0.006ns±0.003	0.005ns±0.002	0.004ns±0.002	0.003*±0.001	60.00
	50%	0.016±0.007	0.012ns±0.005	0.009*±0.002	0.006*±0.003	0.005**±0.001	67.25
	75%	0.025±0.010	0.018ns±0.005	0.014*±0.005	0.008**±0.003	0.007***±0.002	70.67
	100%	0.032±0.010	0.029ns±0.009	0.027ns±0.006	0.025ns±0.006	0.024ns±0.004	25.26
Fe (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.307±0.074	0.285ns±0.066	0.259*±0.065	0.243*±0.053	0.240*±0.054	21.63
	50%	0.615±0.124	0.478*±0.119	0.468*±0.044	0.432*±0.049	0.429*±0.053	30.30
	75%	0.922±0.010	0.707*±0.010	0.614*±0.004	0.310**±0.002	0.308**±0.004	66.58
	100%	1.228±0.039	1.164ns±0.023	1.159ns±0.020	1.128ns±0.006	1.127ns±0.004	8.20
Cr (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.003±0.002	0.002ns±0.002	0.002ns±0.002	0.002*±0.001	0.001*±0.001	60.00
	50%	0.006±0.005	0.003ns±0.002	0.002*±0.001	0.002*±0.001	0.001*±0.001	78.95
	75%	0.010±0.006	0.005*±0.003	0.004*±0.003	0.003*±0.002	0.002***±0.001	79.31
	100%	0.012±0.007	0.010ns±0.004	0.009ns±0.003	0.009ns±0.003	0.009ns±0.003	27.78
Pb (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.008±0.005	0.007ns±0.005	0.006ns±0.004	0.004ns±0.002	0.004*±0.003	56.00
	50%	0.016±0.008	0.012ns±0.005	0.010ns±0.003	0.006*±0.006	0.006*±0.006	64.58
	75%	0.024±0.013	0.017ns±0.006	0.014*±0.007	0.005**±0.004	0.003***±0.002	94.44
	100%	0.032±0.013	0.030ns±0.012	0.029ns±0.012	0.027ns±0.011	0.026ns±0.007	19.79
Zn (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.073±0.032	0.057ns±0.016	0.052ns±0.013	0.049*±0.011	0.046*±0.008	36.82
	50%	0.146±0.065	0.111ns±0.020	0.108**±0.016	0.102**±0.013	0.080**±0.017	45.08
	75%	0.221±0.069	0.117*±0.025	0.105*±0.016	0.048*±0.009	0.045**±0.007	79.70
	100%	0.292±0.054	0.280ns±0.041	0.276ns±0.038	0.272ns±0.036	0.271ns±0.034	7.19
Mn (mg L^{-1})	% (BWW)	-	-	-	-	-	-
	25%	0.297±0.069	0.273ns±0.047	0.239ns±0.042	0.204ns±0.013	0.199*±0.007	33.00
	50%	0.594±0.115	0.476ns±0.119	0.463ns±0.050	0.353*±0.037	0.342*±0.035	42.48
	75%	0.892±0.130	0.671*±0.061	0.510*±0.101	0.243**±0.057	0.240***±0.054	73.13
	100%	1.188±0.160	1.111ns±0.024	1.068ns±0.020	1.059ns±0.014	1.052ns±0.010	11.44

Values are presented in the table are the means ± SD of three replicates; -: Non detectable; BWW (0%): - Bore well water; ns-non significant; *, **, ***- Significantly at $P<0.05$ or $P<0.01$ or $P< 0.001$ level of ANOVA, respectively.

Changes in the physico-chemical parameters of co-substrate

The selected physico-chemical parameters of the substrates slurry *viz.*, TS, COD, VS, TOC and C/N, were significantly reduced after 15 days of anaerobic digestion. The initial values of pH (7.88), TS (64.22%), COD (2865mgL^{-1}), VS (58.88%), TOC (36.54%), TKN (1.36%), and C/N ratio (26.86) and were found reduced to 6.22, 26.98%, 484 mgL^{-1}, 27.44%, 13.99%, 0.76% and 18.41 after 15 days of HRT at 40°C (Table 5). These changes occurred due to the secretion of some acids, breakdown of lignocellulosic contents, reclamation of other organic compounds and formation of other low molecular weight compounds during the anaerobic digestion process by activity of the numerous enzymes (Mathew *et al.*, 2014; Kumar *et al.*, 2017b). Total solid content (%) in substrate was reduced up to 26.98% due to reduction of organic fraction present in the substrate. The most suitable condition for the anaerobic digestion process in context of digestion was at a temperature of 40°C as earlier reported in our another study (Kumar *et al.*, 2018). Manjula and Mahanta (2014) also reported the similar results of total solids and volatile solids in the co-digestion of food waste and pig manure at 37°C. The anaerobic digestion process is very effective to convert large quantities (>50%) of COD present in the substrate slurry into biogas (Wilkie *et al.*, 2000; O'Sullivan *et al.*, 2010). COD of the substrate was decreased from 2865 mgL^{-1} to 484 mgL^{-1} (83.11%) in 15 days of period at 40°C (Figure 14). Manjula and Mahanta (2014) observed the similar removal of COD of co-substrate during the biogas production process. O'Sullivan *et al.* (2010) noted the quite lower, approximately 50% reduction in COD during the utilization of dairy effluents in biogas production. Moreover, the best percentile reduction in COD may be subjected to a higher retention time as the COD reduction increase with increasing HRT (Bhadouria and Sai, 2011). The C/N ratio was altered from 26.86 to 18.41. The ideal C/N ratio for anaerobic digestion is considered to be in the range of 20-30 C/N ratio (Doraisamy *et al.*, 2013; Kumar *et al.*, 2017b). The high C/N ratio refers the rapid consumption of nitrogen by the methanogenic bacteria to meet their protein requirement and as a result, the biogas production was reduced (Wang *et al.*, 2014). When pH value rose higher than 8.5, it begins to exert a toxic effect on the methanogenic bacteria (Wilkie *et al.*, 2000). Generally, to maintain the C/N level of the digester substrate at optimum levels, substrate of high C/N ratio can be co-digested with substrate of low C/N ratio (Sawant *et al.*, 2007). The total Kjeldhal nitrogen (TKN) and total organic carbon (TOC) of the substrate such as 1.36% - 0.76 and 36.54% – 13.39% was observed after digestion process for the production of biogas. The similar results were reported by Kumar *et al.* (2017b).

Prediction analysis for biogas production using modified Gompertz kinetic model

Biogas production was monitored using water displaced method and measured until there was no more biogas production. The modified Gompertz model was used to estimate the fitness for prediction of cumulative biogas production. Results showed that the cumulative biogas production (5195 mL) and using modified Gompertz kinetic model (5238.71 mL) were achieved after 15 days of anaerobic digestion (Figure 15). The value of different kinetic parameters for the model *viz.*, a, xc and k were 6096.12, 7.73 and 0.26, respectively with R^2 (coefficient of determination) value of 0.99. The modified Gompertz model gave the satisfactory result in predicting biogas production for all variables as earlier reported by previous findings by Budiyono *et al.* (2014) and Kumar *et al.* (2018).

Kinetics of co-substrate COD reduction

The kinetics of COD reduction was studied by applying a first order kinetic equation, by plotting log(COD) *vs.* t (HRT) which showed the good of fitting correlation coefficient (R^2= 0.9594) as presented in Figure 15. The reduction pattern of COD indicates the effectiveness of methanogenic activity and the temperature near to 40°C may also be termed as optimum for mesophilic condition where the best condition for maximum COD reduction was observed. The values of the rate constant and R^2 were 0.0337 and 0.9746, respectively. These results are in good agreement with the findings of Samuel *et al.* (2017) who reported the good fitness of first order kinetic equation to evaluate the COD reduction during biogas production.

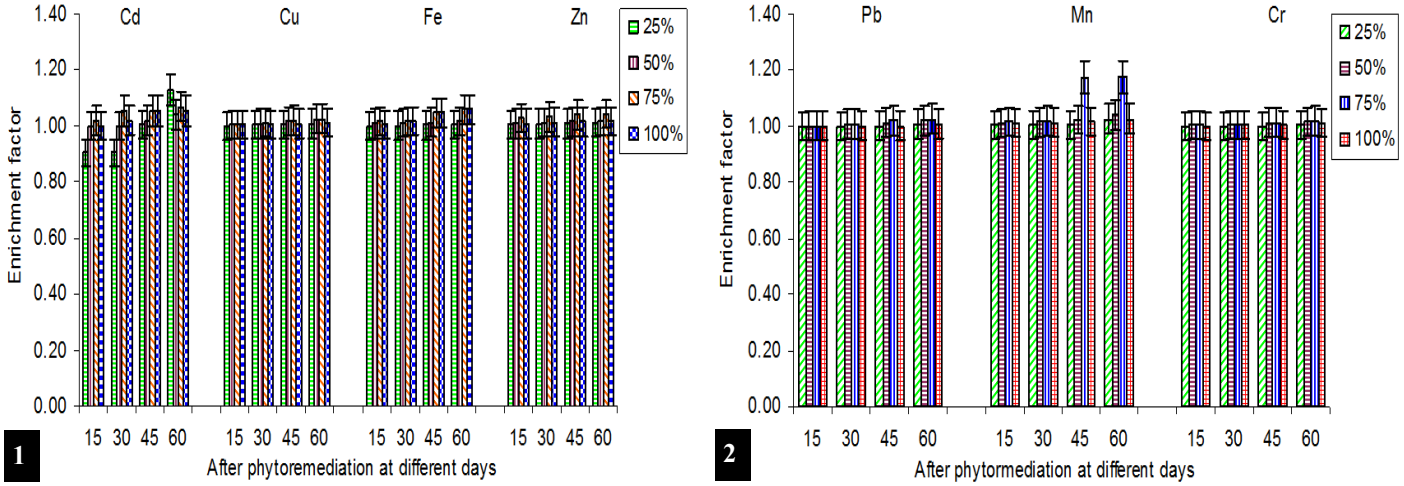

Figure 1-2. *Enrichment factor (E_f) of different heavy metals in the roots of E. crassipes after phytoremediation at different days.*

leaves of *E. crassipes* were progressively enhanced from 15 to 45 days as the maximum uptake was occurred during this period. We observed significant (*P<0.05/P<0.01*) enrichment of different heavy metals in the roots and leaves of *E. crassipes* as presented in Tables 3 and 4. The quantity of different heavy metals in the roots and leaves of *E. crassipes* were observed highest in 75% concentration of the sugar mill effluent. However, the order of their accumulation as per quantity was noted in the order of Fe>Mn>Zn>Cu>Cr>Pb>Cd. The diverse absorption and accumulation of different heavy metals indicated speckled uptake due to self-protection mechanism of the *E. crassipes* against different metals depends on the affinity of these metals in different physiological processes, growth and development. In the similar way, the varied accumulation of different metals in the tissues of *Eichhornia*, *Pistia*, *Lemna*, *and Vallisneria* aquatic plants has also been reported by Sharma *et al*. (2004) and Kumar *et al*. (2016) which supports the data of this study.

Enrichment of heavy metals in tissues of *E. crassipes*

Generally, the hyper accumulator plant having enrichment factor (E_f) values greater than or equal to 1 ($E_f \geq 1$) tells that the selected plant is decent for phytoremediation process. It also indicates that the plant has high capability of to accumulate and tolerate toward higher concentration of heavy metals in its medium. In this study, Figures 1-2 and 3-4 shows the enrichment factor of different heavy metals in the roots and leaves of *E. crassipes* grown in sugar mill effluent amended at different concentrations, respectively. The results revealed that the heavy metals enrichment in the root of *E. crassipes* was observed high as compared to the leaf parts. The order of heavy metal enrichment for roots was observed as Mn>Cd>Fe>Zn>Cu>Pb>Cr; while for leaves it was found as Cd>Fe>Mn>Zn>Pb>Cu>Cr. This defines that *E. crassipes* has varied heavy metal enrichment behavior toward several metals, as; a few of them easily enriched in the root parts while some are not. Similarly, for leaf parts some metals were actively transported to the leaf easily, while, some were retained in the roots. This might be due to the affinity of plant towards the transportation of the heavy metals from roots to leaves, which is related with the biochemistry of the plant, where special plant proteins together bind with heavy metals and further transport them through the plant body. Srivastava *et al*. (2014) reported varied enrichment factor of different heavy metals in the tissues of the plant of *E. crassipes* as Cr>Fe>Cu>Mn>Mg, when grown in Sulem Sarai wetland of Allahabad, Uttar Pradesh, India. They reported that E_f of different heavy metals extended 1.02 to 1.07 in roots of *E. crassipes* whereas 1.02 to 1.85 in the leaves of *E. crassipes* during phytoremediation, which is in good agreement with the results of present study.

Bioaccumulation of heavy metals in the tissues of *E. crassipes*

The bioaccumulation by the plants is defined as their capability to accumulate heavy metals into their different body parts, however, it is strongly affected by numerous external as well as internal factors like; nutrient availability, plant metabolism and microbial growth in the medium (Chandra *et al*., 2017). Figures 5-6 and 7-8 represents the bioaccumulation factor (B_f) of different heavy metals accumulated in the roots and leaves of *E. crassipes*, respectively. We found that maximum bioaccumulation of all selected heavy metals were observed in the 75% concentration of sugar mill effluent. However, the order of their bioaccumulation was varied as Cr>Fe>Cu>Mn>Mg for roots and Cr>Fe>Cu>Mn>Mg for leaves, respectively. The B_f value of all heavy metals was greater than 1 (<1) which showed good potential of *E. crassipes* for the removal heavy metals which supports to consider this as hyper-accumulative plant for phytoremediation purposes (Santillan *et al*., 2010). Mellem (2009) and Chandra *et al*. (2017) reported the B_f values *viz*., 1-2 and conferring *E. crassipes* having high ability to accumulate heavy metals from the contaminated water bodies.

Translocation of heavy metals in the tissues of *E. crassipes*

The transportation of heavy metals from roots to leaves of *E. crassipes* is due to nutritional and metabolic requirements, which is strongly regulated by the several physiological and biochemical processes (Chandra *et al*., 2017). Data in the Figures 9-10 shows the translocation factor (T_f) of selected heavy metals from roots to leaves of *E. crassipes*. It was observed that the translocation factor reached maximum in 75% concentration of the sugar mill effluent with an elemental order of Cd>Zn>Pb>Fe>Cr>Mn>Cu, respectively. Similarly, previous study carried out by Chandra *et al*. (2017) also reported the translocation factor of selected plant greater 1 when used for phytoremediation of pulp and paper industry effluent.

Changes in plant growth parameters of *E. Crassipes* during phytoremediation

Figures 11-13 shows the kinetic growth rate, total plant fresh biomass and total chlorophyll content of *E. crassipes* grown different concentration of sugar mill effluent, respectively. The maximum values of kinetic growth rate (2.56 $gg^{-1}d^{-1}$), total fresh plant biomass (339.87±4.64 g/kg) and total chlorophyll content (4.10±0.10 mg/gfwt) were observed highest in 75% concentration (Figures 11-13). This might be due to the presence of the favorable concentration of different plant nutrients in the sugar mill effluent which trigged the plant growth to reach maximum as earlier reported by (Sooknah and Wilkie, 2014; Kumar *et al*., 2016). However, in the 100% concentration treatment, the plant growth was progressively declined after 45-60 days as compared to other treatments, which may be due to metal induced inhibition of physiological processes and biosynthesis (Mukherjee and Kumar, 2005). A similar type of reduction in the total chlorophyll content of water lettuce was reported in a previous study and concluded that it was due to the presence of Hg toxicity in the wastewater (De *et al*., 1985), Cd and Hg treatment by *Hydrilla verticillata* and *Lemna minor* (Chatterjee and Nag, 1991), Pb treated by the *Salvina natans* (Sen and Bhattacharyya, 1993), Pb and Cr treatment by *Ipomea aquatica* (Alam and Chatterjee, 1994) and Zn, Cu, Cd and Cr treatment of wastewater using water hyacinth and water lettuce (Kouamé *et al*., 2016).

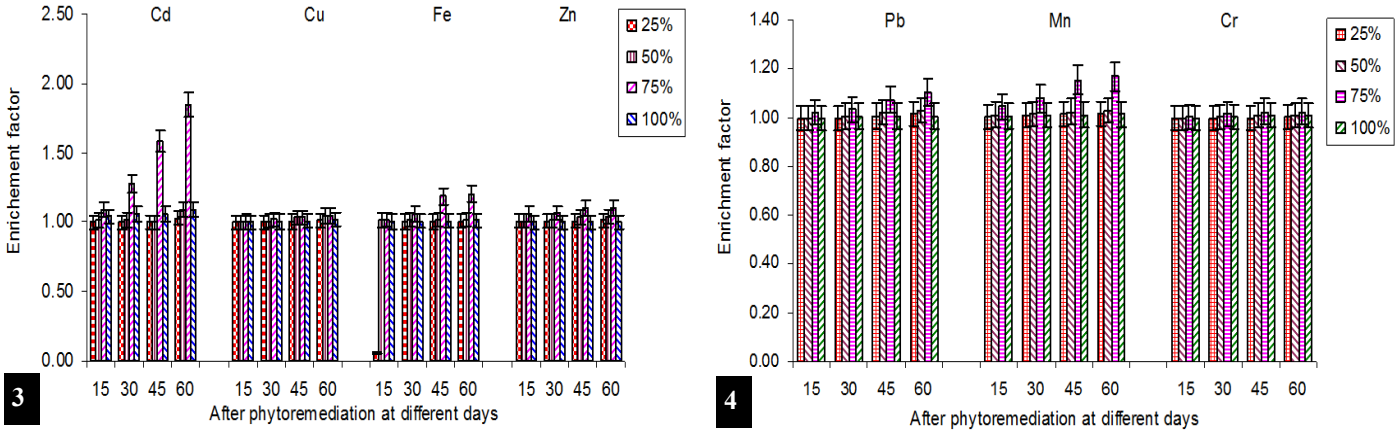

Figure 3-4. *Enrichment factor (E_f) of different heavy metals in the leaves of E. crassipes after phytoremediation at different days.*

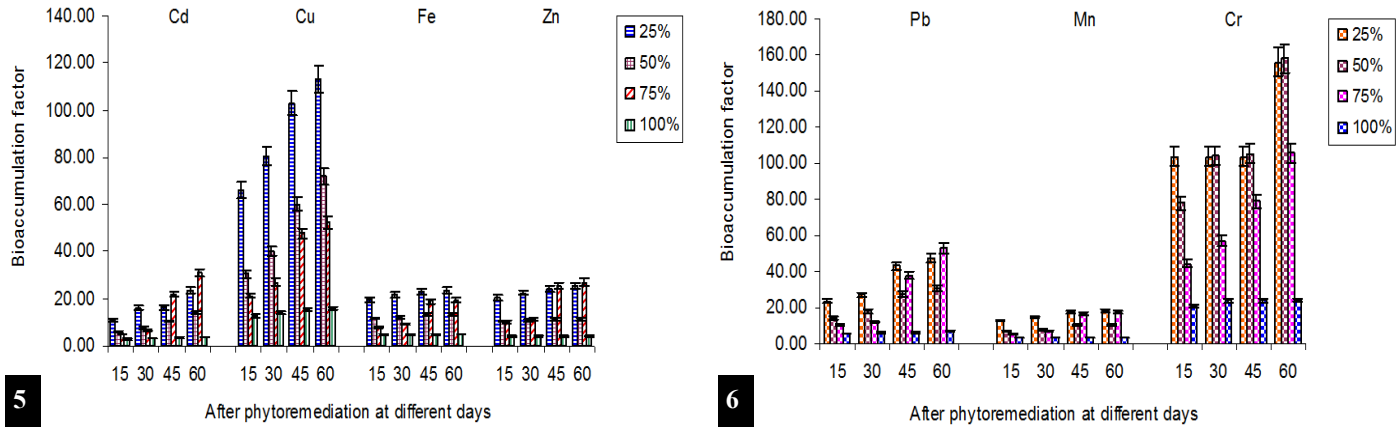

Figure 5-6. *Bioaccumulation factor (B_f) of different heavy metals in the roots of E. crassipes after phytoremediation at different days*

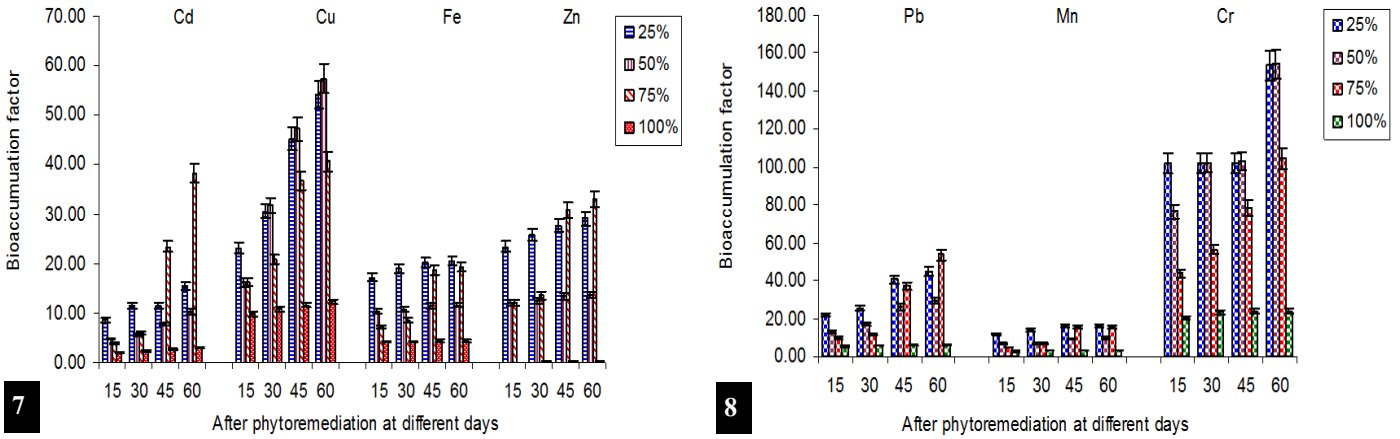

Figure 7-8. *Bioaccumulation factor (B_f) of different heavy metals in the leaves of E. crassipes after phytoremediation at different days.*

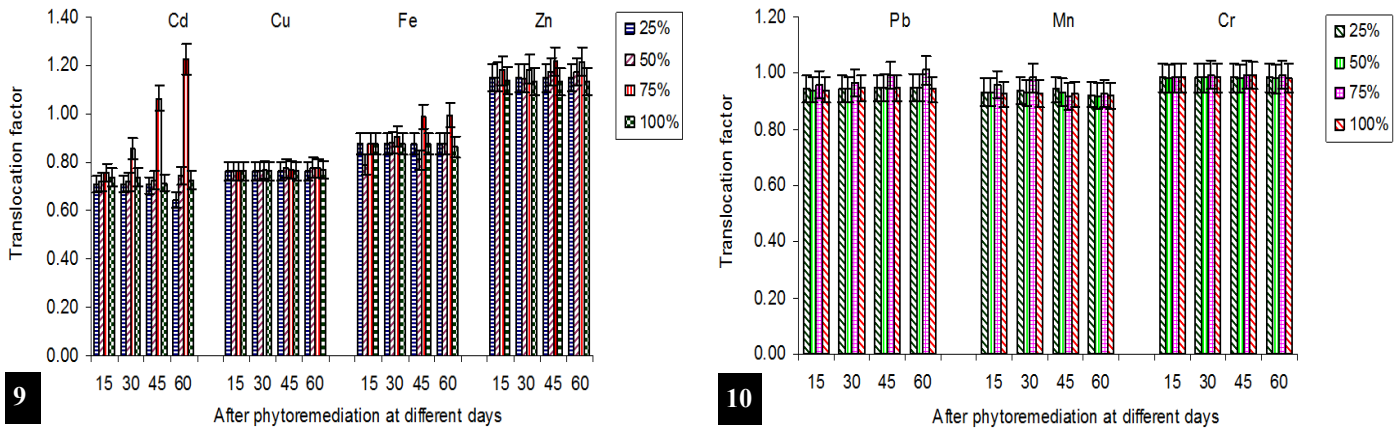

Figure 9-10. *Translocation factor (T_f) of different heavy metals in the roots to leaves of E. crassipes after phytoremediation at different days.*

Table 3. Heavy metals concentration in roots of *E. crassipes* before and after phytoremediation grown in sugar mill effluent.

Parameters	Concentration	After phytoremediation				
		Zero days	15 days	30 days	45 days	60 days
Cd (mg/kg)	% (BWW)	0.049±0.006	-	-	-	-
	25%		0.049ns±0.006	0.049ns±0.006	0.051ns±0.005	0.050ns±0.003
	50%		0.049ns±0.006	0.049ns±0.006	0.050ns±0.005	0.051ns±0.008
	75%		0.050ns±0.005	0.051ns±0.007	0.051ns±0.007	0.052*±0.008
	100%		0.049ns±0.005	0.050ns±0.006	0.051ns±0.008	0.052*±0.008
Cu (mg/kg)	% (BWW)	0.375±0.004	-	-	-	-
	25%		0.375ns±0.004	0.376ns±0.006	0.377ns±0.006	0.378ns±0.008
	50%		0.376ns±0.006	0.378ns±0.007	0.382ns±0.007	0.385*±0.010
	75%		0.377ns±0.006	0.379ns±0.008	0.383ns±0.009	0.384*±0.010
	100%		0.376ns±0.006	0.377ns±0.006	0.378ns±0.007	0.379ns±0.008
Fe (mg/kg)	% (BWW)	5.613±0.057	-	-	-	-
	25%		5.618ns±0.060	5.625ns±0.057	5.630ns±0.053	5.635ns±0.059
	50%		5.663ns±0.058	5.673ns±0.067	5.694ns±0.074	5.703ns±0.083
	75%		5.703ns±0.088	5.713ns±0.098	5.884*±0.077	5.948*±0.069
	100%		5.637ns±0.062	5.639ns±0.064	5.653ns±0.081	5.759ns±0.069
Zn (mg/kg)	% (BWW)	1.164±0.013	-	-	-	-
	25%		1.171ns±0.010	1.173ns±1.346	1.174ns±1.347	1.175ns±1.349
	50%		1.174ns±1.174	1.179ns±1.179	1.181ns±1.181	1.183*±1.183
	75%		1.198*±0.015	1.203*±0.026	1.210*±0.030	1.213*±0.035
	100%		1.173ns±0.012	1.180ns±0.013	1.182ns±0.011	1.183*±0.013
Pb (mg/kg)	% (BWW)	0.172±0.008	-	-	-	-
	25%		0.172ns±0.008	0.172ns±0.008	0.173ns±0.009	0.174ns±0.010
	50%		0.173ns±0.009	0.174ns±0.010	0.175ns±0.011	0.176ns±0.012
	75%		0.173ns±0.009	0.174ns±0.010	0.176*±0.012	0.177*±0.013
	100%		0.173ns±0.009	0.173ns±0.009	0.173ns±0.009	0.174ns±0.010
Mn (mg/kg)	% (BWW)	3.490±0.022	-	-	-	-
	25%		3.502ns±0.087	3.502ns±0.087	3.506ns±0.089	3.581*±0.097
	50%		3.530ns±0.048	3.545ns±0.075	3.573*±0.093	3.636*±0.110
	75%		3.547ns±0.076	3.557ns±0.081	4.090**±0.084	4.101**±0.086
	100%		3.530ns±0.048	3.539ns±0.071	3.555ns±0.077	3.582*±0.084
Cr (mg/kg)	% (BWW)	0.207±0.004	-	-	-	-
	25%		0.207ns±0.004	0.207ns±0.005	0.207ns±0.005	0.208ns±0.006
	50%		0.208ns±0.004	0.208ns±0.005	0.210ns±0.006	0.211*±0.007
	75%		0.208ns±0.005	0.208ns±0.005	0.210ns±0.006	0.210ns±0.006
	100%		0.207ns±0.004	0.208ns±0.004	0.208ns±0.005	0.210ns±0.006

Values are presented in the table are the means ± SD of three replicates; -: Non detectable; BWW (0%): - Bore well water; ns-non significant; *, **, ***- Significantly at P<0.05 or P<0.01 or P< 0.001 level of ANOVA, respectively.

Table 4. Heavy metals concentration in leaves of *E. crassipes* before and after phytoremediation grown in sugar mill effluent.

Parameters	Concentration	After phytoremediation				
		Zero days	15 days	30 days	45 days	60 days
Cd (mg/kg)	% (BWW)	0.035±0.004	-	-	-	-
	25%		0.035ns±0.004	0.035ns±0.005	0.035ns±0.005	0.036ns±0.006
	50%		0.035ns±0.005	0.035ns±0.005	0.036ns±0.006	0.038ns±0.007
	75%		0.038ns±0.007	0.044*±0.010	0.055(±0.011	0.064*±0.013
	100%		0.036ns±0.005	0.037ns±0.005	0.037ns±0.005	0.038ns±0.005
Cu (mg/kg)	% (BWW)	0.285±0.004	-	-	-	-
	25%		0.285ns±0.004	0.286ns±0.004	0.287ns±0.005	0.289ns±0.005
	50%		0.286ns±0.005	0.288ns±0.006	0.295ns±0.007	0.299*±0.010
	75%		0.287ns±0.004	0.292ns±0.005	0.295ns±0.012	0.298*±0.013
	100%		0.286ns±0.005	0.287ns±0.005	0.288ns±0.006	0.291ns±0.004
Fe (mg/kg)	% (BWW)	4.905±0.056	-	-	-	-
	25%		4.921ns±0.049	4.923ns±0.049	4.930ns±0.049	4.944ns±0.049
	50%		4.975ns±0.513	4.998ns±0.461	4.999*±0.462	4.999*±0.462
	75%		5.005*±0.003	5.185*±0.028	5.820*±0.074	5.911**±0.055
	100%		4.930±0.044	4.939±0.044	4.947±0.033	4.956±0.022
Zn (mg/kg)	% (BWW)	1.336±0.003	-	-	-	-
	25%		1.346ns±0.009	1.346ns±0.009	1.347ns±0.007	1.349ns±0.007
	50%		1.346ns±0.009	1.351ns±0.009	1.387ns±0.011	1.390*±0.008
	75%		1.412*±0.035	1.424*±0.055	1.472*±0.062	1.473*±0.065
	100%		1.337ns±0.003	1.338ns±0.005	1.338ns±0.005	1.339ns±0.006
Pb (mg/kg)	% (BWW)	0.162±0.007	-	-	-	-
	25%		0.162ns±0.007	0.162ns±0.007	0.163ns±0.009	0.165ns±0.010
	50%		0.162ns±0.007	0.163ns±0.009	0.166ns±0.011	0.167ns±0.012
	75%		0.166ns±0.011	0.168ns±0.012	0.174*±0.009	0.179*±0.010
	100%		0.162ns±0.007	0.163ns±0.009	0.163ns±0.009	0.163ns±0.009
Mn (mg/kg)	% (BWW)	3.248±0.060	-	-	-	-
	25%		3.263ns±0.071	3.283ns±0.079	3.297ns±0.080	3.302*±0.094
	50%		3.288ns±0.073	3.298ns±0.079	3.328*±0.085	3.340*±0.098
	75%		3.398*±0.076	3.498*±0.097	3.748*±0.088	3.798*±0.101
	100%		3.268ns±0.070	3.278ns±0.095	3.290ns±0.115	3.293ns±0.118
Cr (mg/kg)	% (BWW)	0.204±0.003	-	-	-	-
	25%		0.204ns±0.003	0.204ns±0.003	0.204ns±0.003	0.205ns±0.004
	50%		0.204ns±0.004	0.205ns±0.004	0.206ns±0.004	0.206ns±0.005
	75%		0.205ns±0.004	0.207ns±0.005	0.209*±0.006	0.209*±0.006
	100%		0.204ns±0.003	0.205ns±0.003	0.206ns±0.004	0.206ns±0.005

Values are presented in the table are the means ± SD of three replicates; -: Non detectable; BWW (0%): - Bore well water; ns-non significant; *, **, ***- Significantly at P<0.05 or P<0.01 or P< 0.001 level of ANOVA, respectively.

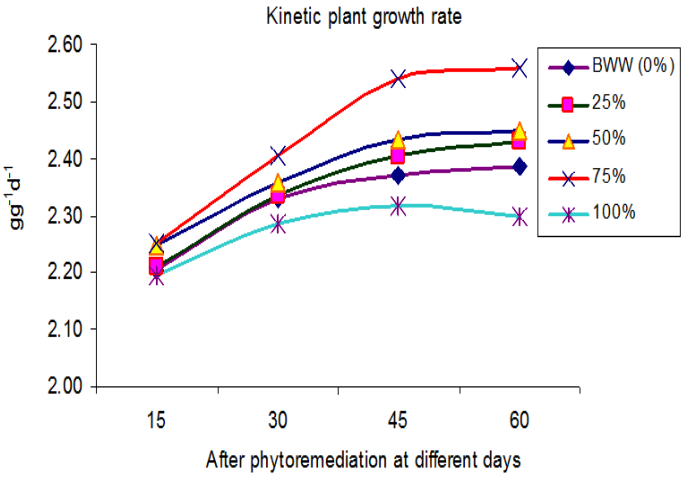

Figure 11. *Kinetic growth rate (KGR) of E. crassipes after phytoremediation at different days.*

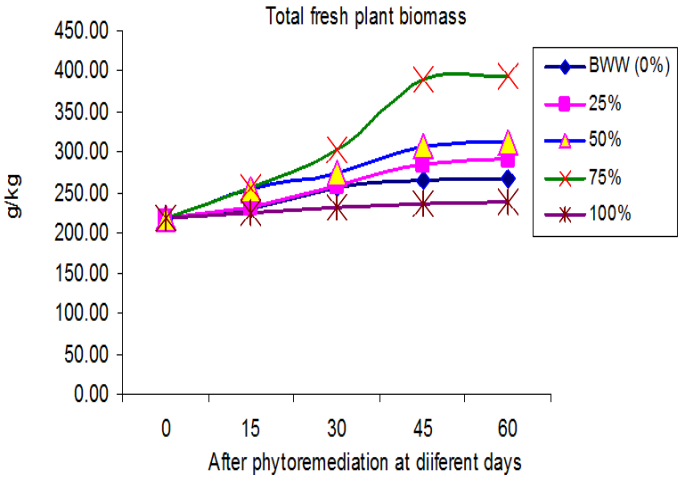

Figure 12. *Total fresh plant biomass of E. crassipes after phytoremediation at different days.*

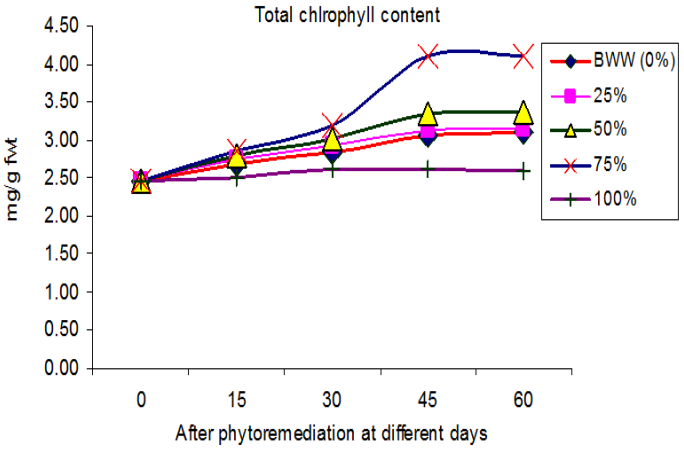

Figure 13. *Total chlorophyll content of E. crassipes after phytoremediation at different days.*

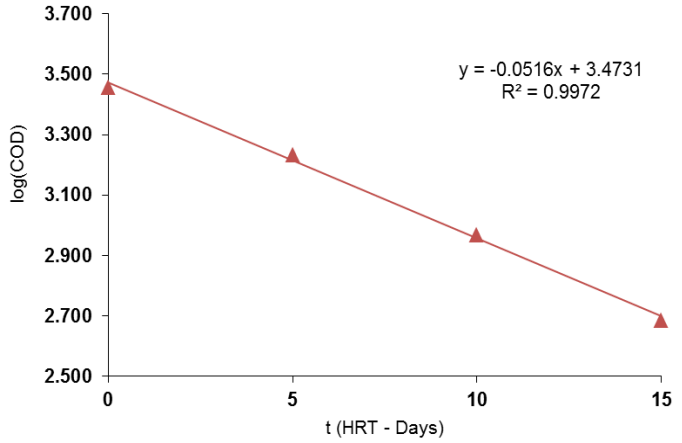

Figure 14. *Plot of log(COD) vs t (HRT) for kinetic reduction of co-substrate at 40°C.*

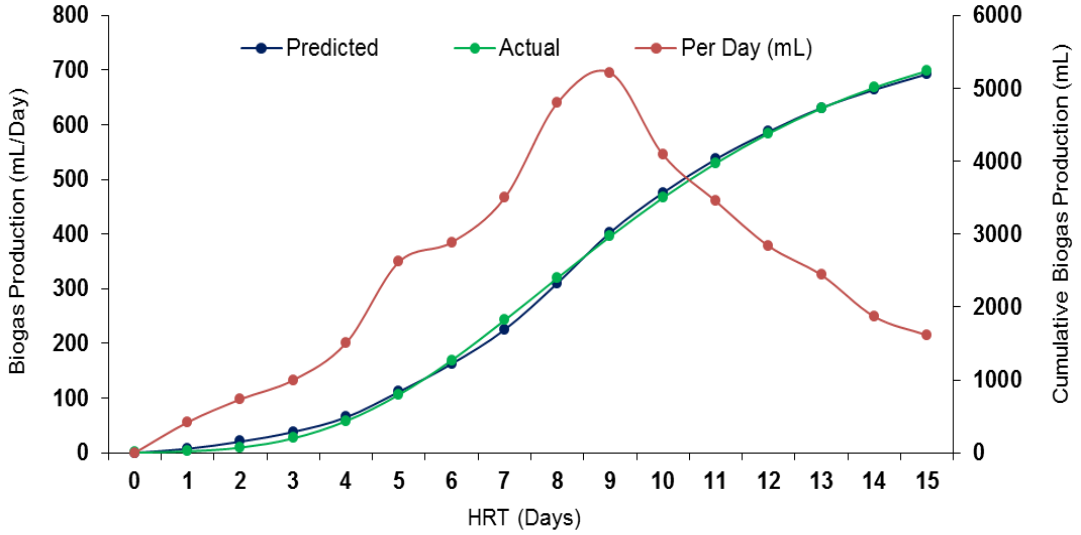

Figure 15. *Biogas production (mL/Day) and cumulative biogas production (actual and predicted by modified Gompertz kinetic model).*

Table 5. Changes in parameters of co-substrate used for biogas production at different digestion days.

Days	Temperature	pH	TS (%)	COD (mg/l)	VS (%)	TOC (%)	TKN (%)	C/N
0 day	40°C	7.88	64.22	2865	58.88	36.54	1.36	26.86
5th day		7.07ns	49.54**	1709*	44.33**	23.76**	1.16*	20.48*
10th day		6.26*	33.21**	932**	35.46**	19.54**	0.99**	19.73*
15 th day		6.22*	26.98**	484**	27.44**	13.99*	0.76**	18.41*

*Level of significant at $P<0.05$; ** Level of significant at $P<0.01$; ns* Not significant

Conclusion

The dual approach of this study is to add benefits to phytoremediation of sugar mill effluent by growing water hyacinth and further evaluation of biomass for biogas production. The results of this experiment concluded that *E. crassipes* significantly reduces both the organic and inorganic pollutants present in the sugar mill effluent. The plant growth attributes of *E. crassipes viz*., fresh weight; total chlorophyll content and kinetic growth rate were found highest in 75% concentration of the sugar mill effluent during 15-60 days. Beside this, the fresh weights, total chlorophyll content and kinetic growth rate of *E. crassipes* was decreased when 100% concentration of sugar mill effluent was used. Additionally, the plant biomass which was grown in the sugar mill effluent was found to have high potential of biogas production. The by-products of the bioreactor residue can be further used as organic fertilizer.

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