Experimental and kinetics study for phytoremediation of sugar mill effluent using water lettuce (*Pistia stratiotes* L.) and its end use for biogas production

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Received: 1 June 2017 / Accepted: 7 September 2017 / Published online: 18 September 2017
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Abstract In present study, the performance of phytoremediation by *Pistia stratiotes* on sugar mill effluent (SME) and its end use for biogas production are investigated. The objectives of the study are to determine the nutrient and pollution reduction efficiency of *P. stratiotes* from SME and evaluation of its biomass as a feedstock for biogas production. Various concentrations of SME (25, 50, 75, and 100%) were remediated by *Pistia stratiotes* (initial weight; 150 g) outdoor for 60 days under batch mode experimental setup. The results showed that *P. stratiotes* achieved marked reduction in nutrient (TKN, 72.86%; TP, 71.49%) and pollutant load (EC, 25.69%; TDS, 57.26%; BOD, 69.40%; COD, 61.80%; Ca^{2+} , 56.79%; Mg^{2+} , 55.01%; Na^+ , 42.86%; K^+ , 54.38%; MPN, 78.13%; SPC, 60.13%) from 75% SME at the end of the experiment. The highest biomass (328.48 ± 2.04 g) and chlorophyll content (3.62 ± 3.04 mg/g) were also achieved with 75% SME. The dried biomass of *P. stratiotes* (from 75% SME) was inoculated with cow dung (10% w/v) and diluted with distilled water (1:10). The whole content was used as a substrate for the biogas production within hydraulic retention time (HRT) of 30 days at room temperature. Substrate parameters such as pH, TS (%), COD (mg/L),

TKN (%), TOC (%), VS (%), and C/N ratio were reduced from 7.85 to 6.0, 66.65 to 28.65%, 12,900 to 2800 mg/L, 0.95 to 0.75%, 45.54 to 19.5%, 76.87 to 28.78%, and 47.94 to 26.00, respectively, in 30 days of HRT. About 8478.6 mL of cumulative biogas production was evaluated by modified Gompertz equation. Thus, the present investigation not only achieved efficient nutrient and pollution reduction from SME but also proved the potential of *P. stratiotes* for biogas production.

Keywords *Pistia stratiotes* · Phytoremediation · Biogas · Modified Gompertz equation · Sugar mill effluent

Introduction

Rapid urbanization and industrialization is placing an unprecedented pressure on water quality and demand. There are numerous other constraints such as inefficient infrastructure, weak urban and municipal regulation, inadequate financial services, which together bring deterioration in environment quality (Kumar and Chopra 2014). Growth of industrial sector has raised the economy of nation but at the same time it has degraded all segment of environment (water, air, and soil) in a significant extent (Brar et al. 2017). The sugar industry is categorized under prime agro-business sector (Sahu 2017). A significantly large amount of waste is generated during the processing of sugar, which contain large amount of pollutants in terms of suspended solids, organic compounds, sludge, press mud, and bagasse (Tiwari and Sahu 2017). In addition to these pollutants, the presence of carbonate ion, polyatomic anion, and oil and grease also increased the complexity of waste generated from sugar industry (Srivastava et al. 2015).

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At present, about 526 sugar mills are operating in India, which contributed in a total production of 33.69 million tons of sugar during the year of 2015–2016 (Indian Sugar Mills Association 2016). It is estimated that approximately 2000 L of water is required to crush one ton of sugar and about 1000 L of wastewater is generated during this process (Kolhe et al. 2009). The highest amount of wastewater is generated during the excess condensation (200 L) followed by hot liquid gland cooling (150 L), spray pond overflow (102 L), external cooling (100 L), daily cleaning and washing (100 L), domestic (90 L), leakage and stream trap (100 L), boiler blowdown (75 L), periodical cleaning (75 L), and laboratory use (6 L) (Kolhe et al. 2009). Various chemicals such as calcium hydroxide ($\text{Ca}(\text{OH})_2$), phosphoric acid (H_3PO_4), and polyelectrolytes are used to enhance the pH of extract, limiting the process for coagulation, desecration and carbonation, respectively (Kushwaha 2013). Gaseous bubbling of carbon dioxide and sulfur dioxide is also carried out to lower the pH of decamped juice and to eradicate the color of raw sugar, respectively (Jadhav et al. 2013). Additions of these chemicals cause the complexity and toxicity in the generated wastewater.

Several methods have been suggested by authors for the treatment of sugar mill effluent such as adsorption, electrochemical, anaerobic treatment, and biological oxidation. These treatment methods are associated with various drawbacks such as inadequate treatment, large chemical input, high-cost input, and high-energy input. Some authors (Pathak et al. 2014; Kothari et al. 2012; Kumar and Chopra 2016) have reported integrated approach for wastewater treatment and energy recovery from the process using aquatic flora. In this context, water lettuce (*Pistia stratiotes* L.) can be used for the phytoremediation process. Water lettuce (*Pistia stratiotes* L.) is a free-floating stoloniferous and small aquatic perennial herb belonging to the aroid family (*Araceae*) and monotypic genus in the subfamily of *Aroideae*. It is dispersed in the tropical and subtropical region of Asia, Africa, and America. *P. stratiotes* possesses potential to absorb the nutrient from the contaminated aquatic environment (Tyagi and Agarwal 2014). This aquatic macrophyte is highly adaptive in diverse environmental conditions and possesses potential to grow in various types of industrial wastewater released from rubber processing industry (Owamah et al. 2014), dairy industry (Victor et al. 2016), chromate mine industry (Saha et al. 2017), and tannery industry (Jahan et al. 2014). Thus, the use of *P. stratiotes* for phytoremediation of industrial wastewater offers a low-cost and solar energy-driven clean-up practice (Kumar et al. 2016; Kumar and Chopra 2016).

Furthermore, the plant biomass after the phytoremediation can be used as a potential feedstock for biogas production. Biogas is a fuel that is produced by anaerobic

digestion of organic matter such as animal residue or plant waste. Some common materials used to inoculate the methanogenic bacteria include cow dung, poultry droppings, pig dung, and digested sewage (Ehiri et al. 2014). Rapid growth rate of *P. stratiotes* ensures the frequent availability of biomass for biogas generation. Previous investigations has shown that *P. stratiotes* contains 49.4% carbohydrates, 16.5% protein, 3.6% lipid, 17.8% fiber, and fiber 23.8% ash, which is an ideal composition for a feedstock for biogas generation (Pantwong et al. 2015).

Therefore, the present investigation was carried out to integrate the phytoremediation and anaerobic digestion process to achieve cost-effective and sustainable practice for treatment of SME using *P. stratiotes*.

Materials and methods

Collection and characterization of wastewater

The sugar mill effluent was collected from the effluent disposal sites of Uttam Sugar Mills Ltd. Libberheri, Roorkee, Haridwar (Uttarakhand). The industrial site was located on ($29^{\circ}44'38''\text{N}$ and $77^{\circ}51'14''\text{E}$) NH 58 about 45 km away from Haridwar (Uttarakhand), India. The effluent was collected in plastic cans (25–30 L capacity) and brought to the laboratory and stored at 4 °C. Physico-chemical parameters of the effluent were immediately measured by following the standard methods prescribed by the AOAC (2005) and APHA (2012). The mean value of the selected parameters such as initial pH (8.6 ± 0.07), EC ($7.26 \pm 0.06 \text{ dS m}^{-1}$), TDS ($1585.77 \pm 7.06 \text{ mg L}^{-1}$), BOD ($472.65 \pm 3.02 \text{ mg L}^{-1}$), COD ($391.55 \pm 2.06 \text{ mg L}^{-1}$), TKN ($144.09 \pm 2.06 \text{ mg L}^{-1}$), *P* ($146.49 \pm 3.07 \text{ mg L}^{-1}$), Ca^{2+} ($226.24 \pm 1.09 \text{ mg L}^{-1}$), Na^{+} ($185.38 \pm 2.01 \text{ mg L}^{-1}$), K^{+} ($230.25 \pm 2.09 \text{ mg L}^{-1}$), MPN ($6.71 \times 10^3 \pm 350/100 \text{ mL}$), and SPC ($14.11 \times 10^3 \pm 900 \text{ mL}^{-1}$) indicated the significant extent of pollution load in collected effluent that can cause adverse impact to flora and fauna of the receiving aquatic body.

Description of selected aquatic plant

Water lettuce is a free-floating aquatic plant and possesses ability to accumulating or absorbs the contaminants by their whole body. *P. stratiotes* is a monotypic kind in the subfamily *Aroideae*. *P. stratiotes*, also known as Jalkumbhi, is a free-floating stoloniferous, slight aquatic returning herb belonging to the aroid family (*Araceae*) floating on lakes, streams, and still water ponds and in lime-rich water throughout India. Water lettuce (*Pistia stratiotes* L.) was collected from the local ponds located at

Jamalpur Kalan village (29.9120°N and 78.1308°E), Haridwar district (Uttarakhand), India.

Phytoremediation of SME (sugar mill effluent)

Experiments for phytoremediation of SME using *P. stratiotes* were conducted in the 25-L glass aquariums. Different concentrations of SME, viz. 0% (Bore well water, BWW), 25% (5 L SME + 15 L BWW), 50% (10 L SME + 10 L BWW), 75% (15 L SME + 5 L BWW), and 100% (absolute effluent) were prepared and used for the phytoremediation treatment. Twenty liter of each selected concentration of SME was filled in the glass jar and healthy plants of known biomass of *P. stratiotes* were transferred in the artificial pond. The whole experiment was observed for 60 days. Wastewater from each experimental aquarium was characterized for reduction in pollution load on each 15th day of interval, and pollution reduction efficiency of plant was calculated by the following equation (Hurst 1997):

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

where C_i is the initial concentration of pollutant and C_e is the final concentration of pollutant.

Observation of growth of *P. stratiotes*

The growth of *P. stratiotes* in the batch study was measured in terms of dry biomass (g) as well as total chlorophyll content (mg/g) every 15 days over a total resident time of 60 days (Tables 1, 2). In order to measure the dry biomass, *P. stratiotes* was harvested and dried over 80 °C in microwave oven for 3 h to remove the moisture content and desiccated for constant weight. The total chlorophyll content was measured by spectrophotometric (Model No. HALO DB-20) technique following the protocol prescribed by Aron (1949). Total chlorophyll content in biomass of *P. stratiotes* was calculated by the following equation:

$$\text{Total Chl (g L}^{-1}\text{)} = 0.0202 \text{ A}_{663} + 0.00802 \text{ A}_{645}. \quad (2)$$

Anaerobic digestion of wastewater grown *P. stratiotes*

The lab-scale experimental plan for anaerobic digestion process was designed and assessed for biogas production process. A batch setup (2 L capacity) was operated for experimental study with 1 L of working volume. Biomass of *P. stratiotes* grown in 75% SME was rinsed with distilled water and grinded with mixer to convert the biomass into granular powdered form. A small portion of substrate was dried over 105 °C for 24 h, then weighted again and put into 550 °C for another two hours for TS and VS analysis. The slurry was prepared from 1:10 proportion of biomass and distilled water inoculated with digested cow dung (10% w/v). Initial characterization of slurry was performed by following the standard method as prescribed by APHA (2012). The substrate used for biogas generation which was initially characterized as pH (7.85), TS (66.65%), COD (12,900 mg/L), TKN (0.95%), TOC (45.54%), VS (76.87%), and C/N ratio (47.94) was found to be reduced in a significant extent within 30 days of HRT (Table 3), which was higher than the optimum value as suggested by Maishanu and Hussaini (1991). Batch experiment for anaerobic digestion was operated at room temperature of 30 °C ± 2 °C and HRT of 30 days (Table 3). Quantification of biogas was performed by water displacement method on per day basis; however, theoretical estimation of methane was performed on the basis of reduction in chemical oxygen demand. The biogas production per day is shown in Fig. 6.

Kinetics of biogas production process

Modified Gompertz equation was employed to calculate the cumulative biogas production. Gompertz equation has been used by many authors (Atlas 2009; Lin and Shei 2008; Li et al. 2008) for the prediction of maximum biogas

Table 1 Total biomass of water lettuce (*P. stratiotes* L.) before and after phytoremediation grown in sugar mill effluent

Parameter	Concentration	Before phytoremediation	After phytoremediation			
			15 days	30 days	45 days	60 days
Total biomass (g)	Control (BWW)	150 ± 1.04	174.17 ± 2.02	189.88 ± 201	211.22 ± 2.03	216.67 ± 2.02
	25%		182.24 ± 2.02	245.27 ± 2.03	297.74 ± 3.02	302.45 ± 3.04
	50%		199.44 ± 2.03	257.74 ± 3.02	289.73 ± 3.04	313.98 ± 4.01
	75%		203.87 ± 2.04	247.67 ± 3.03	299.89 ± 2.02	328.48 ± 2.04
	100%		167.15 ± 2.02	195.27 ± 2.03	217.74 ± 2.02	221.23 ± 3.02

Data are mean ± SD of three replicates

Table 2 Variation in chlorophyll content of water lettuce (*P. stratiotes* L.) during the phytoremediation of sugar mill effluent

Parameter	Concentration	Before phytoremediation	After phytoremediation			
			15 days	30 days	45 days	60 days
Total chlorophyll content (mg/g)	Control (BWW)	2.13 ± 2.03	2.28 ± 2.02	2.36 ± 2.02	2.44 ± 2.03	2.49 ± 2.03
	25%		2.66 ± 2.04	2.98 ± 3.03	3.21 ± 3.05	3.28 ± 3.04
	50%		2.88 ± 2.05	3.21 ± 3.04	3.24 ± 2.04	3.29 ± 3.05
	75%		2.85 ± 3.01	3.45 ± 3.05	3.53 ± 3.03	3.62 ± 3.04
	100%		2.23 ± 2.02	2.34 ± 2.03	2.39 ± 2.03	2.46 ± 2.03

Table 3 Variation in parameters of substrate used for biogas production at different digestion days

Days	Temperature	pH	TS%	COD mg/L	TKN%	TOC%	VS%	C/N
0 day	30 °C	7.85	66.65	12,900	0.95	45.54	76.87	47.94
10th day		6.4	48.5	5400	0.87	28.43	58.3	32.68
20th day		6.1	34.8	3800	0.84	21.5	42.56	25.60
30th day		6.0	28.65	2800	0.75	19.5	28.78	26.00

production potential, specific maximum biogas production, and lag time. This equation can be expressed as

$$Y(t) = P \exp \left\{ - \exp \left[\frac{\mu_m}{P} (\lambda - 1) + 1 \right] \right\}, \quad (3)$$

where $Y(t)$ is the cumulative biogas production; P is the maximum biogas production potential; μ_m is the maximum specific biogas production (mL); λ is the lag time (days); and t is the observed time of biogas production (days).

The modified Gompertz equation was carefully used to fit the experimental data of this study in order to determine some important kinetic parameters necessary for digester design and optimal operation of large-scale anaerobic plants. In the present study, the equation was estimated using nonlinear curve fitting tool, obtained by optimization tools using Origin 9.0 software.

Statistical analysis of data

The values reported in the present study were the mean of three replicates. The means were calculated with the help of MS Excel 2007, while the graphs were plotted with the help of Sigma plot, 2000.

Results and discussion

Growth characteristics of *P. stratiotes* at varying wastewater concentrations

From Table 1, it is revealed that the maximum biomass growth of *P. stratiotes* 328.48 ± 2.04 g was achieved in

75% SME, while a further increase in the concentration reduces the biomass growth (221.23 ± 3.02 g) of *P. stratiotes*. The observation of total chlorophyll content in biomass of *P. stratiotes* also revealed the same information and showed the highest total chlorophyll content (3.62 ± 3.04 mg/g) in biomass grown at 75% SME. At 100% SME, the total chlorophyll content was reduced up to 2.46 ± 2.03 mg/g, which confirms the inhibitory effect of 100% SME as shown in Table 2. Fonkou et al. (2002) reported that water lettuce doubles its biomass in just over 5 days, triples it in 10 days, quadruples it in 20 days, and has its inventive biomass multiplied by a factor of 9 in <1 month under optimum nutrient condition.

Efficiency of *P. stratiotes* for treatment of SME

A significant extent of reduction in physico-chemical parameters was achieved by *P. stratiotes*, which is shown in Table 4. The pH of wastewater was found to be reduced from its initial value and after 15 days of contact time the pH profile was 7.6 ± 0.07 , 7.7 ± 0.07 , 7.9 ± 0.08 , and 8.2 ± 0.08 for 25, 50, 75, and 100% SME concentration, respectively. After 30 days of contact time, the pH profile was 7.5 ± 0.07 , 7.5 ± 0.07 , 7.6 ± 0.06 , and 7.6 ± 0.07 for 25, 50, 75, and 100% SME concentration, respectively. Similar pace of reduction in pH was also observed after 45 days of contact time which remained constant after the 60 days of contact time with a final pH profile of 7.3 ± 0.05 , 7.2 ± 0.06 , 7.1 ± 0.07 , and 7.5 ± 0.07 for 25, 50, 75, and 100% SME concentration, respectively. Electrical conductivity from control was found to reduce from

Table 4 Variations in physico-chemical and microbiological characteristics of sugar mill effluent before and after phytoremediation

Parameters	Concentration	Before phytoremediation	After phytoremediation			
			15 days	30 days	45 days	60 days
pH	Control (BWW)	7.6 ± 0.06	7.5 ± 0.06	7.4 ± 0.06	7.3 ± 0.07	7.3 ± 0.05
	25%	7.7 ± 0.07	7.6 ± 0.07	7.5 ± 0.07	7.4 ± 0.05	7.3 ± 0.05
	50%	7.9 ± 0.08	7.7 ± 0.07	7.5 ± 0.07	7.3 ± 0.07	7.2 ± 0.06
	75%	8.3 ± 0.09	7.9 ± 0.08	7.6 ± 0.06	7.3 ± 0.07	7.1 ± 0.07
	100%	8.6 ± 0.07	8.2 ± 0.08	7.6 ± 0.07	7.5 ± 0.07	7.5 ± 0.07
EC (dS m ⁻¹)	Control (BWW)	1.33 ± 0.02	1.27 ± 0.02	1.24 ± 0.01	1.19 ± 0.02	1.16 ± 0.01
	25%	1.81 ± 0.01	1.71 ± 0.02	1.68 ± 0.02	1.63 ± 0.02	1.61 ± 0.02
	50%	3.63 ± 0.02	3.39 ± 0.04	3.27 ± 0.04	3.11 ± 0.04	3.10 ± 0.04
	75%	5.45 ± 0.06	5.26 ± 0.05	5.01 ± 0.05	4.17 ± 0.05	4.05 ± 0.05
	100%	7.26 ± 0.06	7.11 ± 0.06	7.04 ± 0.07	6.08 ± 0.06	6.06 ± 0.06
TDS (mg L ⁻¹)	Control (BWW)	198.44 ± 2.08	164.66 ± 2.07	151.69 ± 2.08	147.76 ± 2.07	144.58 ± 2.06
	25%	396.87 ± 2.07	277.66 ± 2.08	258.11 ± 2.07	211.91 ± 2.08	202.19 ± 2.07
	50%	792.01 ± 3.06	588.39 ± 3.07	498.88 ± 3.06	401.76 ± 3.07	362.39 ± 3.06
	75%	1188.89 ± 7.07	851.69 ± 7.06	691.33 ± 7.07	609.71 ± 7.06	508.09 ± 7.05
	100%	1585.77 ± 7.06	1351.54 ± 7.07	1222.75 ± 7.08	1101.91 ± 7.07	1044.01 ± 7.06
BOD (mg L ⁻¹)	Control (BWW)	3.87 ± 0.02	3.77 ± 0.04	3.73 ± 0.04	3.72 ± 0.03	3.72 ± 0.03
	25%	118.16 ± 0.05	103.87 ± 0.05	82.54 ± 0.06	68.36 ± 0.05	61.32 ± 0.04
	50%	236.32 ± 2.07	204.93 ± 2.07	151.21 ± 0.05	129.62 ± 2.06	111.71 ± 0.05
	75%	354.49 ± 2.09	251.74 ± 2.08	161.48 ± 2.07	124.77 ± 2.08	108.48 ± 2.09
	100%	472.65 ± 3.02	419.76 ± 3.03	403.28 ± 3.02	371.76 ± 3.02	367.86 ± 3.02
COD (mg L ⁻¹)	Control (BWW)	5.83 ± 0.06	5.7 ± 0.06	5.53 ± 0.05	5.33 ± 0.05	5.23 ± 0.06
	25%	97.88 ± 1.04	83.78 ± 1.04	71.36 ± 1.03	56.66 ± 1.04	54.11 ± 1.03
	50%	195.77 ± 0.05	165.34 ± 0.07	135.47 ± 0.07	129.12 ± 0.05	91.66 ± 0.05
	75%	293.66 ± 2.06	232.59 ± 2.08	192.59 ± 2.08	144.87 ± 2.07	112.19 ± 2.06
	100%	391.55 ± 2.06	332.88 ± 2.07	329.87 ± 2.09	323.56 ± 2.08	310.33 ± 2.07
TKN (mg L ⁻¹)	Control (BWW)	9.58 ± 0.08	8.21 ± 0.08	8.14 ± 0.07	8.09 ± 0.08	8.02 ± 0.09
	25%	36.61 ± 2.04	23.16 ± 2.04	16.54 ± 2.05	14.51 ± 2.06	12.22 ± 2.05
	50%	72.51 ± 3.02	41.03 ± 3.02	39.52 ± 3.02	31.95 ± 3.02	25.92 ± 3.02
	75%	108.03 ± 3.03	71.04 ± 3.04	62.66 ± 3.03	39.76 ± 3.04	29.32 ± 3.02
	100%	144.09 ± 2.06	124.97 ± 2.05	121.88 ± 2.06	118.97 ± 2.05	116.66 ± 2.04
P (mg L ⁻¹)	Control (BWW)	4.68 ± 0.08	3.62 ± 0.08	3.21 ± 0.07	3.06 ± 0.08	3.03 ± 0.09
	25%	36.62 ± 1.04	31.27 ± 1.04	22.54 ± 1.03	16.66 ± 1.03	11.53 ± 1.02
	50%	73.25 ± 1.07	59.12 ± 1.07	51.31 ± 1.07	32.83 ± 1.06	24.08 ± 1.04
	75%	109.87 ± 1.09	81.39 ± 1.09	75.33 ± 1.09	51.39 ± 1.09	31.32 ± 1.07
	100%	146.49 ± 3.07	122.73 ± 1.09	119.92 ± 3.07	114.92 ± 3.06	111.72 ± 3.06
Ca ²⁺ (mg L ⁻¹)	Control (BWW)	17.43 ± 1.04	14.27 ± 1.03	11.48 ± 1.02	9.64 ± 1.02	7.34 ± 1.02
	25%	56.55 ± 2.04	46.77 ± 1.04	42.44 ± 1.05	31.59 ± 2.04	29.29 ± 1.05
	50%	113.11 ± 3.06	92.19 ± 3.05	81.65 ± 3.03	61.87 ± 1.05	48.87 ± 3.03
	75%	169.67 ± 3.07	155.87 ± 1.09	133.34 ± 3.06	92.67 ± 3.07	72.44 ± 3.07
	100%	226.24 ± 1.09	212.07 ± 2.02	210.94 ± 1.09	188.58 ± 2.02	179.14 ± 2.02

Table 4 continued

Parameters	Concentration	Before phytoremediation	After phytoremediation			
			15 days	30 days	45 days	60 days
Mg^{2+} (mg L ⁻¹)	Control (BWW)	14.90 ± 1.04	12.33 ± 1.04	11.75 ± 1.03	10.89 ± 1.02	9.44 ± 1.02
	25%	39.62 ± 1.03	27.02 ± 1.04	25.11 ± 1.04	24.12 ± 1.03	22.91 ± 1.03
	50%	79.25 ± 3.03	64.33 ± 3.03	58.78 ± 3.04	41.89 ± 3.04	38.97 ± 3.04
	75%	118.88 ± 3.07	98.98 ± 2.02	81.78 ± 2.02	62.36 ± 3.03	53.49 ± 3.03
	100%	158.61 ± 2.02	141.33 ± 3.07	138.43 ± 3.07	136.66 ± 3.07	134.98 ± 3.06
Na^{+} (mg L ⁻¹)	Control (BWW)	19.88 ± 1.07	17.23 ± 1.04	15.98 ± 1.07	14.68 ± 1.04	13.11 ± 1.07
	25%	46.34 ± 1.02	37.65 ± 1.02	31.09 ± 1.02	28.98 ± 1.01	27.89 ± 1.01
	50%	92.69 ± 2.07	79.56 ± 1.09	71.53 ± 1.09	48.97 ± 2.07	46.68 ± 1.09
	75%	139.05 ± 1.09	119.78 ± 2.06	117.32 ± 2.07	95.77 ± 2.09	79.45 ± 2.07
	100%	185.38 ± 2.01	171.88 ± 2.07	165.29 ± 1.09	163.79 ± 2.07	155.94 ± 1.09
K^{+} (mg L ⁻¹)	Control (BWW)	9.55 ± 0.09	7.87 ± 0.09	6.32 ± 0.08	5.48 ± 0.09	4.88 ± 1.04
	25%	57.56 ± 1.07	45.33 ± 1.07	41.68 ± 1.04	31.54 ± 1.07	28.43 ± 1.04
	50%	115.13 ± 1.06	88.78 ± 2.07	85.46 ± 2.06	71.89 ± 1.01	55.47 ± 1.01
	75%	172.68 ± 2.07	151.56 ± 1.05	128.13 ± 1.04	101.84 ± 1.07	78.77 ± 1.09
	100%	230.25 ± 2.09	211.73 ± 2.07	208.13 ± 2.07	201.96 ± 2.04	196.89 ± 2.03
MPN (100 mL ⁻¹)	Control (BWW)	–	–	–	–	–
	25%	1.67 × 10 ³ ± 120	1.64 × 10 ³ ± 113	1.61 × 10 ³ ± 102	1.56 × 10 ³ ± 117	1.54 × 10 ³ ± 119
	50%	3.35 × 10 ³ ± 335	3.11 × 10 ³ ± 321	3.04 × 10 ³ ± 304	2.12 × 10 ³ ± 279	2.03 × 10 ³ ± 258
	75%	5.03 × 10 ³ ± 420	4.01 × 10 ³ ± 410	3.37 × 10 ³ ± 379	2.33 × 10 ³ ± 348	2.10 × 10 ³ ± 336
	100%	6.71 × 10 ³ ± 350	6.01 × 10 ³ ± 332	5.97 × 10 ³ ± 314	5.55 × 10 ³ ± 288	5.49 × 10 ³ ± 252
SPC (mL ⁻¹)	Control (BWW)	28.35 ± 3.06	26.18 ± 3.06	24.97 ± 3.05	22.46 ± 3.05	21.54 ± 3.05
	25%	3.53 × 10 ³ ± 150	2.88 × 10 ³ ± 120	2.51 × 10 ³ ± 100	2.05 × 10 ³ ± 90	2.01 × 10 ³ ± 75
	50%	7.07 × 10 ³ ± 485	5.08 × 10 ³ ± 400	4.01 × 10 ³ ± 390	3.37 × 10 ³ ± 376	3.09 × 10 ³ ± 305
	75%	10.61 × 10 ³ ± 655	8.08 × 10 ³ ± 685	6.19 × 10 ³ ± 576	4.41 × 10 ³ ± 510	4.23 × 10 ³ ± 440
	100%	14.11 × 10 ³ ± 900	13.01 × 10 ³ ± 965	12.09 × 10 ³ ± 956	11.78 × 10 ³ ± 925	10.68 × 10 ³ ± 848

Data are mean ± SD of three replicates

–: not detectable

1.33 ± 0.02 dS m⁻¹ to 1.16 ± 0.01 dS m⁻¹ in 60 days of treatment period with a maximum reduction of 12.78%. In case of different concentrations of wastewater, mean reduction in EC was found to be maximum in 75% concentrated wastewater with a reduction efficiency of 25.69%. Total dissolved solid in wastewater was found to be reduced by 49.05, 54.24, 57.26%, and 34.16 in 25, 50, 75, and 100% SME concentration, respectively. Reduction in organic load in terms of BOD (69.40%) and COD (61.80%) was also found to be maximum in 75% concentrated wastewater; however with 25, 50, and 100%, the reduction in BOD was 48.10, 52.73, and 22.17%, while in COD it was 44.72, 53.18, and 20.74%, respectively. The total Kjeldhal nitrogen was reduced by 66.62, 67.01, 72.86, and 25.98% from 25, 50, 75, and 100% wastewater

concentration, respectively. The soluble phosphorous (71.49%) was reduced maximum in 75% concentrated wastewater; however in 25, 50, and 100%, the reduction in soluble phosphorus was 65.78, 67.13, and 23.74%, respectively. A significant extent of reduction in alkaline earth metals such as Ca, Mg, Na, and K was observed in all treatments; however, maximum reduction in Ca (57.31%), Mg (55.01%), and K (54.38%) was again observed in 75% concentrated SME except Na, where maximum reduction of 49.64% was obtained with 50% SME. In addition to the physico-chemical parameters, microbiological parameters provide the information about the pathogenic nature of wastewater. In the present study, the reduction in MPN (78.13%) and SPC (60.13%) was found to be maximum in 75% SME concentration.

Similar to the present study, a number of authors have also reported wastewater treatment via employing different aquatic plant species. Findings of Kumar et al. (2016) also supported the present study and observed a significant reduction in TDS, EC, BOD, COD, TKN, PO_4^{3-} , Na^+ , K^+ , Ca^{2+} , and Mg^{+2} of pulp and paper mill effluent with *T. natans* and *E. crassipes*. They also reported a gradual increase in the removal of pollutant load, while it was found at a slower rate beyond the treatment period of 60 days. They also reported that *E. crassipes* has higher efficiency for the removal of different parameters of pulp and paper mill effluent in comparison to *T. natans*. In another study, Dipu et al. (2011) found that on treatment of dairy effluent with *P. stratiotes*, the alkaline pH changed into neutral and biological oxygen demand and chemical oxygen demand were reduced in the ranges of 65.4–83.07% and 70.4–85.3%, respectively. In another lab-scale experiment on treatment of SME with water hyacinth, it was found that the mechanism behind the change in pH is due to absorption of nutrients and other salts by plants simultaneous release of H^+ ions with an uptake of the metal ion (Mahmood et al. 2005). Lu (2009) revealed that growth of water lettuce in wastewater decreased the EC in the treatment plot due to salt removal from the wastewater through the uptake or root adsorption.

Removal efficiency of *P. stratiotes* after phytoremediation

During the present study, the maximum reduction was observed at 60 days with 75% concentration of SME, with the most reduction being observed in the major physico-chemical, microbiological pollutants of SME, viz. electrical conductivity (EC), total dissolved solid (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldhal nitrogen (TKN), phosphorus (P), calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), most probable number (MPN), and standard plate count (SPC), i.e., 25.69, 57.26, 69.40, 61.80, 72.86, 71.49, 56.79, 55.01, 42.86, 54.38, 78.13, and

Fig. 1 Removal efficiency of physico-chemical parameters of sugar mill effluent after phytoremediation using water lettuce (*P. stratiotes* L.)

60.13%, respectively, at 60 days of phytoremediation (Figs. 1, 2, 3, 4, 5). Kumar et al. (2016) also supported the present study in term of the reduction in the physico-chemical parameters, viz. TDS, EC, BOD, COD, TKN, PO_4^{3-} , Na^+ , K^+ , Ca^{2+} , and Mg^{+2} of the pulp and paper mill effluent with aquatic plant *T. natans* and *E. crassipes* at 60 days. Dar et al. (2011) also reported that the aquatic plant water hyacinth has the potential for the removal of nitrogen, phosphorus, calcium, and magnesium of the sewage wastewater.

Fig. 2 Removal efficiency of physico-chemical parameters of sugar mill effluent after phytoremediation using water lettuce (*P. stratiotes* L.)

Fig. 3 Removal efficiency of physico-chemical parameters of sugar mill effluent after phytoremediation using water lettuce (*P. stratiotes* L.)

Fig. 4 Removal efficiency of physico-chemical parameters of sugar mill effluent after phytoremediation using water lettuce (*P. stratiotes* L.)

Fig. 5 Removal efficiency of microbiological parameters of sugar mill effluent after phytoremediation using water lettuce (*P. stratiotes* L.)

Variation in substrate characteristics in relation to biogas production

In comparison to the initial substrate characteristics, significant changes were reported in the selected parameters. The pH of the substrate was found to be reduced from an initial value of 7.85 to 6.0 in 30 days of HRT. It might be due to the formation of organic acids during the acidogenesis process which is a key step in the biogas production. In a study by Mathew et al. (2015), it was reported that pH plays a major part in anaerobic biodegradation by impelling the activity of the hydrolytic enzymes. Moreover, it has also been observed that methanogenesis in an anaerobic digester occurs proficiently at pH 6.5–8.2, while hydrolysis and acidogenesis occurs at pH 5.5 and 6.5, respectively, as reported by Lee et al. (2009). The total solid content (%) in substrate was reduced up to 28.65%, which showed the efficacy of process for the breakdown of substrate ingredients. The reduction in total solid shows the high degradability of organic fraction of substrate. For this reason, the most suitable condition for the anaerobic digestion process in the context of digestion was 30 °C. The reduction in volatile solid content occurs due to its engrossment in methanogenic process, which is found to be reduced by 28.78% in 30 days of contact time. Similar findings are also reported by Annop et al. (2007), who observed a reduction in TS and VS by 67.1 and 75.2%, respectively, in anaerobic digestion of municipal solid waste in a stirred tank reactor. In a recent study by Conor et al. (2016), the TS and VS were reduced by 37.5 and 25.6% from co-digestion of food waste and pig manure at 37 °C.

Chemical oxygen demand (COD) of the substrate is used as a measure of the oxygen equivalent of the organic matter content. In this situation, COD is removed by converting organic compounds to methane (CH_4), a significant amount of CO_2 , H_2 , and negligible amounts of other gases like H_2S (Manariotis et al. 2010; Mathew et al. 2015). So

the methane produced of a waste (by microorganisms) is related to the concentration of organics (COD) in it and in the effectiveness of the system. In present study, the COD of substrate was decreased from 12,900 to 2800 mg/L in 30 days of contact time. Similar to the present study, Manjula and Mahanta (2014) also reported the removal in COD by 78.3% during the biogas production process.

In the present study, variation in C/N ratio from 47.94 to 26 represents suitable ratio for biogas production. The carbon/nitrogen (C/N) ratios represent the relationship between the amount of carbon and nitrogen present in organic substrate. The ideal C/N ratio for anaerobic digestion is considered to be in the range of 20–30 C/N ratios (Wang et al. 2014). If the C/N ratio is too high, the nitrogen is consumed rapidly by the methanogenic bacteria to meet their protein requirement and is no longer available to react on the left-over carbon content in the material. As a result, the biogas production is reduced (Wang et al. 2014). If the C/N ratio is too low, nitrogen is liberated and accumulates in the form of ammonia. This increases the pH of the digestates. When pH value rises higher than 8.5, it begins to exert a toxic effect on the methanogenic bacteria (Wang et al. 2014). 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 (Wang et al. 2014).

Kinetic evaluation of anaerobic digestion

The kinetic behavior of biogas production is shown in Fig. 6. There was an overall agreement between the model and experimental data, which determine the optimum equation to fit with these kinds of substrate to evaluate the parameters that influenced the anaerobic digestion process. Experimental biogas yield is clearly depended on the removal of COD at 30 °C temperature. The values of kinetic constants such as μ_m , x_c , and k were 8478.6, 7.79, and 0.342, respectively, with R^2 (coefficient of determination) value of 0.99. The biogas potential of *Pistia*

Fig. 6 Relationship between per day and cumulative biogas production mL/per day

stratiotes in the present study was found to be in line with the results of previous investigations. Pantawong et al. (2015) harvested water lettuce in fresh water and achieved about 9677.33 mL of biogas production in 45 days of HRT. However, in the present study wastewater is utilized for the cultivation of water lettuce with no additional input of fertilizer. On the other hand, biogas yield in the present investigation is found to be higher than other aquatic weeds such as *Lemna minor* and *salvinia* (Mathew et al. 2015).

Conclusion

Utilization of *P. stratiotes* was found to have the potential to remove major pollutants such as BOD, COD, N, and P from SME, which pose serious impact in aquatic bodies by means of eutrophication. For sustainable treatment process, energy recovery from the end product (biomass of *P. stratiotes*) is essential, which is successfully achieved in the present study. Utilization of *P. stratiotes* plant biomass after phytoremediation process was found to have high potential to produce biogas. In conventional practices, manure left after composting process releases nitrous oxide and methane which possess 310 times and 21 times more potential for global warming when compare to CO₂. Thus, anaerobic conversion of manure into biogas can be a green solution and would help to achieve energy security of the nation.

Acknowledgements The Meritorious Rajiv Gandhi National Fellowship (RGNF) F1-17.1/2015-16/RGNF-2015-17-SC-UTT-5597/ (SA-III/Website) provided by the University Grants Commission, New Delhi, India to Jogendra Singh is acknowledged.

Conflict of interest The authors of this manuscript declare that there is no conflict of interest.

References

- AOAC (2005) Official methods of analysis of the association of official analytical chemists, 13th edn. AOAC, Washington, pp 545–567
- APHA (2012) Standard methods for the examination of water and waste water. American Public Health and Association (APHA), Washington, D.C, p 2462
- Aron D (1949) Copper enzymes isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–15
- Atlas L (2009) Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. J Hazard Mater 162:1551–1556
- Brar A, Kumar M, Vivekanand V, Pareek N (2017) Photoautotrophic microorganism and bioremediation of industrial effluents: current status and future prospects. 3Biotech 7(18):1–8
- Conor D, Peadar GL, Thomas C, Yan J, Liam M, Gillian EG, Xinmin Z (2016) Synergism and effect of high initial volatile fatty acid concentrations during food waste and pig manure anaerobic co-digestion. Waste Manag 56:171–180
- Dar SH, Kumawat DM, Singh N, Wani KA (2011) Sewage treatment potential of water hyacinth (*Eichhornia crassipes*). Res J Environ Sci 5(4):377–385
- Dipu S, Kumar AA, Thanga VSG (2011) Phytoremediation of dairy effluent by constructed wetland technology. Environmentalist 31:263–278
- Ehiri RC, kelle II, Mgbabor C, Ogbuanu CC (2014) Kinetics of biogas production from a mixture of water hyacinth (*Eichhornia crassipes*) and fresh rumen residue. IOSR J Appl Chem 7(7):36–39
- Fonkou T, Agendia P, Kengne I, Akoa A, Nya J (2002) Potentials of water lettuce (*Pistia stratiotes*) in domestic sewage treatment with macrophytic lagoon systems in Cameroon.. In: Proceedings of international symposium on environmental pollution control and waste management tunis, pp 709–714
- Hurst CJ (1997) Water microbiology in public health. Manual of Environmental Microbiology. ASM Press, Washington, DC
- Indian Sugar Mills Association (2016) Indian sugar year book. Indian Sugar Mills Association, Calcutta
- Jadhav PG, Vaidya NG, Dethle SB (2013) Characterization and comparative study of cane sugar industry wastewater. Int J Chem Phys Sci 2(2):19–25
- Jahan MAA, Akhtar N, Khan MS, Roy CK, Islam R, Nurunnabi M (2014) Characterization of tannery wastewater and its treatment by aquatic macrophytes and algae. BCSIR 49(4):233–242
- Kolhe AS, Sarode AG, Ingale SR (2009) Study of effluent from sugar cane industry. Sodh Samiksha Mulyankan 2:303–306
- Kothari R, Pathak VV, Kumar V, Singh DP (2012) Experimental study for growth potential of unicellular algal *Chlorella pyrenoidosa* on dairy wastewater: an integrated approach for treatment and biofuel production. Bioresour Technol 116:466–470
- Kumar V, Chopra AK (2014) Ferti-irrigation effect of paper mill effluent on agronomical practices of *Phaseolus vulgaris* (L.) in two seasons. Commun Soil Sci Plant Anal 45:2151–2170
- Kumar V, Chopra AK (2016) Reduction of pollution load of paper mill effluent by phytoremediation technique using water caltrop (*Trapa natans* L.). Cogent Environ Sci 2:1153216. doi:10.1080/23311843.2016.1153216
- Kumar V, Chopra AK, Singh J, Thakur RK, Srivastava S, Chauhan RK (2016) Comparative assessment of phytoremediation feasibility of water caltrop (*Trapa natans* L.) and water hyacinth (*Eichhornia crassipes* Solms.) using pulp and paper mill effluent. Arch Agric Environ Sci 1(1):13–21
- Kushwaha JP (2013) A review on sugar industry wastewater: sources, treatment technologies, and reuse. Water Treat, Desal. doi:10.1080/19443994.2013.838526
- Lee DH, Behera SK, Kim J, Park HS (2009) Methane production potential of leachate generated from Korean food waste recycling facilities: a lab scale study. Waste Manag 29:876–882
- Li M, Zhao Y, Guo Q, Qian X, Niu D (2008) Bio-hydrogen production from food waste and sewage sludge in the presence of aged refuse excavated refuse landfill. Renew Energy 33:2573–2579
- Lin CY, Shei SH (2008) Heavy metals effect on fermentative hydrogen production using natural mixed microflora. Int J Hydrogen Energy 33:587–593
- Lu Q (2009) Evaluation of aquatic plant for phytoremediation for phytoremediation of eutrophic stormwaters. Ph.D. Thesis, University of Florida, Florida
- Mahmood Q, Zheng P, Islam E, Hayat Y, Hassan MJ, Jilani G, Jin RC (2005) Lab scale studies on water hyacinth (*Eichhornia crassipes marts solms*) for bio treatment of textile wastewater. Casp J Environ Sci 3(2):83–88
- Maishanu SM, Hussaini HBN (1991) Studies on factors affecting biogas generation from *Pistia stratiotes*. In: Paper presented at

- the 32nd annual conference of Nigeria society of microbiology, university of Ilorin, April, 7–11
- Manariotis ID, Grigoropoulos SG, Hung YT (2010) Anaerobic treatment of low-strength wastewater by a biofilm reactor. In: Wang LK, Tay JH, Tay ST, Hung YT (eds) Handbook of environmental engineering, vol 11. Humana Press, New York. doi:[10.1007/978-1-60327-031-1.id](https://doi.org/10.1007/978-1-60327-031-1.id)
- Manjula DK, Mahanta P (2014) Comparison of kinetic models for biogas production rate from saw dust. Int J Res Eng Technol 3(7):248–254
- Mathew K, Bhui I, Banerjee SN, Goswami R, Shome A, Chakraborty AK, Balachandran S, Chaudhury S (2015) Biogas production from locally available aquatic weeds of Santiniketan through anaerobic digestion. Clean Technol Environ Policy 17:1681–1688
- Nopharatana A, Pullammanappallil PC, Clarke WP (2007) Kinetics and dynamic modelling of batch anaerobic digestion of municipal solid waste in a stirred reactor. Waste Manag 27:595–603
- Owamah HI, Enaboito MA, Izinyon OC (2014) Treatment of wastewater from rubber processing industry using water lettuce macrophyte pond and the reuse of its effluent as biofertilizer. Agric Water Manag 146:262–269
- Pantawong R, Chuanchai A, Thipbunrat P, Unpaprom Y, Ramraj R (2015) Experimental investigation on biogas production from water lettuce, *Pistia stratiotes* L. Emer Life Sci Res 1(2):41–46
- Pathak VV, Singh DP, Kothari R, Chopra AK (2014) Phycoremediation of textile wastewater by unicellular microalga *Chlorella pyrenoidosa*. Cell Mol Biol 60:35–40
- Saha P, Shinde O, Sarkar S (2017) Phytoremediation of industrial mines wastewater using water hyacinth. Int J Phytoremediation 19(1):87–96. doi:[10.1080/15226514.2016.1216078](https://doi.org/10.1080/15226514.2016.1216078)
- Sahu O (2017) Treatment of sugar processing industry effluent up to remittance limits: suitability of hybrid electrode for electrochemical reactor. MethodsX 4:172–185
- Srivastava S, Chopra AK, Kumar V, Sehgal D (2015) Agro fertigational response of sugar mill effluent and synthetic fertilizer (DAP) on the agronomy of crop *Vigna unguiculata* L. Walp in two seasons. Res J Agric Environ Sci 2(3):5–17
- Tiwari A, Sahu O (2017) Treatment of food-agro (sugar) industry wastewater with copper metal and salt: chemical oxidation and electro-oxidation combined study in batch mode. Water Resour Ind 17:19–25
- Tyagi TR, Agarwal MH (2014) Aquatic plants *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms: a sustainable eco-friendly bioresources a review. Int J Pharm Res Sch 2:540–550
- Victor KK, Seka Y, Norbert KK, Sanogo TA, Celestin AB (2016) Phytoremediation of wastewater toxicity using water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*). Int J Phytorem 18(10):949–955
- Wang X, Lu X, Li F, Yang G (2014) Effects of temperature and carbon–nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. PLoS ONE 9(5):e97265