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Exploring integrated methodology for phytoremediation and biofuel production potential of *Eichhornia crassipes*

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Eichhornia crassipes (EC) is a well-known invasive weed in different aquatic ecosystems. Its effective and complete eradication remains a challenge. The plant is a heavy metal (HM) hyperaccumulator in water bodies; however, studies regarding its biomass utilisation post-phytoremediation remain limited. The abundant growth rate and biochemical composition make EC a promising lignocellulosic feedstock for biofuel production; hence could be a deterministic approach for solving the twin problems of water pollution and higher energy demand, which are the global pressing issues in today's scenario. The present study aimed at evaluating the phytoremediation potential of EC followed by proximate and biochemical analysis to investigate its suitability for biofuel production. After two weeks, the EC removed above 90% of Lead (Pb) and 60% of Cadmium (Cd) at all experimental doses. Lower doses of HMs, especially Pb, showed stimulatory effects on *E. crassipes* leaf biomass (ECLB). The recovered ECLB from Pb contaminated water (1 mg L^{-1}) was further analysed for moisture (89.23±0.86%), dry matter (10.77±0.60%), ash (11.91±1.20%), organic carbon (51.56±1.08%), cellulose (21.89±0.64%), hemicellulose (26.50±1.13%), lignin (5.62±0.83%), total carbohydrate (32.00±1.58%), and protein (20.83±0.52%) content. SEM imaging of harvested ECLB confirmed compact and rigid structure. The recorded peaks in FTIR-spectra (1015.21, 1153.71, 1246.01, 1339.63, 1419.71, 1540.71, 1646.80, 1736.73, 2933.03, and 3263.72 cm^{-1}) indicate the presence of lignocellulosic biomass. XRD peak at 21.55° confirmed the crystalline fraction of cellulose in ECLB. The results of theoretical yields of H_2 and CH_4 co-generation (HMG) (210.85 mLH_2/g DW and 150.28 mL CH_4/g DW) and Bioethanol (0.278 g/g DW) derived from cellulose and hemicellulose content of ECLB were comparable to those in reported studies. Overall, this work demonstrates an integrated methodology of phytoremediation followed by biofuel production from the recovered phytobiomass.

Keywords: Aquatic weed, Bioenergy feedstock, Heavy metals, Lignocellulose, Wastewater treatment

EC, a free-floating macrophyte, is known to invade tropical and subtropical regions worldwide[1]. This plant presents a big threat to biodiversity[2] and causes serious environmental and socioeconomic concerns[3]. The quest of this plant for nutrients and proliferative growth behaviour in waterbodies renders it a promising tool for phytoremediation; however, secondary pollution caused by the generated phytobiomass aids in additional complexity to the environment. As a solution to this, EC biomass recovered post-phytoremediation can be further explored as a potential bioenergy feedstock[4] to meet the world energy requirement, estimated to increase 27% by 2040 as per the available reports[5]. Fossil fuels alone cannot meet such demands[6], and the search for sustainable and alternative energy sources has become the need of the hour.

Biomass-derived fuel appears to be a feasible alternative[7]; however, first-generation biofuels (FGB) raise debate over Food Vs Fuel[8,9], and second-generation biofuels (SGB) have higher production costs[9] and increasing demand for labour, land, and other resources[8]. The cultivation of terrestrial phytobiomass for SGB has resource requirements such as arable land, irrigation water, *etc*., which applies to FGB as well. In the category of lignocellulosic phytobiomass, EC represents a promising source having no risk of food-energy conflict[10]. The holocellulose profile of EC (~50% or more) favoured the plant's utility as an efficient feedstock material for Bioethanol, Hydrogen, and Methane production[11,12]. The Lipid content and fatty acid composition reported by Shanab *et al*[13] further promote the biodiesel production potential of EC, and studies advocated utilization of EC oil blended with petroleum diesel fuels in diesel engines[14].

HMs contamination adversely affects the aquatic ecosystems and human well-being; hence recognized

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as a global environmental concern. The significant reduction in HMs (Cd, Cu, Fe, Mn, Pb, and Zn) from industrial effluent utilizing EC has been reported[15]. Integrated studies coupling EC-inspired phytoremediation and bioenergy production are encouraged[4] and hold great promise in searching sustainable routes for environmental clean-up and bioenergy production. The techniques promoting utilisation of wastewater as a suitable growth media for EC can serve as a cohesive platform for phytoremediation and ensure a steady biomass supply for biofuel generation. However, studies related to the exploitation of ECB post-phytoremediation in this context remain limited[4,16]. In the present study, we have investigated the HM contaminated wastewater reclamation using EC followed by possibilities of utilizing the harvested biomass for biofuel production. To meet the aforementioned objective, the phytoremediation potential of EC was assessed by exposing the plant to different experimental doses of HMs (Cd and Pb). The effect of seasonal variation and culture period has also been studied on BOD, COD, and TH removal by EC in sampled wastewater. The next section of the study entails proximate, biochemical, and structural characterisation of the ECLB from the selective experimental harvest, and the theoretical fuel yields (Hydrogen, Methane, Bioethanol) derived from complex carbohydrate composition (cellulose and hemicellulose content) was compared with the available reports to determine the plant's potential for biofuel production.

Materials and Methods

Young plants of EC were obtained from the Hindon river at Mohan Nagar Barrage, Ghaziabad district of Uttar Pradesh. The plants were inspected for any adhering debris, cleaned with tap water, and taken carefully in reusable polyethylene bags to the departmental laboratory. For phytoremediation studies, plants were given one-week acclimatisation under controlled conditions, after which they were given different treatments. For HM removal, EC plants cultured in Hoagland solution (Hoagland No. 2, Basal salt mixture, modification no. 1, Himedia) were treated with known concentrations (0.5, 1.0, 1.5, and 2.0 mg L^{-1}) of Cd and Pb. Stock solutions of $Cd(NO_3)_2.4H_2O$ (Cadmium nitrate) and $Pb(NO_3)_2$ (Lead nitrate) of desired molarity were prepared in deionised water and diluted further to get the experimental doses. The HM removal by the plants was analysed for three consecutive weeks using the Atomic Absorption Spectrophotometer. EC is known to flourish in water bodies receiving different effluents; therefore, experiments were also performed to investigate the BOD, COD, and TH removal from Hindon river wastewater (HRWW). The said parameters were analysed as per APHA standard methods[17], provided in the CPCB Guide Manual on Water and Wastewater analysis[18]. The HRWW procured from the sampling site has BOD, COD, and TH values of 27.5, 70, and 183 mg L^{-1}, respectively (indicates average values from water sampled during pre-monsoon, monsoon, and post-monsoon seasons).

For proximate analysis, the plant's leaves from the selective experimental batch were separated and processed for Moisture[3], Dry matter[19], Ash[3], and Organic Carbon content[20]. Furthermore, for biochemical parameters, the leaves were shade dried at room temperature and then grounded to obtain the fine powder and analysed for Cellulose, Hemicellulose, Lignin (Zheng *et al*[21] with slight modifications), total Carbohydrate (Anthrone method as described by Chavan *et al*[22]), and total protein (Lowry method as described by Aziz *et al*[23]) content.

The surface morphology of the plant sample was studied by Scanning Electron Microscopy (SEM) (Zeiss, V5.05 Sigma) operated at an accelerating potential of 5 kV, and the observations were made based on the micrographs recorded at different magnifications. The sample's surface properties and composition were analyzed using X-ray Diffraction (XRD) (Rigaku, Ultima IV X-ray Diffractometer), and the spectra were recorded over a 2θ angle up to 80^o. Fourier-transform Infrared (FTIR) spectroscopy (Bruker, Tensor 37) was carried out in the spectral range of 4000-500 cm^{-1} to determine the functional groups present in the plant sample. The calculated values of Cellulose and Hemicellulose from harvested ECLB have been transformed into theoretical yields of HMG[24] and Bioethanol[25]. All the chemicals were of analytical grade, and double-distilled water (DDW) was used to prepare reagent and standard solutions. The experiments were assigned in a complete randomised design with three triplicates of each, and data represented as Mean ± Standard error.

Results and Discussion

Phytoremediation Potential of EC

Taking account of Cd removal, over 70% reduction was observed up to 1 mg L^{-1} concentration after the second week (SW), and the highest removal was recorded at 0.5 mg L^{-1} followed by 1 mg L^{-1} after the third week (TW) (Fig. 1A). In the case of Pb, over 90%

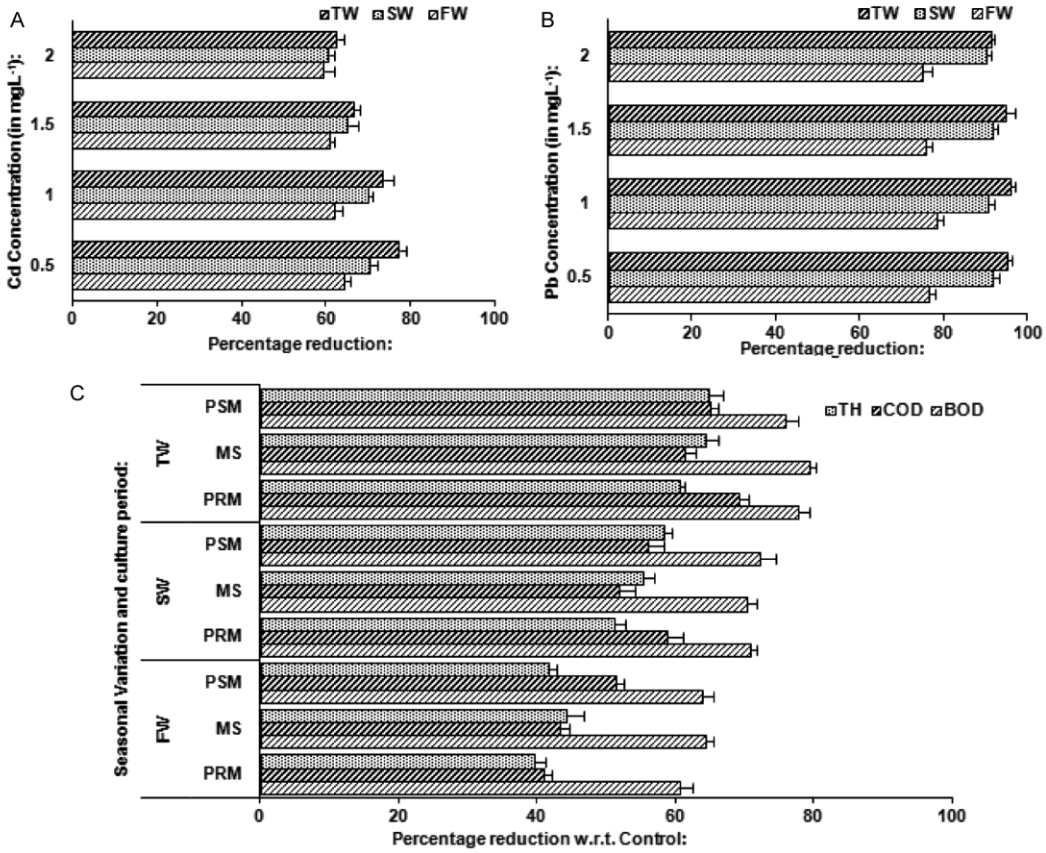

Fig. 1 — Phytoremediation effect of ECon: (A) Cd removal; (B) Pb removal; and (C) BOD, COD, and TH levels

reduction was observed following the SW at all the experimental doses, with the highest removal recorded at 1 mg L^{-1}concentration (Fig. 1B). Hence, the metal uptake efficiency of EC was comparatively reduced at higher concentrations (*i.e.*, 2 mg L^{-1}), which is more visible in Cd treatments than Pb. Overall, the maximum relative removal rate was seen after the first week (FW) for both HMs. Similar observations were made by Zaida *et al*[26], wherein EC showed tolerance to Cd at 1 mg L^{-1} (up to 7 days) and Pb at concentrations of 1 and 3 mg L^{-1} (14 days study). In a 14-day study, they reported the highest removal at a lower initial metal concentration of 1 mg L^{-1}, *i.e.*, 98.2% for Pb and 50.2% for Cd. Aquatic plants are reported to have different metal uptake efficiency that primarily depends upon the type of HMs[27], their concentration in solution[28], and exposure time[29], which has also been observed in the present study. Nwe *et al* recorded 79.83% Cd and 95.78% Pb removal after 10 days[30]. Zhou *et al*, using EC, reported the highest removal of Pb (92.69±0.10%) after eight days at an initial Pb concentration of 1 mg L^{-1}, whereas, for Cd, the highest removal (*i.e.*, 50.00±0.18%) was seen at 0.02 mg L^{-1} after 24 days[31].

Figure 1C shows the trend in variation of BOD, COD, and TH over the period of time, and the results were presented as relative percentage (%) with respect to the initial concentration of the parameters. The maximum reduction in BOD, COD and TH levels were recorded during Monsoon (MS), Pre-monsoon (PRM), and Post-monsoon (PSM) seasons, respectively, after TW. The BOD values showed over 60% reduction after the FW, while COD and TH parameters were found to have over 50% reductions following the SW at all the three seasonal variations. Panneerselvam and Priya treated diluted wastewater (20% wastewater + 80% tap water) using EC and after one week they observed 40-50% reductions in BOD and COD, and TH values were reduced by 30-50%[32]. Kumar and Deswal using EC reported 71.87% of BOD removal in 15 days study[33]. As observed from the preliminary findings and cited literature, the two-week period can be considered for the treatment of wastewater contaminated with HMs (such as Cd, Pb), BOD, COD, *etc*.

Effect of HM treatment on ECLB

The biomass harvested from HM contaminated water can be a crucial indicator for estimating a plant's growth performance. ECLB at different treatment doses of Cd and Pb have been recorded, and the obtained results were compared with biomass of plants raised in Hoagland media without HM treatment (ECC) to get a better understanding of the role of Cd and Pb on the growth performance of the plant. Compared to ECC, there was an increase in leaf biomass content (LBC) at lower doses of both the HMs (up to 1 mg L^{-1}). Comparing the LBC at HM treatment of 1 mg L^{-1}, results favoured exposure of Pb over Cd. Stimulatory effects of Cd at lower doses on EC growth have also been reported[28]. The plant was treated with 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1} of Cd for 7 and 14 days, and an increase in plant growth was observed up to 2.0 mg L^{-1} concentration[28]. Pereira *et al*[34] observed 11.29% higher photosynthetic efficiency of EC at Pb treatment of 1.0 mg L^{-1}, when compared with control. Previous studies reported a higher accumulation of Cd and Pb in the roots of EC as compared to leaves[26,28]. Melignani *et al* studied the accumulation and growth effects of high Cd concentration on EC and found slight stimulation in growth of the plant at lower doses (*i.e.*, 1 mg L^{-1})[35]. They also reported low translocation of Cd from roots to leaves, and in all the treatments, the leaf biomass did not differ significantly from that of control. Referring to the above reports, the effect of HM treatments was more pronounced in roots than leaves. Hence, leaves of EC harvested post-phytoremediation from HM contaminated water can serve as a potential bioenergy feedstock. To further investigate the suitability for biofuel production, ECLB harvested from Pb (1 mg L^{-1}) contaminated water was analysed for proximate, biochemical, and structural properties.

Proximate and Biochemical Characterisation of ECLB

The amount of energy present in the biomass-derived fuel depends upon the chemical composition of biomass, and factors such as ash, carbon, moisture content, *etc*., are also imperative[36].The present study recorded the proximate and biochemical composition of harvested ECLB, and results were shown as percentage content (Table 1). As observed from the previous studies and the present findings, the moisture content in EC biomass was 89.23±0.86%. The reported ash and organic carbon content values in ECLB were 11.91±1.20% and 51.56±1.08%, respectively. The relative abundance of complex sugars, *i.e.*, cellulose, hemicellulose, and lignin, is the key factor that helps determine the phytobiomass potential for bioenergy generation[37]. We have observed that the ECLB showed higher cellulose and hemicellulose with lower lignin content. As observed from (Table 1), the Cellulose, Hemicellulose, and Lignin contents usually lie ≥ 20%, ≥30%, and ≤10% respectively for EC biomass, which is in concordance with the values cited by Singh *et al*[15]. The obtained values appear in favour of the biofuel production potential of ECLB as the lower Lignin content increases the biomass digestibility and fastens the process of delignification, while Cellulose and Hemicellulose rich biomass makes an aquatic weed more susceptible for hydrolysis to fermentable sugars[38].

Table 1 — Proximate and Biochemical characterisation of ECB

S. No.	Parameters	Previous Studies	*Present study (%)
1.	Moisture	$^{\#}$91.00%[3]; $^{\$}$90.84%[44]	89.23±0.86
2.	Dry Matter	*6.37±0.02%[1]; $^{\$}$9.16%[44]	10.77±0.60
3.	Ash Content	*15.60±0.20%[1]; $^{\$}$16.95%[44]	11.91±1.20
4.	Organic Carbon	$^{\#}$49.33%[45]	51.56±1.08
5.	Cellulose	$^{\#}$21.00%[11]; *24.77%[43]; $^{\#}$22.30±1.12%[12]	21.89±0.64
6.	Hemicellulose	$^{\#}$36.00%[11]; *22.72%[43]; $^{\#}$34.20±1.24%[12]	26.50±1.13
7.	Lignin	$^{\#}$7.00%[11]; *2.34%[43]; $^{\#}$10.2±0.52%[12]	5.62±0.83
8.	Total Carbohydrate	35.00±0.02%[3]	32.00±1.58
9.	Total Protein	*20.13±0.19%[1]; $^{\#}$12.80±0.03%[3]; $^{\$}$9.26 %[44]	20.83±0.52

Note: *Leaves, $^{\#}$Whole plant, $^{\$}$ Rootless plant

Table 2 — Characterisation of harvested ECLB using FTIR for the presence of Cellulose, Hemicellulose, and Lignin

Reference	FTIR spectra range (cm^{-1})						
	1050-1000	1200-1150	1250-1200	1350-1300	1450-1400	1550-1500	1650-1600
Adapa *et al*[39]	1050$^{\#}$	1157$^{\$}$ 1166$^{\#}$	1203* 1213$^{\#}$	1319* 1338*	1429$^{\$}$ 1431*	1511$^{\$}$	1606$^{\#}$
Present Study	1015.21	1153.71	1246.01	1339.63	1419.71	1540.71	1646.80

*Pure Cellulose (Microcrystalline powder); $^{\#}$Pure Hemicellulose (Xylan from Birchwood); $^{\$}$Pure Lignin (Hydrolytic powder)

FTIR, XRD, and SEM analysis

The recorded FTIR spectra on harvested ECLB showed characteristic peaks owning to the presence of cellulose, hemicellulose and lignin as evident when compared with the relevant literature (Table 2). Adapa *et al* recorded FTIR peaks at 1203 cm^{-1}, 1319 cm^{-1}, 1338 cm^{-1}, 1431 cm^{-1} (for Pure Cellulose); 1050 cm^{-1}, 1166 cm^{-1}, 1213 cm^{-1}, 1606 cm^{-1} (for Pure Hemicellulose); 1157 cm^{-1}, 1429 cm^{-1}, 1511 cm^{-1} (for Pure Lignin)[39]. In our study, the FTIR analysis of ECLB also showed peaks in the range of 1000-1650 cm^{-1} *i.e.*, at 1015.21 cm^{-1}, 1153.71 cm^{-1}, 1246.01 cm^{-1}, 1339.63 cm^{-1}, 1419.71 cm^{-1}, 1540.71 cm^{-1}, and 1646.80 cm^{-1}. Soeprijanto *et al*[40] also recorded characteristic peaks within these ranges for natural, pre-treated and digested EC samples *i.e.*, 1006.19-1022.94 cm^{-1}, 1245.75 cm^{-1}, 1313.63-1315.97 cm^{-1}, 1377.86-1420.58 cm^{-1}, 1538.38 cm^{-1}, and 1604.11-1632.54 cm^{-1}. In addition to this, they have also found peaks at 1729.90 cm^{-1}, 2917.32-2922.96 cm^{-1}, and 3272.63-3278.49 cm^{-1}. FTIR peaks were also recorded at 1736.73 cm^{-1}, 2933.03 cm^{-1}, and 3263.72 cm^{-1} for ECLB in the present study.

Microscopic studies such as SEM on recovered plant biomass post-phytoremediation allow an analysis of surface morphology (such as appearance, fibrillar arrangement, structural organisation, *etc*.), which can facilitate evaluation in terms of structural attributes owning to the suitability of phytobiomass for processing as potential biofuel substrate. SEM imaging was performed at different magnifications (Fig. 2) to get a better overview of the surface morphology. The structure appeared to be compact at magnifications up to 20000 X, while from images recorded at further higher magnifications, the fibrillar arrangement was found to be well-shaped, the surface being smooth and rigid[41].

XRD analysis determines the degree of crystallinity of the lignocellulosic material and the efficiency of treatments in removing lignin and hemicellulose henceforth[15]. For native cellulose, the peak intensity between 22° and 23° is reported to represent the crystalline fraction (002 crystalline plane), while the amorphous part has been shown with observed peaks between 18° and 19°[15]. The XRD peak at 21.55° in our study (Fig. 3) confirmed the crystalline fraction, and the peak recorded at 14.87° was in concordance with the findings of Bronzato *et al*[42]. The characteristic peak at 1419.71 cm^{-1} in FTIR-spectra further supports the cellulose-rich fraction in the studied sample[15].

Theoretical Biofuel yields from ECLB

Theoretical evaluation of biofuel yields facilitates preliminary assessment of the phytobiomass for its suitability as a potential bioenergy feedstock. Referring to the study of Cheng *et al*[24], it has been found that the theoretical biofuel (HMG) yields of EC leaves were higher than that of stem and roots, owing to their higher cellulose and hemicellulose contents. Adding to this, lower values of lignin and ash in EC leaves than stem

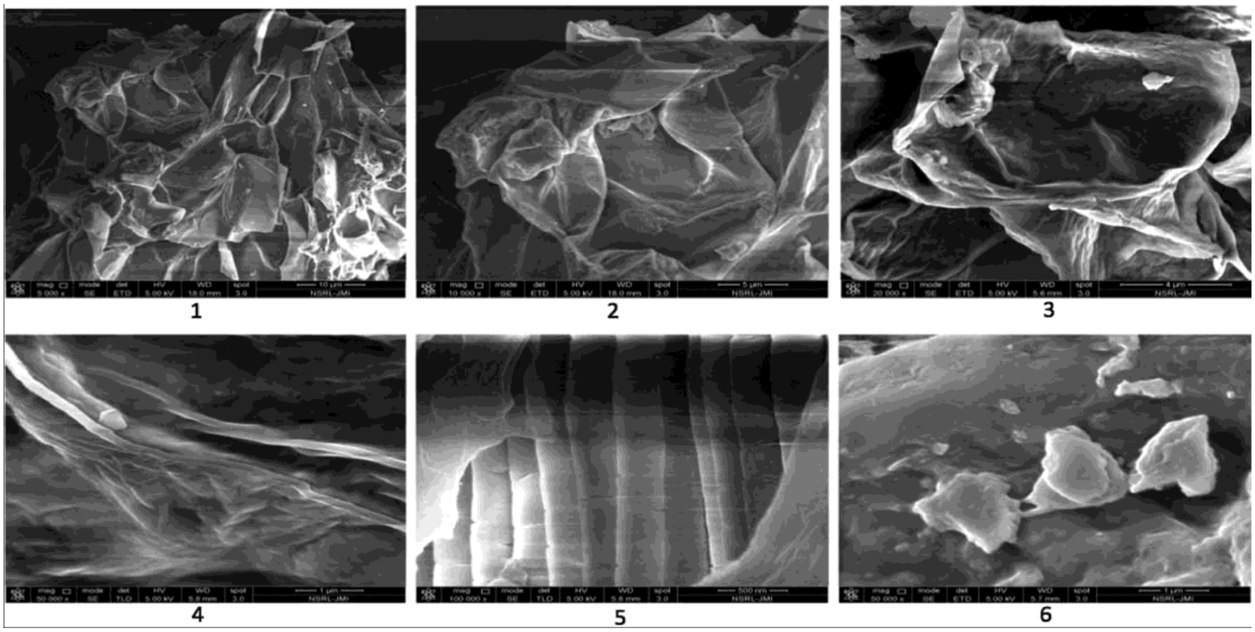

Fig. 2 — SEM micrographs of harvested ECLB (number 1 to 6 indicates micrographs obtained at different magnifications: 1-5000X, 2-10000X, 3-20000X, 4-50000X, 5-100000X, 6-50000X)

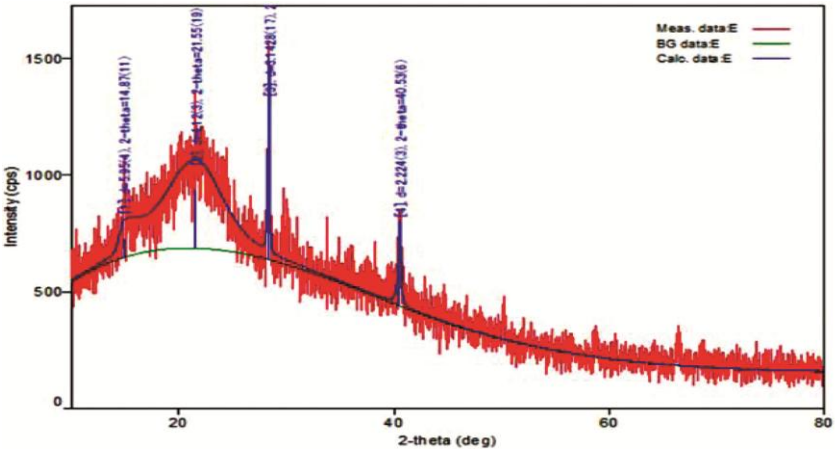

Fig. 3 — X-Ray Diffractogram of harvested ECLB

Table 3 — Theoretical Biofuel yields from Cellulose and Hemicellulose content of ECB^

Cellulose (%)	Hemicellulose (%)	Reducing Sugar (%)	HMG		Bioethanol Yield	Reference
			H_2 yield (mL/g DW)	CH_4 yield (mL/g DW)	(g/g DW)	
21.00	36.00	64.24	238.11	180.01	0.328	#Ratnani *et al*[11]
24.77	22.72	53.34	214.00	145.47	0.272	*Poomsawat *et al*[43]
22.30	34.20	63.64	239.21	177.51	0.323	#Masto *et al*[12]
21.89	26.50	54.43	210.85	150.28	0.278	*Present Study

*Leaves biomass, #WPB: Whole plant biomass, ^Theoretical biofuel yields were derived from the cellulose and hemicellulose content reported in present and previous studies

and roots also complement their overall biofuel production potential. Theoretical yields of HMG (210.85 mL H_2/g DW and 150.28 mL CH_4/g DW) and Bioethanol (0.278 g/g DW) calculated in the present study were comparable with those derived from the reported Cellulose and Hemicellulose values of ECB by other researchers[11,12,43] (Table 3).

Conclusion

EC known to grow luxuriantly, creating trouble in the waterbodies, is either removed for disposal or controlled using the various chemical and biological agents. However, such efforts are practiced with limited success, and studies promoting sustainable management of this weed are encouraged. In the present study, efforts have been made to exploit the proliferative growth behaviour of EC for phytoremediation and characterisation of recovered phytobiomass as a potential bioenergy substrate. The obtained results demonstrate a significant reduction in all the tested parameters (BOD, COD, TH, and HMs, *i.e*., Pb and Cd), and ECLB was found to have a stimulatory effect at lower doses of HMs. SEM characterisation confirmed the intact and smooth surface of ECLB harvested from Pb contaminated water. The biochemical investigations on ECLB revealed higher cellulose and hemicellulose with lower lignin content, and results of theoretical yields of HMG and bioethanol were comparable to the reported studies, demonstrating the characteristics of a promising bioenergy source. Overall, this work attempts towards sustainable management of EC by proposing an integrated methodology for wastewater reclamation and utilisation of resultant phytobiomass as a potential bioenergy feedstock.

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Conflict of interest

All authors declare no conflict of interest.

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