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RESEARCH ARTICLE

Effect of thermal and NaOH pretreatment on water hyacinth to enhance the biogas production

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Abstract
Water hyacinth (WH) is used as the substrate for biogas production due to its high lignocellulosic composition and natural abundance. The present study used thermal and chemical (alkali) pretreatment techniques to enhance biogas production from water hyacinth used as a substrate by anaerobic digestion. Thermal pretreatment was done using an autoclave at 121 °C and 15 lb (2 bar) pressure and alkali pretreatment by NaOH at two concentrations (2% and 5% w/v). The inoculum:substrate ratio for biogas production was 2:1, where cow dung was used as inoculum. Results indicated that the pretreatments increased biomass degradability and improved biogas production. Water hyacinth pretreated with 5% NaOH produced the highest amount of biogas (142.61 L/Kg VS) with a maximum methane content of 64.59%. The present study found that alkali pretreatment can modify the chemical structure and enhance WH hydrolysis, leading to enhanced energy production.

Keywords Anaerobic digestion · COD · Chemical pretreatment · Lignocellulose · Methane · Bioenergy

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Introduction

Researchers have mainly concentrated on the bioenergy sector as a step towards a low carbon future due to its rising energy demand. It is noteworthy to mention that the biogas sector is receiving greater attention compared to other renewable sources because of the availability of feedstocks, effectiveness in greenhouse gas (GHG) mitigation, and value addition to biomass waste (Show et al. 2022; Chan et al. 2019; Loy et al. 2018; Luo and Zhou 2012). Yan and Guo (2017) reported that water hyacinth (WH), also known as *Eichhornia crassipes*, is an invasive plant species native to the Amazon Basin of South America (Ilo et al. 2021) and is linked to its distinguishing biological structures apart from intense eutrophication of water bodies.

WH is considered a promising feedstock for anaerobic digestion (AD) due to its high reproductive capability, no risk to food security worldwide, easy hydrolyzable sugars, and lower lignin content (Koley et al. 2023a, b; Basu et al. 2021; Ilo et al. 2021). Numerous researches have been carried out on AD of WH using various types of inoculums such as cow dung (Bhui et al. 2018; Ali et al. 2022), buffalo dung, poultry litter (Castro and Agblevor 2021), and anaerobic sludge (Barua and Kalamdhad 2019) in various inoculum:substrate (I:S) ratios (Show et al. 2023). The availability of nutrients is directly related to the degradation of substrates (Zhou et al. 2017). The addition of cow dung ensures the carbon-nitrogen (C/N) balance, enriches the microbial consortia, and enhances the degradation kinetics. This method needs future and critical investigations. A challenge encountered in using lignocellulosic biomass for the bio-conversion process is the structural resistance of lignocellulosic biomass cell wall components, which needs to be pretreated before the bioconversion process (Banerjee et al. 2022; Show et al. 2022; Sinha et al. 2021).

Pretreatment is essential when utilizing biomass (Sheng et al. 2021) as a feedstock for the bioenergy process. It is vital for accelerated biomass conversion into hydrolyzable sugars (Sankaran et al. 2020). Bioenergy and biofuels derived from lignocellulosic and microalgal biomass are substitute renewable energy sources (Bajpai and Nemade 2023; Roy et al. 2022). Previously, WH was pretreated with a wide variety of methods like acid (Sathyanagalakshmi et al. 2011), alkali (Aswathi et al. 2013), biological (Sinegani et al. 2005), hot water (Saha et al. 2014), microwave-alkali (Zhang et al. 2016), ultrasound combined alkali (Soontornchaiboon et al. 2016), catalytic hydrothermal liquefaction (Singh et al. 2015), calcium peroxide (Cheng et al. 2015), thermo-chemical conversion (Huang et al. 2016), and microwave-assisted alkali-organosolvent (Das et al. 2016) to increase their biodegradability for enhancing bioenergy production capability with increasing their methane yield (Patil et al. 2011; Lin et al. 2015; Zhang et al. 2018). Mathew et al. (2011) suggested that alkaline pretreatment is more energy efficient than acid pretreatment. NaOH pretreatment has shown an effective ability to remove amorphous substances than other techniques (Singh and Singh 2021). The biogas production from WH can be accelerated by the thermal process (Barua and Kalamdhad 2017a, b) using an autoclave (Kurniawan et al. 2014). It is still necessary to fine-tune pretreatment technologies for various biomass types and improve an economically feasible technique (Sankaran et al. 2020). The objective of the present study encompasses the effect of thermal (autoclave) and alkali (5% and 2% NaOH) pretreatment on WH to enhance the biogas production through various analytical techniques, such as pH, COD, reducing sugar, production of volatile fatty acids (VFA) like acetic acid, and methane concentration.

Materials and methods

Feedstock collection and preparation

In this study, fresh WH was collected from ponds in Santiniketan, West Bengal, India (23.6800° N, 87.6800° E). The ponds were clean and received no pollutants from the nearby area. The harvested WH plants were carried to the laboratory in polyethylene plastic bags and cleaned to eliminate impurities, like dead plant parts, and the root portions were also removed before milling before the pretreatment. Wet biomass was milled in a mixer blender and kept at 4 °C for 48 h prior to anaerobic digestion. Locally collected and stored cow manure was used for anaerobic digestion. WH and cow dung's total solids (TS) and volatile solids (VS) contents were separately calculated, and the digester was loaded based on the volatile solids concentration (Table 1) (Mathew et al. 2015). The TS content of pretreated and untreated WH varied between 3.7 and 4.8%. The pH was determined employing the Thermo Scientific Orion ROSS electrode and electrical conductivity by DuraProbe 4-Electrode Conductivity Cells using the Thermo Scientific ORION Star A329 meter. Total nitrogen content, in the form of Kjeldahl nitrogen, was determined using the N-Kjeldahl method developed by Kjeldahl (1883). Organic carbon content was measured through the Walkley and Black method (Walkley and Black 1934).

Pretreatments of water hyacinth

Chemical pretreatment

Sodium hydroxide (NaOH) was used for chemical pretreatment. The NaOH was purchased from Sigma-Aldrich with a purity of ≥ 97%. Two different strengths of NaOH were

Table 1 TS and VS concentration

Sample	VS%	Moisture content (%)	Weight of substrate in the digester (g)	Weight of inoculum in the digester (g)	pH	EC (mho/cm)	TN (Kg/Ha)	C/N ratio
Pretreatment of WH 2% NaOH*	61.19	96	117.84	235.68	8.3	7.637	616	19.3 ± 1.3
Pretreatment of WH 5% NaOH*	51.19	95.6	141.22	282.44	11.07	12.50	616	18.7 ± 1.3
Pretreatment of WH autoclave*	67.61	95.2	106.92	213.84	5.82	6.087	308	25.7 ± 1.3
Control*	72.97	96.3	99.08	198.16	7.16	4.441	184.8	29.0 ± 1.3
Cow dung*	72.3	87	00	200	6.89	4.936	739.2	17.0 ± 2.6

*500 mL of distilled water was added additionally in each reactor used: 2% (w/v) and 5% (w/v). The pretreatment was conducted for 48 h. This chemical pretreatment aims to break down and solubilize organic matter in the WH, making it more accessible for bacterial degradation and subsequent biogas generation.

Thermal pretreatment

An autoclave was used for thermal preparation. The thermal treatment was carried out at 121 °C (approximately 250 °F). The pressure during thermal preparation was 15 lb (2 bar) for 15 min. The thermal preparation involves subjecting the WH to high temperature and pressure conditions. This helps in sterilizing the material and further breaking down complex organic compounds.

Anaerobic digestion of aquatic weeds

Cow dung was employed as an inoculum and WH as a substrate in a biogas generation process with a 2:1 inoculum to substrate ratio using the Mathew et al. (2015) technique, employing a 2-L digester bottle (Tarsons, India) (Fig. 1) in a batch culture. The anaerobic environment in the digesters was created by flushing them with nitrogen gas for 5 min and then sealing them. A temperature-regulating water shaking bath at 37 ± 2 °C was used to maintain the anaerobic digesters. Experiments were carried out in triplicate for each treatment.

Analytical methods

The COD was analyzed by the method of Yadav et al. (2006). COD tests were conducted using the reflux method and determined by oxidizing the organic matter in the sample with the $K_2Cr_2O_7$ solution. Titration with a ferrous ammonium sulfate solution determines the oxygen required for this oxidation, comparable to the COD. The procedure guarantees that all organic matter is oxidized, and the findings are represented as COD, which measures the quantity of organic and inorganic compounds in a sample that chemical agents may oxidize.

Fig. 1 Laboratory biogas setup

The biogas volume was measured by the water displacement method (Sunarso et al. 2010). The volume of gas produced was equal to the volume of water displaced from the measuring cylinders.

The methane and carbon dioxide percentage in the biogas was measured by gas chromatograph (GC) with a thermal conductivity detector (Mathew et al. 2015) using helium as the carrier gas. The column used was HP-molesieve; its dimensions were 30 m in length, a diameter of 0.32 mm, and a film thickness of 12 m. The injector and detector temperatures were maintained at 200 °C and 250 °C, respectively. The temperature range was set to rise from 40 °C for 5 min to 250 °C at a rate of 20 °C for 10 min. The GC was calibrated using a synthetic gas combination of 3 standards like (i) 75% methane, 5% carbon dioxide, 5% hydrogen, and 15% nitrogen; (ii) 50% methane, 10% carbon dioxide, 2% hydrogen, and 38% nitrogen; and (iii) 25% methane, 20% carbon dioxide, 1% hydrogen, and 54%.

The VFA sample was extracted using the method of Manni and Caron (1995). The volatile fatty acid (VFA) was analyzed by GC-FID using an EBX-70 column (length of 60 m, a diameter of 0.25 mm, and a film thickness of 0.25 m) with helium as a carrier gas and hydrogen as a combustion gas. One microliter of the sample was purged using an auto-injection system. The injector and detector were set at a temperature of 250 °C. The column temperature was designed to start at 70 °C with a holding period of 3 min, which was raised to 180 °C at a rate of 10 °C with a holding period of 6 min. The VFA standard (Volatile Free Acid Mix) was procured from Sigma-Aldrich (Supelco Bellefonte, USA).

Results and discussion

Changes in pH in various treatments

The pH value decreased significantly over the first few days of incubation in all treatments. It recovered as a result of the hydrolysis/acidogenesis process using VFA (Wang et al. 2017). The pH range of 6.5–8.2 is known to be optimum for methane generation (Kothari et al. 2014). In the present study, the pH range was found to be optimum in all the treatments. The pH range for pretreatment with 5% NaOH was 8.32 to 6.86; for pretreatment with 2% NaOH, it was 8.13 to 6.92; for thermal pretreatment, it was 7.51 to 6.9 (Fig. 2). Adding NaOH will increase the concentration of hydroxide ion which raises the pH, so that 5% NaOH has

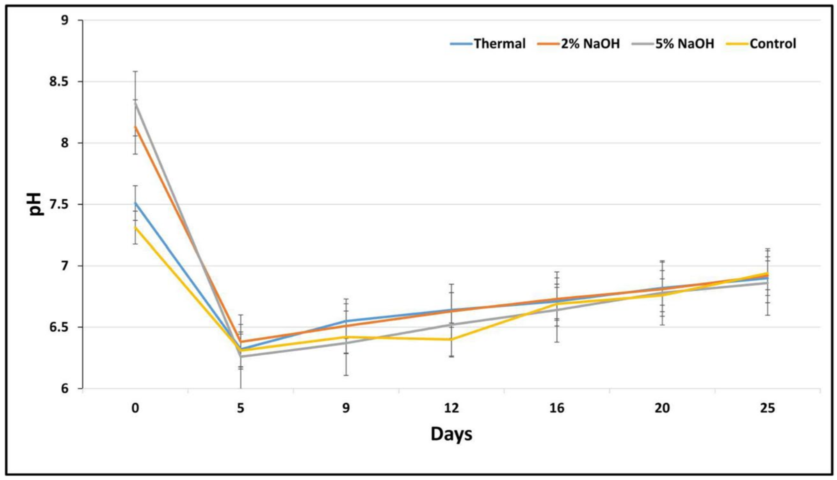

Fig. 2 Comparison of day-wise pH change in various treatments

the maximum pH value in the present study. In all the treatments, the maximum pH was reduced on day 5 as organic acids were produced. After that, the pH again increased as the concentration of ammonia increased due to the digestion of nitrogen (Angelidaki and Ahring 1993). A similar trend in pH change was observed in a study conducted by Dai et al. (2018), where maximum pH reduction was found on day 6 and increased pH.

Changes in COD in various treatments

COD is a crucial metric in the AD process. Syaichurrozi and Sumardiono (2013) state that biogas production is directly proportional to COD removal. There, the maximum removal of COD was 38%, where vinasse was used as a substrate. The present study found a maximum COD reduction in 5% NaOH alkali pretreatment (68.17%). The initial COD was 208.65 mg/L; at the digestion end, the COD was 66.4 mg/L. The COD reduction in 2% NaOH pretreatment was from 198.35 to 72.56 mg/L (63.41%), and in thermal pretreatment, it was 190.5 to 90.44 mg/L (52.52%) (Fig. 3). In a previous study, WH pretreated with 121 °C and pH 13 (pH adjusted by NaOH) can solubilize 66.64% of COD (Patel et al. 1993). However, the previous study suggests that biological pretreatment can help in more COD reduction (Sinha et al. 2021). Studies suggested that COD removal depends upon various physiological parameters like pH, temperature, hydraulic retention time, and organic loading (Chen et al. 2014; Jiang et al. 2014; Fu et al. 2015; Wu et al. 2016).

Changes in day-wise reducing sugar production in various treatments

In the initial process of biogas production, complex polymers of lignocellulosic material are converted into reducing sugars which play a crucial role in the hydrolysis and acidogenesis step in AD (Xu et al. 2019). Finally, the concentration of these reducing sugars aids in determining the degree of hydrolysis (Vanegas et al. 2015), which leads to VFA formation and the final product as biogas. Across the present

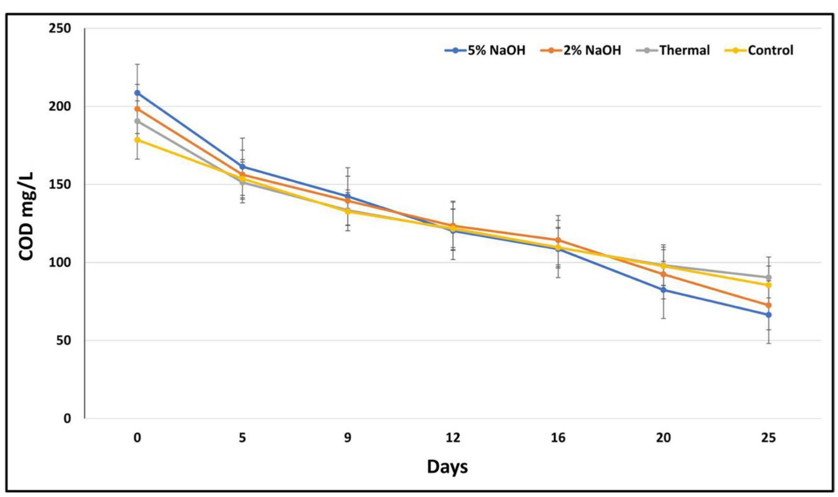

Fig. 3 Comparison of day-wise COD change in various treatments

study, maximum reducing sugar production was observed at day 2 in 5% NaOH alkali pretreatment, leading to maximum biogas production (294.74 mg/mL). In 2% NaOH pretreatment, the reducing sugar production was (212.42 mg/mL), while in thermal pretreatment, it was (261.58 mg/mL) (Fig. 4).

Changes in day-wise VFA production in various treatments

VFA concentration plays a vital role as a sensitive indicator in the AD process. Accumulation of VFA could inhibit methanogenesis. The present study monitored the total VFA production during the anaerobic digestion of WH pretreated with different concentrations of NaOH and autoclaving. A maximum VFA accumulation was found in 5% NaOH alkali pretreatment (1876.12 ppm), followed by thermal (1641.82 ppm), and 2% NaOH pretreatment (1614.32 ppm) (Fig. 5). Maximum amount of VFA was found at day 9, and the beginning of the methanogenesis phase might indicate the progressive decrease of VFA. According to Barua and Kalamdhad (2017a, b), the highest VFA production levels for untreated and hot air oven-pretreated WH were found on day 14 in concentrations of 1491 mg/L and 1758 mg/L, respectively. The present study reports higher VFA production than WH pretreated with dilute acid-thermal pretreatment and cattle dung biochar (Suthar et al. 2022). This study justified the methanogenic activity as the previous study suggests a VFA concentration of more than 6000–8000 mg/L inhibits methanogenesis (Karthikeyan and Visvanathan 2013). In this study, acetic acid production with alkali and thermal (autoclave) pretreated WH has been monitored; it was observed that with the 5% NaOH pretreatment, the maximum accumulation of acetic acid was 380 ppm and 350 ppm, 326 ppm with the 2% alkali and thermal pretreatment on the 9th day with the gradual decrease till the end of the fermentation period (Fig. 6). Although the AD process took 25 days, no significant changes were seen after day 9. The

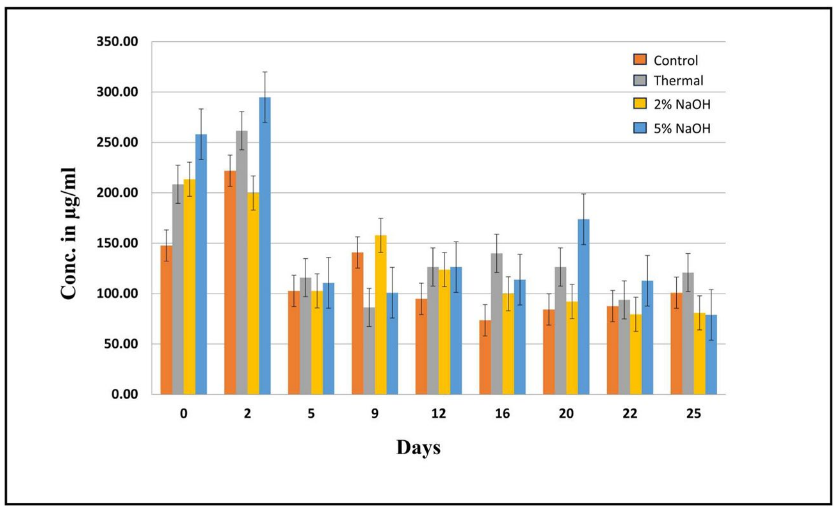

Fig. 4 Comparison of day-wise reducing sugar production in various treatments

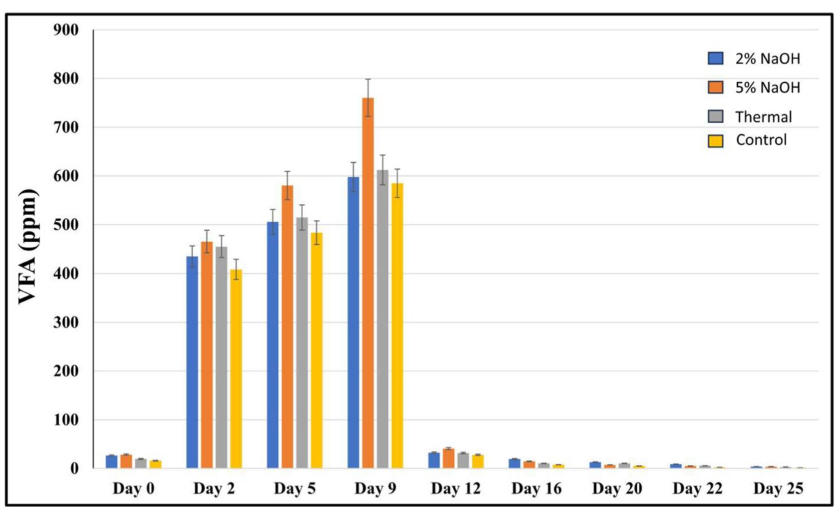

Fig. 5 Comparison of day-wise VFA production in various treatments

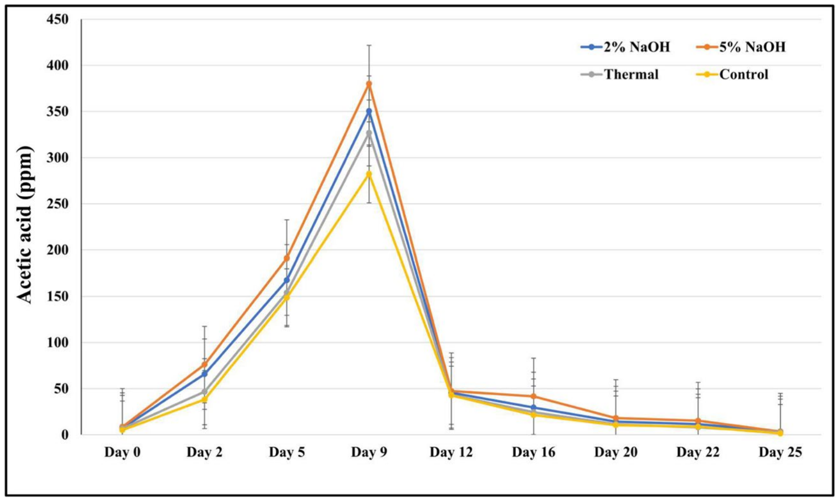

Fig. 6 Comparison of day-wise acetic acid production in various treatments

considerable decline in acetic acid suggests that the methane produced from the VFAs is created during hydrolysis (Sinha et al. 2021). Consequently, the reactors' VFA production was within the available range; thus, there was no operational imbalance brought on by VFA buildup.

Comparison of methane and biogas production in various treatments

Biogas and methane production from WH with different pretreatment techniques was monitored for 25 days. Barua and Kalamdhad (2017a, b) previously reported that WH pretreated with hot water produced 193 ± 22 mL CH_4/g VS on the 14th day with 67.4 ± 0.3% methane content. In this present study, the pretreatment process with the most significant methane concentrations was 5% NaOH alkali (64.59%), followed by thermal pretreatment (54.24%), and 2% NaOH alkali (51.47%) (Fig. 7). On day 12, the methane concentration was highest in 5% alkali treatment. Still, the methane concentration peaked on day 25 for the other treatments, which was not more significant than the control. Pretreated WH also shows maximum biogas production with 5% NaOH (142.61 L/Kg VS), while 2% NaOH alkali treatment results in lower biogas production (137.47 L/Kg VS) than thermally treated WH (139.11 L/Kg VS) (Fig. 8). The model's variance analysis (ANOVA) result produces an F value of 300.091 and predicted R^2 values of 0.995 and 0.998, indicating a well-fitted model. Table 2 provides a summary of the ANOVA results that were used to determine the regression validation for modifying the biogas production. The significance of regression, as determined by the F test, indicates the model's suitability, reliability, and accuracy. According to the present analysis, total biogas production has increased considerably within 25 days. Overall, 5% NaOH and thermal treatment enhanced biogas production significantly ($P \leq 0.01$) compared to 2% NaOH treatment.

The structure of the WH cell wall was altered by alkali pretreatment using 5% NaOH; the organic matter content is

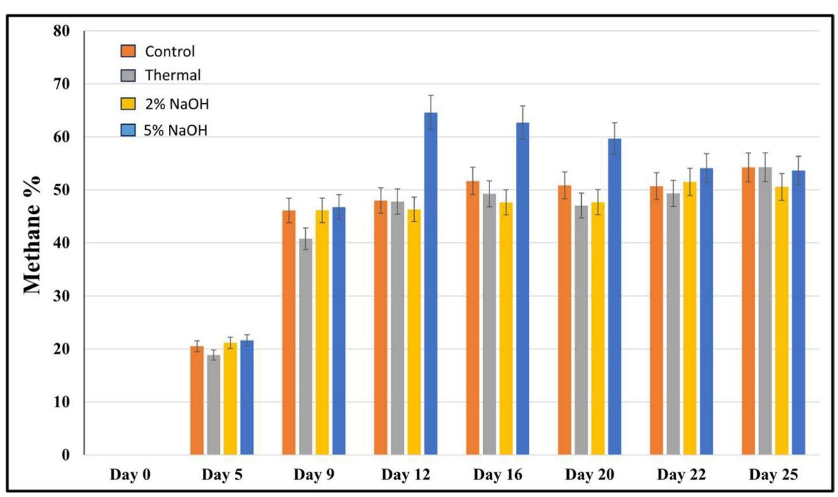

Fig. 7 Comparison of methane production in various pretreatments

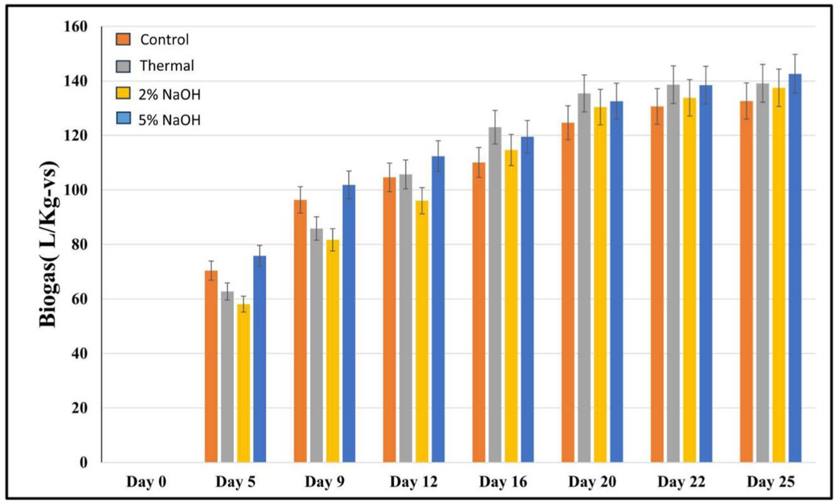

Fig. 8 Comparison of biogas generation across different treatments

Table 2 Analysis of variance (ANOVA) between days and pretreatments (thermal and NaOH)

Time	Sum of squares	Mean square	*F*	Sig.
Model	14.971	2.139	300.091***	0.000
Day 5	558.854	186.285	41.480***	0.000
Day 9	775.044	258.348	94.963***	0.000
Day 12	405.225	135.075	48.441***	0.000
Day 16	275.194	91.731	24.641***	0.000
Day 20	185.730	61.910	16.014***	0.001
Day 22	135.266	45.089	11.654***	0.003
Day 25	154.178	51.393	20.288***	0.000
R square	0.998			
Adjusted *R* square	0.995			
Significance level	<0.000			

***Significant level: < 0.01

made more vulnerable to bacterial assault, which enhances the biogas generation process (Martin and Hadiyanto 2020). An increase in the intensity of NaOH pretreatment can lead to higher lignin reduction (Jung et al. 2020). However, it is important to note that an increase in the intensity of NaOH pretreatment can also cause a greater loss of total biomass (Fahmayanti and Abtokhi 2018). A similar trend was in Barua and Kalamdhad (2017a, b), where hot water pretreated WH produced maximum biogas and methane at day 14. This performance supports past findings by other researchers that pretreatment of WH with NaOH catalyzes the production of biogas, which results in a higher yield (Patil et al. 2011). In this study, the final methane concentration was higher in 5% alkali treatment than the untreated WH reported by Chanakya et al. (1993) and pretreated with dilute acid-thermal pretreatment and cattle dung biochar (0.5 and 1.5% of biochar) reported by Suthar et al. (2022). Yet, methane yield was similar, as reported by Ferrer et al. (2010). However, a study by Bhui et al. (2018) suggested that inoculum to substrate ratio of 3:1 can produce a maximum biogas of up to 383 L/Kg. Still, the methane concentration was lower than in this current study with the 5% NaOH alkali pretreatment technique.

Energy equivalent: an environmentally friendly strategy

Using the fundamental energy equivalents, the potential of WH-derived biogas for power generation with alkali treatment was estimated. In this present study, the total biogas production from 5% NaOH pretreated WH was 142.61 L/Kg VS with 64.59% methane content. To estimate the electrical potential, it was assumed that 1 m^3 of biogas has a calorific value of 22 MJ, and 1 m^3 of methane has a calorific value of 36 MJ. One cubic meter of biogas will produce 2.14 kWh of power, and 1 m^3 of methane will produce 10 kWh of electricity under the premise that the electrical conversion efficiency is 35%. Therefore, according to this current study, it was estimated that 0.305 kWh of electricity from biogas and 0.92 kWh of electricity from the methane content could be generated from 5% NaOH pretreated WH. Families may benefit from using the generated biogas instead of the polluting firewood, kerosene, and dung cake, and the electricity will help in human welfare.

Conclusion

The research emphasized the potential of the aquatic weed *Eichhornia crassipes*, used to produce biogas. The pretreatment process with alkali and thermal was utilized to increase biogas generation. Results show that the pretreatments boosted gas generation and increased biomass degradability.

Thermal pretreatment did not result in any additional enhancement or appreciable changes to WH digestion. The methane concentration is highest in the 5% NaOH alkali pretreatment (64.59%), which can produce 0.92 kWh of electricity, followed by thermal pretreatment (54.22%) and 2% NaOH alkali pre-treatment (51.47%). Pretreated WH with 5% NaOH shows maximum biogas production (142.61 L/Kg VS) than WH pretreated with thermal treatment (137.56 L/Kg VS).

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Binoy Kumar Show, Gaayathri Shivakumaran, Apurba Koley, and Anudeb Ghosh. The first draft of the manuscript was written by Binoy Kumar Show and Gaayathri Shivakumaran, and conceptualisation, methodology, validation, project administration, supervision, and writing—review and editing were performed by Shibani Chaudhury, Amit Kumar Hazra, and Srinivasan Balachandran. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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