https://doi.org/10.48047/AFJBS.4.4.2022.167-189

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Production and analysis of biofuel from *Chenopodium album*

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Volume 4, Issue 4, Dec 2022

Received: 15 Nov 2022

Accepted: 05 Dec 2022

Published: 25 Dec 2022

doi: 10.48047/AFJBS.4.4.2022.167-189

Abstract

Climate scientists promote eco-friendly fossil fuel alternatives. Bioethanol from weeds might solve lignocellulosic biomass concerns. This study examined the potential of *Chenopodium album* for biofuel production. EHG eliminates 18.4-20.2 moles H_2 per kg organic carbon. AF and EHG produced 27–33.4 moles (0.5–0.70 dm3/g TOCR). The 48-hour anaerobic fermentation method produced 30.88-45.2 moles of biogas per kilogram of total organic carbon removed (TOCR) by converting acetate and formate (AF → MG). Methane production ranged from 27.34 to 93.02 moles. The three-stage anaerobic fermentation comprising acidogenic fermentation (AF), methanogenesis (MG), and electrohydrogenesis (EHG) produces 81.4% more biogas than the single-stage acidogenic fermentation. Highly efficient hydrogen-methane biogas production yielded 35.27 to 45.31 moles per kilogram of TOCR. Thermochemical process converted cellulose into glucose and xylose. Enzymatic biomass breakdown improved cellulose hydrolysis by 95.1%. This method produced 45 g/l glucose. The maximal weed saccharification glucose yield was 93%. Furthermore, biofuel analysis revealed 91% bioethanol and 0.0021 mg/L methanol. The water, Copper and Chlorine content was found to be 0.201%, 0.056 mg/kg and 19.33 mg/l respectively. The gum concentration was 2.1 mg/100 mL. In addition to this, pH of biofuel was found to be 8.8, with 6021 kcal/kg heating value, 0.75 g/cm3 density, 2.11 cSt viscosity, and a flash point of 13. Each reading showed bioethanol levels produced from the *Chenopodium album* within the permissible limits.

Keywords: Anaerobic fermentation, *Chenopodium album*, Enzymatic breakdown, Flash point

1. Introduction

Bioethanol is a sustainable biofuel produced from plant matter, largely utilized as a substitute for non-renewable fuels in the transportation industry [1]. Bioethanol is derived

from several sources, encompassing food crops like corn and sugarcane, non-food biomass like agricultural leftovers and woody crops, and even algae. The production of bioethanol consists of multiple stages, including pre-treatment, hydrolysis, fermentation, distillation, and dehydration [2]. It has a wide range of applications, including as being mixed with gasoline to produce fuel blends like E10 and E85, as well as being used as an industrial solvent and a key component in chemical manufacturing. Bioethanol provides environmental advantages through the mitigation of greenhouse gas emissions and the promotion of renewable resource utilization. Moreover, it stimulates the development of rural economies by generating fresh opportunities for the marketing and trade of agricultural products [3]. Nevertheless, it is imperative to tackle other obstacles such as the ongoing dispute regarding the allocation of resources between food and fuel, issues related to land and water usage, concerns about maintaining energy equilibrium, and the technological complexities involved in manufacturing second and third-generation bioethanol. Despite the obstacles, progress in biotechnology and sustainable practices instill hope for the future of bioethanol as a significant contributor to the shift towards more environmentally friendly energy sources [4].

To evaluate the suitability of *Chenopodium album* as a potential biofuel source, it is imperative to quantify its biomass productivity, chemical composition, and conversion efficiency. Chenopodium album, renowned for its fast growth and versatility, possesses the capacity to generate significant biomass. Under ideal circumstances, it can yield an annual production of 10-15 tons per hectare [5]. The majority of the plant's biomass consists of lignocellulosic material, with cellulose and hemicellulose accounting for around 60-70% of its weight when dry. This characteristic makes it a suitable option for bioethanol production. Pre-treatment techniques, such as steam explosion or alkaline treatment, can effectively eliminate fermentable sugars from the lignocellulosic structure, resulting in conversion efficiencies of approximately 80-90%. In addition, thermochemical techniques like as pyrolysis and gasification can be employed to convert Chenopodium album biomass into bio-oil and syngas, respectively. The energy conversion efficiency of these methods ranges from 40% to 60%. By optimizing the costs associated with the collection, transportation, and processing of Chenopodium album, its total biofuel production can be made comparable to that of other non-food biomass sources [6]. The measurement demonstrates the plant's substantial potential to make a large contribution to sustainable biofuel production, while simultaneously providing a method for controlling an invasive species.

2. Methodology

In the previous paper, we studied the process optimization studies of the *Chenopodium album,* which confirmed it to be a potential candidate for biofuel production. Therefore, in the current study, we have discussed the production and analytical techniques of biofuel produced from the *Chenopodium album.*

2.1 Acidogenic fermentation

Anaerobic reactors with a total volume of 125 ml and a working volume of 100 ml were used to conduct acidogenic fermentation for biohydrogen generation [7]. The aquatic biomass, pre-treated at a concentration of 6% (w/v), was injected with 5 ml of heat-treated mixed culture at a concentration of 5% (v/v) under anaerobic conditions. The entire material was completely blended, and then the pH was adjusted to 6.0 using a 1 M ortho-phosphoric acid. The reactors were hermetically sealed to prevent any gas exchange and then purged with nitrogen (N2) to establish an anaerobic atmosphere. The experiments were conducted at a temperature of 37 °C in an incubator shaker, with a rotation speed of 120 rpm, for a duration of 7 days in the single-stage operation. However, in the two and three-stage operations, the duration was limited to 48 hours (2 days). The bioprocess parameters, including H2 generation, total organic carbon (TOC), pH, and volatile fatty acids (VFA), were regularly analyzed to monitor the performance of the process [8]. The effluents from the acidogenic stage were sent to either the electrohydrogenesis or methanogenesis stages, as specified in the experimental combinations.

2.2 Electrohydrogenesis

The electrohydrogenesis process was carried out in a dual-chambered microbial electrochemical cell (MEC) with a standard H-type configuration. The MEC comprised of two Scott-Duran glass bottles, with a combined volume of 65 ml and a usable volume of 50 ml. A proton exchange membrane served as the barrier between the two chambers. The counter electrode employed was composed of a graphite rod that was coated with activated carbon cloth (ACC) [9]. The working electrode utilized consisted of a graphite rod that was covered with stainless steel mesh. The electrodes were connected to the potentiostat using stainless steel wire, which acted as the current collector. In order to increase the current density, the counter electrode of the microbial electrolysis cell (MEC) was infected with a selectively enriched exoelectrogenic inoculum (10% v/v) acquired from a running microbial fuel cell (MFC) that was treating synthetic wastewater. The first culture was enhanced with a concentration of 10 mM acetate in a 50 mM phosphate buffer. The electrode was adjusted to a potential of +0.2 V compared to Ag/AgCl (3 M KCl) [10]. After the biofilm stabilization phase was finished, the

anodic chamber received pre-treated biomass slurry either directly or through the effluent from acidogenic fermentation/methanogenesis in integrated systems. In contrast, the cathodic chamber was filled with a solution of phosphate buffer saline containing 1% (w/v) NaCl to guarantee electrical conductivity [11]. The experiment was carried out by applying a constant potential of -1 V to the working electrode while positioning a reference electrode (Ag/AgCl, with a potential of +0.195 V compared to the standard hydrogen electrode) near the working electrode. The experiment was carried out in an oxygen-free environment at normal room temperature for a period of 7 days in a single phase, and for 48 hours using different combined methods. The MEC's performance was assessed by regularly monitoring the current consumption, total organic carbon (TOC), volatile fatty acids (VFAs), and hydrogen (H2) generation [12].

2.3 Methanogenesis

The methanogenesis tests were conducted using the identical methods as the acidogenic fermentation studies, except replacing the heat-treated mixed culture with untreated anaerobic mixed culture as the inoculum. Furthermore, the pH of the reactor was adjusted to a value of 7.0 instead of 6.0. Methane was synthesized at a temperature of 37 °C for 7 days in the single stage but was limited to a duration of 48 hours in the two and three-stage integrations [13]. The tests were overseen using qualitative and quantitative methods, specifically focusing on the biogas content and output, as well as the levels of total organic carbon (TOC) and volatile fatty acids (VFA).

2.4 Ethanol fermentation

The fermentation process took place in 250 ml conical flasks at a pH of 5.0 and a temperature of 32 °C, while ensuring anaerobic conditions. The biomass slurry was enhanced with S. cerevisiae at a concentration of 10% (v/v), and the resulting mixture was subjected to incubation at a temperature of 32 °C [14]. The experiment was conducted for 120 hours. At regular 24-hour intervals for 5 days, samples were taken and examined

2.5 Analytical Methods

2.5.1 Biochemical Analysis

The quantity of reducing sugar produced after acid pre-treatment was measured using the DNS (dinitro salicylic acid) assay. In this experiment, 3 ml of the filtrate obtained after pre-treatment was mixed with 3 ml of DNS reagent (Sigma-Aldrich) in a test tube with a loosely fitted cap

[15]. The solution underwent heating in a water bath, which was kept at a constant temperature of 95 °C, for a period of 10 minutes. The operation was halted when a reddish-brown coloration emerged, and the absorbance was measured at a wavelength of 540 nm using a spectrophotometer. The concentration of decreasing sugar was quantified by employing a standard curve consisting of known glucose values. The Hewlett Packard Gas Chromatograph (GC), specifically the HP 5890 series II model, was utilized to analyze the volatile fatty acids (VFAs) present in the collected samples. The total organic carbon (TOC) was quantified utilizing a TOC analyzer. During this procedure, the organic carbon and inorganic carbon present in the sample undergo combustion facilitated by platinum and acidification, respectively, resulting in the conversion of these forms of carbon into carbon dioxide (CO2) [16]. The carbon dioxide concentration is measured using an infrared analyzer, which gives a reading of the overall carbon content in the sample. The pH of the sample was determined using a pH meter (S400 Seven Excellence™ pH/mV, Mettler Toledo). The ethanol content was quantified using gas chromatography (GC). The monomeric sugars and inhibitory furfurals generated during the pre-treatment stage, enzyme hydrolysis, and fermentation were measured using High-Performance Liquid Chromatography (HPLC) with an Agilent 1100 instrument. The samples were filtered using a 0.25 µm syringe filter before analysis [17].

2.5.2 Analysis of Alcoholic Content

The studied features of chemical bioethanol will include its ethanol, methanol, water, Cu, Cl, and gum levels. The physical qualities of the substance include its heating value, density, viscosity, and flash point. The chemical qualities of the substance are tested using ASTM standards, specifically D 5501, D 1744, D 1688, D 512, D 2622, and D 381. The physical properties of the substance are tested using ASTM standards, namely D 1613, D 240, D 1298-99, D 445, and D 93 [18].

2.5.3 Biostatistical Analysis

Biostatistical Analysis refers to the application of statistical methods and techniques in the field of biology and medicine. The experimental sets were conducted in triplicates. The means and standard deviations were calculated using descriptive statistics. An analysis of variance (ANOVA) was conducted to determine the differences in the means of biogas and ethanol yield derived from various aquatic weeds in different experimental combinations [19]. The Tukey post hoc test was employed to detect the statistically significant differences ($p < 0.05$).

The experimental data sets were analysed using Graphpad Prism, version 5, developed by Graphpad Software, Inc. in San Diego, USA.

3. Results

3.1 A comprehensive method for producing gaseous biofuel from *Chenopodium album*

In this study, we aimed to create a sustainable and profitable solution for treating weeds and producing bioenergy [20]. To achieve this, we selected the *Chenopodium album* to produce gaseous biofuel using various integrated biological methods. At first, we assessed the effectiveness of three different methods, namely AF, MG, and EHG, in producing biogas (consisting of H2 and CH4) and degrading the substrate. In addition, the experiments were conducted using various combinations of two-stage and three-stage processes to enhance both energy recovery and carbon conversion efficiency, as shown in Tables 1, 2, and 3. The remaining biomass slurry recovered from the first bioprocess was transferred to the second bioprocess for each set of experiments. Experiments were conducted in a single stage for 10 days [21]. However, the duration of each experiment was limited to 48 hours depending on the data obtained.

Table 1: Experimental design and the operational conditions adopted during the single-stage process

Experimental Conditions	**Reactor Design**	**Catalyst**	**Work Conditions**		
			pH	**Temp (℃**	**HRT (h)**
Acidogenic Fermentation (AF)	Anaerobic Reactor	Heat-treated anaerobic mixed culture	5.8	37	48
Electrohydrogenesis (EHG)	Dual Chamber Microbial Electrolysis Cell	Exoelectrogens	6.2	37	48
Methanogenesis (MG)	Anaerobic Reactor	Untreated mixed culture	6.8	37	48

Table 2: Experimental design and the operational conditions adopted during the two-stage process

	Reactor Design	**Catalyst**	**Work Conditions**

Experimental Conditions			**pH**	**Temp (℃**	**HRT (h)**
Acidogenic Fermentation to methanogenesis	Anaerobic Reactor	Heat-treated anaerobic mixed culture to Untreated mixed culture	5.8-6.8	37	96
Acidogenic Fermentation to Electrohydrogenesis	Anaerobic Reactor to Dual Chamber Microbial Electrolysis Cell	Heat-treated anaerobic mixed culture to Exoelectrogens	5.8-6.2	37 to room temperature	96

Table 3: Experimental design and the operational conditions adopted during the three-stage process

Experimental Conditions	**Reactor Design**	**Catalyst**	**Work Conditions**		
			pH	**Temp (℃**	**HRT (h)**
Acidogenic Fermentation to methanogenesis to Electrohydrogenesis	Anaerobic Reactor to Dual Chamber Microbial Electrolysis Cell	Heat-treated anaerobic mixed culture to Untreated mixed culture to Exoelectrogens	5.8 to 6.8 to 6.2	37 to 37 to room temperature	144
Acidogenic Fermentation to Electrohydrogenesis to methanogenesis	Anaerobic Reactor to Dual Chamber Microbial Electrolysis Cell	Heat-treated anaerobic mixed culture to Exoelectrogens to Untreated mixed culture	5.8 to 6.2 to 6.8	37 to room temperature to 37	144

3.2 Single-Stage Approach for hydrogen and methane production

Research on single-stage methods including acidogenic fermentation (AF), electrohydrogenesis (EHG), and methanogenesis (MG) for H2/CH4 has shown that biogas generation and organic fraction degradation vary [22]. At 24 h, acidogenic fermentation of weed biomass yielded 6.10-6.87 mol/kg TOCR, rising to 8.11-11.32 at 48 h. After 48 h, substrate degradation was 33.1-46.1%. Hydrogen generation and substrate degradation increased little after 48 h.

To match TOC content, decreasing sugar degradation was 29.72–31.7% in 48 h. However, due to persistent acidogenesis, which degrades organics and H2, the content of soluble metabolites/VFA (volatile fatty acids) increased from 603-866 mg/l at 24 h to 1567-2876 mg/l at 48 h. With increasing VFA concentration, pH drops from 5.8 to 5 in 48 h and farther. Acetate and butyrate made up 88% of the VFAs, with propionic and valeric acids in small amounts. EHG had higher substrate clearance (47.0-51.3%) and RS than AF after 48 h (figure 1).

EHG operation showed little VFA content. This may be attributed to cathode H2 generation and VFA and soluble metabolite degradation [23]. Methane production was absent for the first 168 h of MG. After 192 h, 0.1-1.2 mmol methane was generated, yielding 2.7-3.0 mol/kg TOCR. The VFA concentration initially increased due to the acidogenesis pathway (from 356-621 mg/l at 24 h to 1453-2134 mg/l at 96 h), but after 144 h, methanogens consumed VFAs for methane production, lowering the VFA content to 408-503 mg/l and stabilizing the pH to 6.8. In the first 48 h, substrate degradation was 33.3-41.6 percent, rising to 50.3-63.3% after 192 h. Similar to TOC content, decreasing sugar degradation was 40.1-51.4% after 48 h and 57.8-66.4% after 192 h.

In conclusion, all three single-stage processes failed to degrade the substrate and convert it to energy [24]. Thus, sequential bioprocess integration improved energy conversion efficiency.

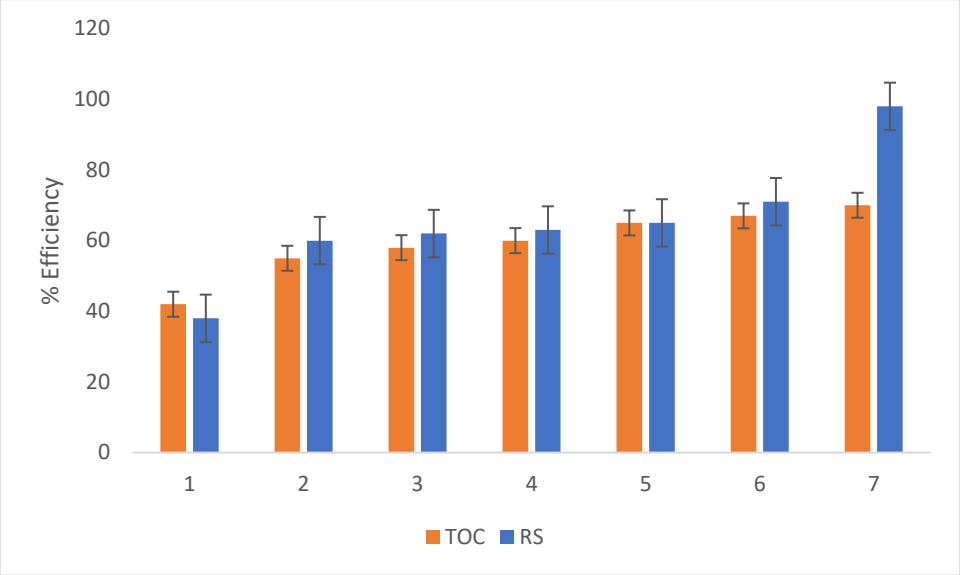

Figure 1: Total Organic Content (TOC) and Reducing Sugars (RS) removal efficiency of different experimental approaches. 1-AF, 2-EHG, 3-MG, 4- AF+ EHG, 5- AF+ MG, 6- AF+ EHG+ MG, 7- AF+ MG+ EHG

3.2 Two Stage Approach for hydrogen and methane production

In two-stage integration, the effluent/residual slurry of (AF) were further employed in methanogenesis or electrohydrogenesis (AF → EHG or AF → MG) to utilize the residual carbon source towards additional H_2/CH_4 production [25]. Both the integration strategies maximized the energy recovery in the form of H_2/CH_4 production and increased substrate removal efficiency. The effluent from AF reactor (5800-7935 mg/l TOC) was fed into the cathode chamber of EHG under constant voltage of -1 V for 48 h. Integration of EHG showed a marked increment in H_2 yield single stage. The hydrogen yield in EHG alone was 18.4-20.2 moles H_2/kg TOC_R while the cumulative hydrogen yield (AF + EHG) accounted for 27 – 33.4 moles H_2/kg TOC_R (0.5-0.70 dm3 /g TOCR). The combined hydrogen production process in the present study also facilitated significant TOC removal, contributed to about 30.09-33.1% alone in EHG and about 68- 73.3% cumulative of both the processes [26].

Similarly, the effluent generated during AF (5800-7935 mg/l TOC) was also evaluated for methanogenesis for 48 h. The integrated process (AF → MG) showed additional bioenergy generation with the methane production of 27.34- 93.02 moles CH_4/kg TOCR in 48 h accounting for a total biogas yield of 30.88-45.2 moles/kg TOCR [27]. However, the total substrate degradation (5643-8054 mg/l TOC) was higher than the single stage operation (MG).

Besides that, higher methane concentration (86%) was observed in two-stage system as compared to MAD (65%) alone.

3.3 Three-Stage Approach for hydrogen and methane production

In the first combination (AF → EHG → MG), the overall biogas yield was 29.45-.5 moles biogas/ kg TOC_R with substrate degradation of 7554-9084 mg TOC/l (76%). In second combination (AF → MG → EHG), the overall biogas yield was 35.1-40.9 moles biogas/kg TOC_R with substrate degradation 6558-8010 mg TOC/l (82%) On the whole, third-stage integration was found to be effective for both energy production and substrate removal wherein the first combination (AF → EHG → MG) depicted to perform better in increased substrate degradation while a slightly higher biogas yield was observed in the second integration (AF → MG → EHG) [28]. The maximal experimental biogas yield was achieved for AF → MG → EHG which was 81.4% significantly higher ($p < 0.05$) compared to single stage AF. (Figure 2)

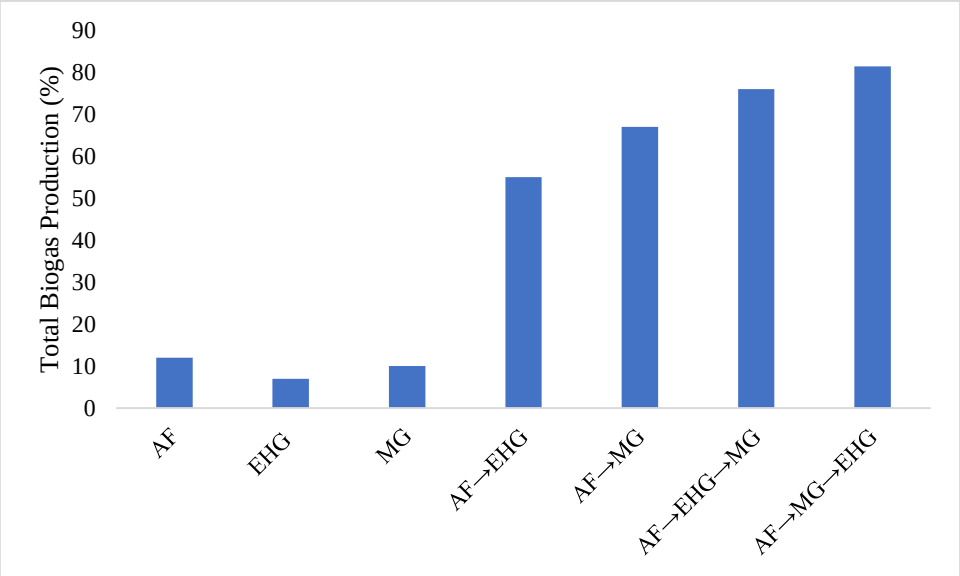

Figure 2: A consolidated graph depicting the total biogas produced from *Chenopodium album*

Table 4: Assessment of the efficiency of energy recovery and substrate removal in studies that integrate process integration.

Experimental Combinations	Biogas production (mmol)			Total Biogas (mmol)	TOC$_R$ (%)	RS$_R$ (%)	SHY (moles/kg TOC$_R$)	SHY (moles/kg TOC$_R$)	SHY (moles/kg TOC$_R$)	Total Biogas Yield (Moles/kg TOC$_R$)
	AF	EHG	MG				AF	EHG	MG	
AF	1.2	-	-	1.3	40.01	37.6	11.3	-	-	-
EHG	-	1.3	-	1.5	51.50	65.21	-	10.1	-	-
MG	-	-	0.57	1.4	61.30	70.30	-	-	5.4	3.1
AF+EHG	1.2	2.5	-	7.1	68.07	83.14	11.3	19.8	-	-
AF+MG	1.2	-	4.7	7.4	67.01	82.12	11.3	-	31.7	31.2
AF+EHG+MG	1.2	2.5	4.2	8.1	80.12	95.55	11.3	19.8	12	12
AF+MG+EHG	1.2	2.3	5.6	8.2	83.11	97.41	11.3	14.1	31.2	35.4

3.4 Evaluation of the Energy Efficiency of Integrated Approaches

Energy security and environmental benefits must be considered while assessing biofuels' long-term viability [29]. Each bioprocess's energy efficiency (KJ per kilogram of TOCR-produced biogas) was calculated to evaluate the measurements. The retrieval of H2 and CH4 from aquatic biomass anaerobic breakdown in each bioprocess was used to evaluate energy conversion efficiency [30]. The assessment of substrate degradation was conducted by calculating the total organic carbon removal efficiency (TOC_R) using the equation stated in Eq. 1.

$TOC_R = [(TOC_I - TOC_E)/TOC_I] \times 100$

Where, TOC_I is initial concentration of total organic carbon (mg/l) and TOC_E is the total organic carbon concentration (mg/l) found in the effluent.

Hydrogen yield (mol H_2/kg TOC_R) and CH_4 yield (mol CH_4/kg TOC_R) represent the H_2 or CH_4 produced in a given time with the function of total substrate removed (TOC in kg) in that specific time in each process

Specific Hydrogen Yield= (moles H_2)/(TOC_I-TOC_E)

Specific Methane Yield= (moles CH_4)/(TOC_I-TOC_E)

The total biogas yield from aquatic biomass (mol/kg TOC_R) is a measure of the total amount of biogas produced [31].

Total Biogas yield= (moles H_2+ moles CH_4)/(TOC_I-TOC_E)

The total energy yield of the produced biogas was calculated using the theoretical calorific values of H_2 (141790 kJ/kg) and CH_4 (55530 kJ/kg).

When comparing individual trials (AF, EHG, and MG), it was found that AF had a greater energy production (ranging from 2541.0 to 3206.6 KJ/kg TOC_R) followed by MG (ranging from 2008.7 to 2987.3KJ/kg TOC_R) and EHG (ranging from 1876.9 to 3182.1 KJ/kg TOC_R) [32].

A significant increase in energy output was reported in both two-stage and three-stage integration methods. Among the two-stage process integrations, the AF → MG integration demonstrated significantly greater energy efficiency (20155.1-29671.3 KJ/kg TOC_R) compared to the AF → EHG integration (6003.2-7103.5 KJ/kg TOC_R) [33]. The integration of electrohydrogenesis as the last process (AF → MG → EHG) in three-stage integration techniques resulted in higher energy recovery (22423.1-31326.2 KJ/kg TOC_R) compared to the integration of methanogenesis as the final process (AF → EHG → MG) (15178.3-18101.4 KJ/kg TOC_R). (Figure 3)

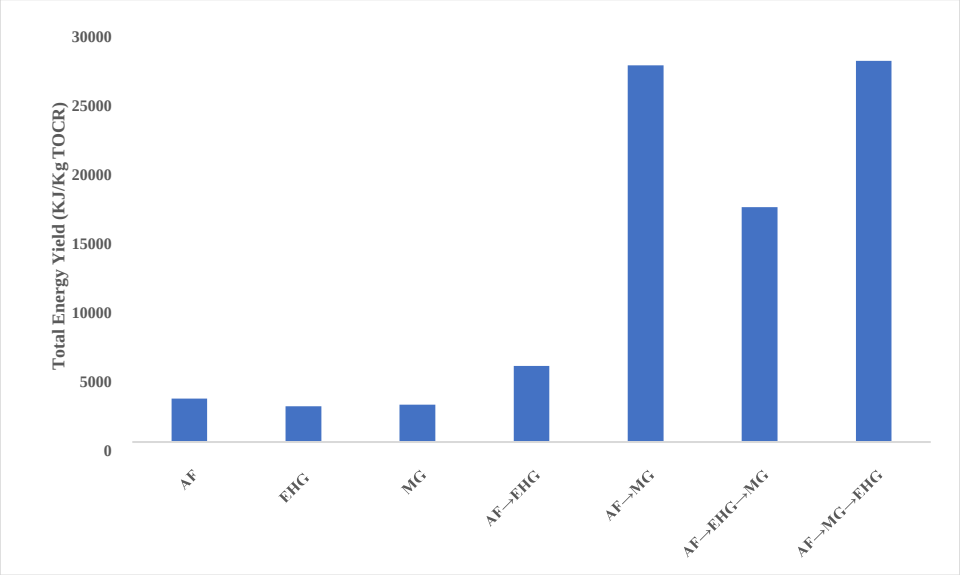

Figure 3: Total energy efficiency of different experimental combinations for *Chenopodium album*

The study showcased the practical viability of combining acidogenic fermentation, methanogenesis, and electrohydrogenesis to efficiently extract the maximum amount of energy from weed in the form of biohydrogen and biomethane [34]. The system achieved a very efficient production of hydrogen and methane, resulting in a total energy yield of (35.27-45.31) moles biogas/kg TOC_R). This yield is much greater than the yield produced from each bioprocess step tested in the study. In addition to the increase in energy conversion efficiency, the three-stage system also demonstrated a significant reduction in organic load.

3.5 Production of liquid biofuel

3.5.1 Biomass pretreatment

Thermal pretreatment of 10% w/v dehydrated weed with 1% H2SO4 at 121 °C/15psi for 20 minutes was studied. The acid-hydrolyzed slurry was neutralized with 10% v/v ammonium hydroxide and filtered with Whatman paper discs [35]. DNS detected hydrolysate sugar decreases. The entire pre-treated slurry was then saccharified enzymatically. Cellulase enzyme from Trichoderma reesei (Sigma Aldrich, at least 700 units/g) at 10 FPU/g biomass converted 10% (w/v) solids into simpler sugars. In an incubator-shaker at pH 4.8, hydrolysis took 72 hours at 50 °C, 120 rpm.

To test thermochemical pretreatment, weeds were treated with 1% dilute sulphuric acid. To assess the impact, total reducing sugar (TRS) per gram of aquatic biomass was measured [36].

Sugar reduction peaked at 0.44 g/g biomass. Much hemicellulose became xylose after dilute acid pretreatment. Commercial cellulase hydrolysed the cellulose-rich material. Acid pretreatment dissolves hemicellulose but not cellulose. Xylose is the primary monomeric sugar in pre-treated hydrolysate [37]. Best enzyme hydrolysis of pre-treated biomass transformed cellulose into glucose.

3.5.2 Characterization

The analysis of the hydrolysate fraction obtained during pretreatment revealed that xylose was the predominant reducing sugar component, with concentrations ranging from 75% to 81%. Glucose, on the other hand, accounted for only 19% to 25%. Enzyme hydrolysis is the process of breaking down weed biomass using enzymes [38]. The study found that thermochemical pretreatment significantly improved the degradation of cellulose, resulting in a glucose concentration of around 75% and xylose accounting for just 13%. The enzymatic breakdown of treated biomass demonstrated enhanced cellulose hydrolysis, leading to a greater glucose output (45g/l) and a saccharification rate of 95.1%. (Table 5)

Table 5: Characterization of the chemical composition of pretreatment hydrolysate, pretreated hydrolysate, and stillage during the ethanol manufacturing process

	Pretreated Hydrolysate	**Enzyme Hydrolysate**	**Residual Biomass and stillage**
Total Reducing sugar (g/g dry weight)	0.44	0.54	0.03
Glucose (g/l)	4.3 ± 0.01	44.2 ± 0.02	2.12 ±0.02
Xylose (g/l)	15.1 ±0.02	11.01 ± 003	8.9 ± 0.01

3.5.3 Bioethanol Production and Energy Output

The fermentation process was carried out in 250 ml conical flasks at a temperature of 32°C and a pH of 5.0, by employing *Saccharomyces cerevisiae*. The experiment was conducted for a duration of 120 hours. After 24, 48, 72, 96, and 120 hours, samples were collected to analyse monomeric sugars and sugar breakdown products such as HMF and furfurals. The saccharification of aquatic biomass led to a substantial increase in glucose yield, reaching up to 93%. This, in turn, resulted in the immediate generation of ethanol [39]. The findings

indicated that 65% of the expected ethanol production was achieved during the initial 48 hours. This value increased to 84% after 120 hours. An inverse relationship was detected between the glucose concentration and fermentation time, with the glucose concentration dropping as the fermentation duration increased [40]. (Fig. 4 and 5)

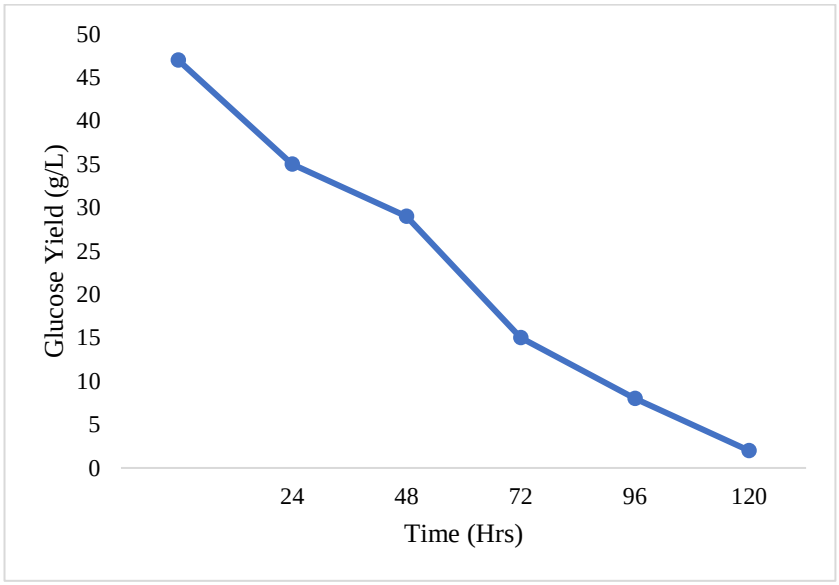

Figure 4: Glucose yield during Bioethanol production from *Chenopodium album*

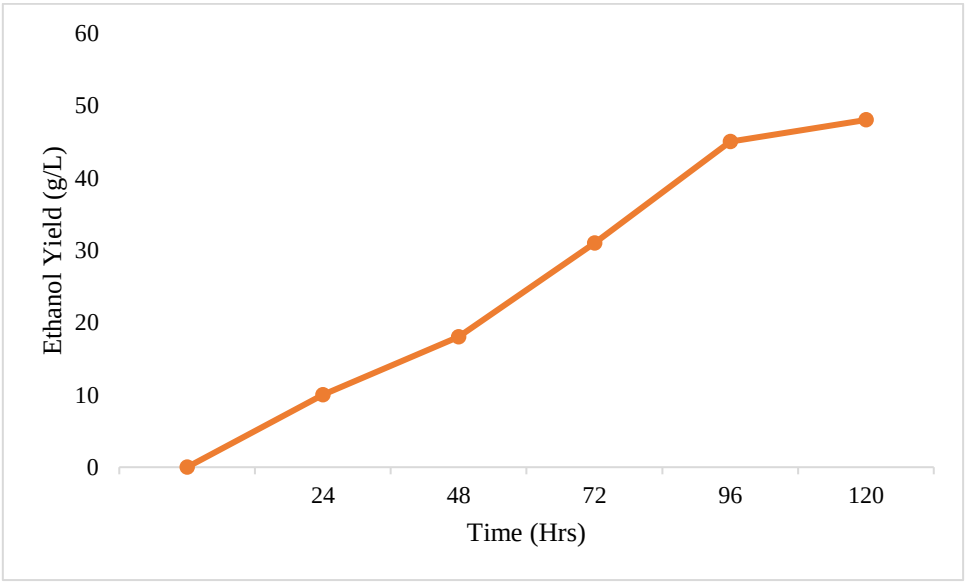

Figure 5: Ethanol yield during Bioethanol production from *Chenopodium album*

3.5.4 Analysis of Alcoholic Content

The biofuel had 91% bioethanol and 0.0021 mg/L methanol. The volume content of water was 0.201%. Copper and chlorine concentrations were 0.056 and 19.33 mg/kg and liter, respectively [41]. The gum content was 2.1 mg/100 ml. Biofuel physical parameters: pH 8.8, heating value 6021 kcal/kg, density 0.75 g/cm3, viscosity 2.11 cSt, and flash point 13℃.

4. Discussion

Mohan et al. (2008) investigated an integrated technique that increases biogas generation and accelerates substrate degradation to improve energy conversion efficiency. Hydrogen-methane generation boosts energy efficiency by 20%–60%. More study is needed to improve biorefinery energy conversion efficiency with these integrated methods. Lignocellulosic biomass produces most biogas [42]. The high lignin content (20–35%) of lignocellulosic biomass makes biofuel production expensive and energy-intensive.Weed bioethanol could assist lignocellulosic biorefineries overcome their problems. Insufficient substrate breakdown and energy conversion were discovered in all three single-stage procedures.

Acidogenic fermentation (AF) was chosen as the first stage in following research because it produces the most VFAs and H2 and can be used to harness energy. In this investigation, EHG alone yielded 18.4-20.2 moles H2/kg TOCR, while AF + EHG yielded 27–33.4 moles (0.5-0.70 dm3 /g TOCR). In the combined process (AF → MG), methane production increased to 27.34-93.02 moles CH4/kg TOCR in 48 hours, resulting in a total biogas yield of 30.88-45.2 moles/kg TOCR. Previous studies found the same tendency [43]. The coupled system's higher methane concentration would reduce CO2 separation, making biogas upgrading easier. Thus, a two-stage system with optimal operating conditions may reduce hydraulic retention time (HRT) and improve biogas methane concentration. This would reduce upgrade costs compared to single-stage systems. The three-stage strategy resulted in 81.4% higher biogas yield ($p < 0.05$) for AF → MG → EHG compared to single-stage AF. The study showed that acidogenic fermentation, methanogenesis, and electrohydrogenesis can maximize weed biohydrogen and biomethane generation. Hydrogen and methane production was efficient, generating 35.27-45.31) moles biogas/kg TOCR. The yield was far higher than any bioprocess step studied [44].

The three-stage technique lowered organic load and increased energy conversion efficiency. Pretreatment of biomass improves valorization and reduces sugar release 2013). Dry aquatic weeds (10% w/v) were thermally treated for 15 min at 121 °C/15psi with 1% H2SO4. Acid hydrolyzed slurry was neutralized with 10% v/v ammonium hydroxide and

filtered on Whatman paper discs. DNS detected hydrolysate lowering sugars (ADESINA, 2014).

Enzyme saccharification followed pretreatment of the slurry. Pretreatment hydrolysate contained 75%–81% xylose. It was 19%–25% glucose [45]. The stiff, resistant cellulose component may not solubilize, reducing glucose hydrolysis. Weeds go down with hydrolysis enzymes.

A thermochemical preparation improved cellulose breakdown to 75% glucose and 13% xylose. Enhanced biomass enzymatic breakdown increased cellulose hydrolysis, glucose production (45g/l), and saccharification (95.1%). Saccharification of weed biomass increased glucose production to 93%. This produced ethanol immediately. This work proposes a cost-effective bioethanol alcohol quantification method. Biofuel is determined physically and chemically [46]. Biofuel analysis showed 91% bioethanol and 0.0021mg/L methanol. Water volumetrically was 0.201%. Copper was 0.056 mg/kg and chlorine 19.33 mg/L. The gum content was 2.1 mg/100 ml. The biofuel's physicochemical properties were measured: Muhaji and Sutjahjo (2018) report a pH of 8.8, heating value of 6021 kcal/kg, density of 0.75 g/cm3, viscosity of 2.11 cSt, and flash point of 13℃. Thus, Chenopodium fermentation provides more ethanol due to its high carbohydrate content [47]. Therefore, Chenopodium album is recommended for bioenergy generation.

5. Conclusion

Scientists are motivated to transition to environmentally friendly fossil fuels due to the impact of climate change. Utilizing bioethanol derived from cannabis plants could potentially address the challenges associated with lignocellulosic biomass. Weeds possess a favorable combination of high glucose levels and fast development, rendering them suitable for use as biofuel. The study focused on the production of biofuels derived from Chenopodium album. EHG eliminates 18.4-20.2 moles of H2 per kilogram of organic carbon. AF and EHG generated a quantity of 27–33.4 moles (0.5–0.70 dm3/g TOCR). The process of anaerobic fermentation, which leads to the generation of methane (AF → MG), resulted in the production of 30.88-45.2 moles of biogas per kilogram of TOCR and 27.34-93.02 moles of methane within a 48-hour period. The three-stage anaerobic fermentation process (AF → MG → EHG) yields an 81.4% increase in biogas production compared to the one-stage AF process . Statistically significant change ($p < 0.05$). The findings of this study indicate that acidogenic fermentation,

electrohydrogenesis, and methanogenesis significantly increased the production of biohydrogen and biomethane from weeds. The hydrogen-methane biogas generation achieved a high level of efficiency, with a range of 35.27-45.31 moles/kg TOCR. This stage of the bioprocess produces the highest amount. Three solutions enhanced energy conversion and decreased organic load. The process of thermochemical conversion was used to transform cellulose into glucose and xylose. The activity of biomass degradation enzymes resulted in a 45 g/l increase in glucose production and a 95.1% cellulose hydrolysis rate. The saccharification of weed glucose achieved a 93% conversion rate. The study of the biofuel revealed a bioethanol content of 91% and a methanol concentration of 0.0021mg/L. The water content was 0.201%. The concentration of copper was 0.056 milligrams per kilogram, whereas the concentration of chlorine was 19.33 milligrams per liter. The gum content measured 2.1 milligrams per 100 milliliters. The pH of biofuel is 8.8, its heating value is 6021 kcal/kg, its density is 0.75 g/cm3, its viscosity is 2.11 cSt, and its flash point is 13℃. Every reading was within the usual range for bioethanol. This attribute renders Chenopodium an efficient source of biofuel.

Author Contribution

Neeraj Sethi and Vivek Srivastava proposed the concept of the manuscript; Vivek and Bhawna investigated the study, collected data, and prepared the initial draft of the manuscript; Sushila Kaura contributed to manuscript writing and data analysis; Ikbal Shah refined the drafts of manuscripts. All authors have read and agreed to the published version of the manuscript

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