International Journal of Sustainability and Advanced Integrated Research
Volume 2, Issue 2 (April-June 2026) | ISSN: 3107-7854
DOI: https://doi.org/10.65339/ijsair.V2.I2.529

Bioethanol Production from Matured Leaves of *Eichhornia Crassipes* through Separate Hydrolysis and Fermentation (SHF): Basis for Instructional Laboratory Manual Development

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Publication history: Received: May 2, 2026; Revised: May 27, 2026; Accepted: May 31, 2026
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Abstract

This study investigated the production of bioethanol from matured leaves of water hyacinth (*Eichhornia crassipes*) using the Separate Hydrolysis and Fermentation (SHF) process and developed an instructional laboratory manual based on the experimental findings. Grounded in constructivist and experiential learning principles, the study employed an experimental research design under controlled laboratory conditions. Matured water hyacinth leaves collected from Balzain, Tuguegarao City, Cagayan were subjected to wet and dried pretreatment conditions, followed by acid pretreatment, enzymatic hydrolysis, fermentation using Saccharomyces cerevisiae, and ethanol quantification through Gas Chromatography (Shimadzu GC-2010). Results revealed that wet pretreated biomass produced a higher average bioethanol yield (3.870 g/L) than dried pretreated biomass (2.396 g/L). Statistical analysis indicated a significant difference between the two biomass conditions, confirming that moisture content influences ethanol production efficiency. The findings demonstrated the effectiveness of SHF in converting water hyacinth into bioethanol and highlighted the potential of this invasive aquatic plant as a renewable lignocellulosic feedstock. The study also resulted in the development of an experimental protocol procedure that serves as the basis for an instructional laboratory manual designed to support experiential, inquiry-based, and sustainability-oriented science learning. It is recommended that future studies optimize process parameters, explore alternative pretreatment methods and microbial strains, and evaluate pilot-scale applications of bioethanol production from water hyacinth. The study supports Sustainable Development Goal (SDG) 4 (Quality Education), SDG 7 (Affordable and Clean Energy), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action) through the integration of renewable energy research and contextualized science instruction. Its sustainability impact includes promoting environmental management through the utilization of invasive biomass, supporting renewable energy development, and strengthening sustainability-focused science education.

Keywords: Bioethanol, Eichhornia Crassipes, Fermentation, Hydrolysis, Instructional Manual, Renewable Energy, Water Hyacinth

1. Introduction

Bioethanol has emerged as a promising renewable energy source in response to increasing global energy demand, fossil fuel dependence, and rising carbon emissions. Although conventional bioethanol production relies on food-based feedstocks, concerns regarding food security and land-use competition have encouraged the exploration of lignocellulosic biomass as a sustainable alternative. Water hyacinth (*Eichhornia crassipes*), an invasive aquatic plant with high cellulose and hemicellulose content, presents potential as a bioethanol feedstock while simultaneously addressing environmental management concerns. This study investigates bioethanol production from matured water hyacinth leaves using Separate Hydrolysis and Fermentation (SHF) and develops an experimental protocol procedure as a basis for an instructional laboratory manual.

1.1 Water Hyacinth-Based Bioethanol Production Using Separate Hydrolysis and Fermentation (SHF)

Growing industrialization and population expansion continue to increase global energy demand, reinforcing dependence on fossil fuels despite their environmental consequences (Patil et al., 2014). Carbon emissions reached approximately 38.1 billion tons in 2025, contributing to climate change, environmental degradation, and public health risks (Global Carbon Budget, 2025a). Renewable energy alternatives such as bioethanol offer opportunities to reduce greenhouse gas emissions and support energy diversification (Hu et al., 2008; IEA, 2023). Traditional bioethanol production depends largely on food-based feedstocks such as corn and sugarcane, raising concerns regarding food security and agricultural resource competition (Zhao & Xia, 2010; Das et al., 2015). Consequently, research attention has shifted toward second-generation bioethanol derived from lignocellulosic biomass, including agricultural residues and aquatic plants (Hossain et al., 2022). Despite their abundance and sustainability advantages, efficient conversion remains challenging because lignin restricts

enzymatic access to fermentable sugars (Taherzadeh & Karimi, 2008). Although pretreatment technologies have improved lignin removal and hydrolysis efficiency (Kaur et al., 2022), economic and operational limitations continue to constrain large-scale implementation (Zabed et al., 2023).

1.2 Themes and Insights from Literature on Lignocellulosic Biomass and Bioethanol Yield

Lignocellulosic biomass is composed primarily of cellulose, hemicellulose, and lignin, which form a rigid structure that limits hydrolysis efficiency (Kumar & Murthy, 2011). Effective pretreatment is therefore necessary to improve sugar release and ethanol production. Studies have reported lignin removal efficiencies of up to 85%, resulting in improved fermentation performance (Kaur et al., 2022). However, feedstock characteristics remain a major determinant of bioethanol yield and economic feasibility. Water hyacinth has gained attention as a promising feedstock because of its rapid growth, minimal cultivation requirements, and high cellulose content. At the same time, its uncontrolled proliferation contributes to oxygen depletion, biodiversity loss, and obstruction of waterways (Malik, 2007). Research has demonstrated its suitability for bioethanol production through efficient enzymatic conversion processes (Rezania et al., 2016; Begam et al., 2024). These findings highlight its dual role as both an environmental challenge and a renewable energy resource.

1.3 Local, National, and Global Context of Water Hyacinth Utilization and Renewable Energy

Globally, the transition toward renewable energy technologies is driven by climate change mitigation and sustainable development objectives. Bioethanol derived from non-food biomass supports this transition by reducing dependence on fossil fuels and minimizing competition with food production systems. Within the Philippines, water hyacinth infestation in freshwater systems presents environmental and socio-economic challenges while also providing opportunities for biomass utilization. The widespread occurrence of water hyacinth in major water bodies demonstrates the need for locally adaptable technologies that can convert invasive biomass into useful energy products. Nationally, renewable energy initiatives promoted by the Department of Energy and environmental management efforts led by the Department of Environment and Natural Resources encourage the exploration of sustainable biomass resources. Educational reforms under the Commission on Higher Education and the Department of Education likewise emphasize inquiry-based, contextualized, and outcomes-based learning approaches that support the integration of sustainability-related scientific investigations into laboratory instruction.

1.4 Theoretical and Educational Basis for SHF-Based Laboratory Instruction

Bioethanol production involves pretreatment, hydrolysis, fermentation, and distillation processes (Canilha et al., 2012). Among available production approaches, Separate Hydrolysis and Fermentation (SHF) allows hydrolysis and fermentation to occur under independently optimized conditions, improving substrate conversion and reducing enzyme inhibition (Choudhary et al., 2016). The use of Saccharomyces cerevisiae further enhances ethanol production due to its fermentation efficiency and tolerance characteristics (Bai et al., 2008). Continued advances in enzyme engineering and process optimization contribute to improved bioethanol yields and process reliability (Zabed et al., 2023). The educational component of this study is grounded in constructivist and experiential learning principles. Constructivism emphasizes active learner engagement in knowledge construction through inquiry and experimentation, while experiential learning promotes learning through cycles of experience, reflection, conceptualization, and application. The use of locally available water hyacinth in laboratory activities enhances contextualized learning, environmental awareness, scientific reasoning, and practical laboratory competence.

1.5 Research Gaps and Rationale for Optimizing Bioethanol Yield from Water Hyacinth

Despite advances in lignocellulosic bioethanol production, significant challenges remain in improving process efficiency, reducing production costs, and optimizing feedstock utilization (Zabed et al., 2023). Although water hyacinth has demonstrated bioethanol potential (Rezania et al., 2016; Begam et al., 2024), studies investigating bioethanol yield optimization from matured leaves under localized and controlled conditions remain limited. Furthermore, the application of SHF specifically for comparing wet and dried pretreated water hyacinth biomass has received insufficient attention. Existing studies also rarely integrate renewable energy research with the development of contextualized instructional laboratory materials. These gaps justify the need to examine bioethanol production from matured water hyacinth leaves using SHF and to develop an experimental protocol procedure that supports science education and sustainability-oriented learning.

1.6 Alignment with Relevant Sustainable Development Goals (SDGs)

This study aligns with several United Nations Sustainable Development Goals. It supports SDG 4 (Quality Education) through the development of an instructional laboratory manual that promotes inquiry-based and experiential learning. It contributes to SDG 7 (Affordable and Clean Energy) by exploring bioethanol production from a renewable and non-food biomass source. The utilization of water hyacinth as a feedstock advances SDG 12 (Responsible Consumption and Production) through resource recovery and sustainable biomass utilization. By promoting renewable energy alternatives that may reduce greenhouse gas emissions, the study also contributes to SDG 13 (Climate Action).

1.7 Importance of the Study to Multidisciplinary Sustainability

The study contributes to environmental sustainability by promoting the utilization of an invasive aquatic species as a renewable energy resource, thereby supporting ecosystem management efforts. It advances energy sustainability through the investigation of alternative biofuel production pathways using locally available biomass. The development of an instructional laboratory manual strengthens educational sustainability by enhancing contextualized, inquiry-driven, and competency-based science education. The study also supports community sustainability by providing alternative strategies for managing water hyacinth infestations and encouraging resource utilization. For policymakers, educational institutions, and energy stakeholders, the findings offer evidence that may inform sustainable environmental management, renewable energy initiatives, and science education practices. Through its integration of renewable energy research and instructional innovation, the study contributes to broader goals of sustainable development and interdisciplinary knowledge advancement.

2. Material and methods

2.1 Research Design

The study employed an experimental research design to determine the bioethanol yield from matured leaves of *Eichhornia crassipes* using the Separate Hydrolysis and Fermentation (SHF) process. Wet and dried pretreated biomass served as the independent variables, while bioethanol yield was the dependent variable. Both treatment conditions were subjected to identical pretreatment, hydrolysis, fermentation, and analytical procedures to ensure that differences in bioethanol yield could be attributed to biomass condition. The experiment was conducted under controlled laboratory conditions to maintain consistency, reliability, and accuracy. The findings were subsequently utilized in developing an experimental protocol procedure for an instructional laboratory manual.

2.2 Locale of the Study

Matured leaves of water hyacinth (*Eichhornia crassipes*) were collected from Balzain, Tuguegarao City, Cagayan, where the plant is abundantly available in freshwater environments. All laboratory procedures, including biomass preparation, pretreatment, SHF, and bioethanol analysis, were conducted at Green Future Innovations, Inc. (GFII). The laboratory provided the necessary facilities and equipment for bioethanol production and enabled the controlled regulation of temperature, pH, and fermentation conditions throughout the experiment.

2.3 Respondents of the Study

This study did not involve human respondents. The primary experimental material consisted of matured leaves of water hyacinth (*Eichhornia crassipes*) collected and processed as biomass feedstock for bioethanol production.

2.4 Sample and Sampling Procedure

Matured water hyacinth leaves were collected from the study site and thoroughly washed to remove dirt and impurities. The leaves were drained and chopped into approximately one-inch pieces before processing. The biomass was oven-dried at 60°C until reaching a moisture content of approximately 10–15% on a dry basis. For wet-pretreated biomass, the oven-dried leaves were soaked in water for 24 hours before being powdered and sieved to obtain a 1 mm substrate size. For dried-pretreated biomass, the dried leaves were directly powdered and sieved to the same particle size. Both biomass preparations were stored in sealed glass containers prior to pretreatment.

2.5 Research Instrument

The study utilized laboratory equipment including an oven, analytical balance, blender, spatula, Erlenmeyer flasks, beakers, stirring rod, pressure cooker or autoclave, filter cloth, incubator, wash bottle, pH meter, aluminum foil, cotton, micropipette, and Gas Chromatography (Shimadzu GC-2010). Reagents used in the experiment included distilled water, 2% sulfuric acid, peptone AR, yeast extract AR, phosphate buffer solution, cellulase enzyme, and Saccharomyces cerevisiae broth. These instruments and materials facilitated biomass processing, pretreatment, hydrolysis, fermentation, and quantitative analysis of ethanol concentration.

2.6 Data Gathering Procedure

Following biomass preparation, acid pretreatment was conducted using a liquid-to-solid ratio of 10:1. Twenty grams of wet and dried pretreated biomass were mixed with 200 mL of 2% (v/v) sulfuric acid in 250 mL Erlenmeyer flasks. The flasks were cotton-plugged, covered with aluminum foil, and autoclaved at 15 psi for 15 minutes. The treated biomass was filtered, rinsed with distilled water until clear, and manually squeezed to remove excess moisture. For enzymatic hydrolysis, twenty grams of each pretreated biomass sample were transferred into 500 mL Erlenmeyer flasks. Phosphate buffer solution was added to achieve a pH range of 6.0–6.5, with 60 mL used for wet-pretreated biomass and 100 mL for dried-pretreated biomass. Subsequently, 1.35 mL of cellulase enzyme was added, and the flasks were wrapped in aluminum foil and incubated in a shaker incubator at 45–50°C for three days. The hydrolysis conditions were based on the optimum requirements of cellulase as recommended by the National Institute of Molecular Biology and Biotechnology (BIOTECH),

UPLB. Following hydrolysis, enzyme activity was terminated by heating the mixture in a boiling water bath at 100°C for five minutes. The mixture was cooled, filtered, and centrifuged at 10,000 rpm for five minutes to separate residues from the liquid fraction. Fermentation was then carried out using the liquid fraction transferred into 500 mL Erlenmeyer flasks. Phosphate buffer solution was added to adjust the pH to 6.0, and 1% v/v Saccharomyces cerevisiae broth was inoculated based on the collected liquid volume. The flasks were wrapped in aluminum foil and incubated at 35°C for 72 hours. Bioethanol concentration was determined using Gas Chromatography (Shimadzu GC-2010) following the protocol of the National Institute of Molecular Biology and Biotechnology (BIOTECH) UPLB 2020. Ethanol standards with concentrations of 0.1%, 0.5%, 1%, 3%, and 5% were prepared using isopropanol as an internal standard. Experimental samples were centrifuged, diluted with isopropanol and distilled water, and thoroughly mixed. One microliter of each prepared sample was injected into the gas chromatograph. Ethanol concentration was determined using a standard calibration curve generated from the ethanol-to-isopropanol concentration ratio.

2.7 Data Analysis Techniques

Bioethanol yield and ethanol concentration served as the primary data for analysis. Gas Chromatography (Shimadzu GC-2010) was used to identify and quantify ethanol concentration in each sample. Ethanol concentration was determined by comparing sample peak ratios against the ethanol standard curve. The resulting concentration values were expressed as percentage volume per volume (% v/v) and converted into grams per liter (g/L) using ethanol density calculations. The bioethanol yields obtained from wet and dried pretreated biomass were compared to determine differences in production efficiency under the SHF process.

2.8 Ethical Considerations

The study complied with institutional and environmental ethical standards throughout the research process. Appropriate permissions were secured before collecting water hyacinth biomass to ensure compliance with environmental regulations and to avoid ecological disruption. The utilization of water hyacinth was intended to support responsible environmental management through the productive use of an invasive species. All laboratory activities were conducted at Green Future Innovations, Inc. following established laboratory safety protocols. Chemicals and reagents were properly handled, stored, and disposed of to ensure researcher safety and minimize environmental impact. Laboratory instruments, including the Gas Chromatography (Shimadzu GC-2010), were operated according to standard procedures to maintain accuracy and reliability. The study also adhered to principles of academic integrity through proper acknowledgment of sources, accurate data recording, and truthful reporting of findings without fabrication, falsification, or misrepresentation.

3. Results and Discussion

3.1 Bioethanol Production from Wet and Dried Pretreated Water Hyacinth Using SHF

3.1.1 Ethanol Yield from Wet and Dried Pretreated Biomass

The analysis using Gas Chromatography (Shimadzu GC-2010) showed that wet pretreated matured leaves of *Eichhornia crassipes* produced an average bioethanol yield of 3.870 g/L, while dried pretreated biomass produced 2.396 g/L. The results indicate that wet biomass consistently generated higher ethanol concentrations than dried biomass, demonstrating the influence of biomass condition on SHF performance. The higher yield obtained from wet biomass may be attributed to the retention of natural moisture, which enhances enzyme-substrate interaction and improves cellulose accessibility during hydrolysis. Moisture also supports nutrient diffusion and microbial activity during fermentation, leading to more efficient sugar conversion into ethanol. In contrast, drying may alter the lignocellulosic structure, increasing rigidity and reducing enzyme penetration, thereby limiting sugar release and ethanol production. These findings are comparable with the work of Sornvoraweat et al. (2010), who reported a maximum ethanol yield of 3.42 g/L from acid-pretreated water hyacinth using SHF. The results further support the viability of SHF for converting *Eichhornia crassipes* into renewable biofuel and demonstrate the importance of biomass condition in optimizing production efficiency.

3.1.2 Consistency of Bioethanol Yield Across Experimental Trials

Across three experimental trials with two replicates each, wet biomass yielded ethanol concentrations ranging from 3.4307 g/L to 4.3588 g/L, whereas dried biomass produced values ranging from 1.9634 g/L to 2.8144 g/L. The computed mean yields were 3.8708 g/L for wet biomass and 2.3963 g/L for dried biomass. Replicate values within each trial were relatively close, indicating consistency in the experimental procedure and reproducibility of the results. The observed consistency demonstrates the reliability of the SHF process for bioethanol production from *Eichhornia crassipes*. Minor variations among trials may be associated with natural differences in biomass composition and slight fluctuations during hydrolysis and fermentation. Nevertheless, the overall trend remained unchanged, with wet biomass consistently outperforming dried biomass. The enhanced performance of wet biomass supports previous findings that moisture facilitates enzymatic hydrolysis and microbial fermentation, while drying may reduce porosity and sugar availability by altering the lignocellulosic structure (Kumar et al., 2011; Taherzadeh & Karimi, 2008). Furthermore, dried biomass may not completely regain its original properties after rehydration, resulting in reduced fermentation efficiency (Zabed et al., 2017; Sun &

Cheng, 2002). The suitability of water hyacinth as a lignocellulosic feedstock is also supported by its high cellulose content and rapid biomass accumulation (Rezania et al., 2016; Ruan et al., 2016).

3.2 Statistical Comparison of Bioethanol Yield Between Wet and Dried Biomass

3.2.1 Significance of Biomass Condition on Ethanol Production

Statistical analysis revealed that wet biomass produced a mean ethanol yield of 3.9588 g/L, while dried biomass yielded 2.4829 g/L. The variance values for both treatments were low, indicating that the data were closely clustered around their respective means and that the experimental outputs were stable across replicates. The computed p-value of 4.09654×10^{-5} was substantially lower than the 0.05 level of significance. The statistical result indicates a significant difference between wet and dried pretreated biomass in terms of ethanol production, leading to the rejection of the null hypothesis. This finding confirms that biomass condition significantly affects bioethanol yield when using SHF. The superior performance of wet biomass can be explained by the role of moisture in facilitating cellulose breakdown and supporting microbial fermentation. Moisture enhances enzyme accessibility and sugar release, while drying can increase biomass rigidity and reduce substrate availability (Kumar et al., 2011; Taherzadeh & Karimi, 2008). Adequate moisture also promotes efficient microbial metabolism during fermentation, whereas rehydrated dried biomass may not fully recover its original structure (Zabed et al., 2017; Sun & Cheng, 2002). These findings provide strong evidence that wet pretreated biomass is more suitable for maximizing ethanol yield from *Eichhornia crassipes* through SHF.

3.3 Development of an Experimental Protocol Procedure as Basis for Instructional Laboratory Manual

Based on the findings of the study, an Experimental Protocol Procedure (EPP) for the analysis of bioethanol production from matured leaves of Eichhornia crassipes through the Separate Hydrolysis and Fermentation (SHF) process was developed as the basis for an instructional laboratory manual. The protocol was designed to translate the validated experimental procedures into a structured learning resource that promotes experiential, inquiry-based, and sustainability-oriented learning in chemistry and science education. The developed instructional laboratory manual consists of five major components: (1) learning objective, (2) chemicals, materials, and equipment, (3) experimental procedures, (4) data analysis and interpretation, and (5) reporting and reflection activities. The chemicals, materials, and equipment included fresh or dried water hyacinth biomass, sulfuric acid, cellulase enzyme, Saccharomyces cerevisiae, distilled water, fermentation vessels, pH meter, autoclave, pipettes, graduated cylinders, thermometer, centrifuge, analytical balance, gas chromatograph, and other laboratory supplies necessary for conducting the experiment. These components were systematically organized to provide learners with a comprehensive understanding of bioethanol production while developing laboratory, analytical, and scientific process skills. The experimental protocol consists of seven major stages: (1) preparation of wet and dried water hyacinth biomass, (2) biomass pretreatment, (3) enzymatic hydrolysis, (4) fermentation, (5) ethanol quantification, (6) data analysis, and (7) reporting. During biomass preparation, fresh water hyacinth was collected, thoroughly washed, chopped into approximately one-inch pieces, and oven-dried at 60°C until a moisture content of approximately 10–15% was achieved. The dried biomass was subsequently powdered and sieved to obtain a 1-mm substrate size. For wet-pretreated biomass, the substrate was rehydrated by soaking in water for 24 hours, whereas dried-pretreated biomass was directly utilized for subsequent processing. Biomass pretreatment was performed by accurately weighing 20 g of wet and dried biomass and mixing each sample with 200 mL of 2% sulfuric acid. The mixtures were autoclaved at 121°C and 15 psi for 15–20 minutes to facilitate biomass breakdown and improve cellulose accessibility. Following pretreatment, the biomass was cooled, filtered, and rinsed with distilled water until clear washings were obtained. For enzymatic hydrolysis, 20 g of pretreated biomass was transferred into a 500 mL Erlenmeyer flask and adjusted to a pH range of 6.0–6.5 using a phosphate buffer solution. Subsequently, 1.35 mL of cellulase enzyme was added, and the flasks were incubated at 45–50°C for three days to convert cellulose into fermentable sugars. The resulting hydrolysate was subjected to fermentation through inoculation with Saccharomyces cerevisiae under controlled conditions for approximately 72 hours. This process facilitated the microbial conversion of fermentable sugars into bioethanol. Upon completion of fermentation, the fermented broth was centrifuged to separate the liquid fraction from residual biomass. Ethanol quantification was then performed using Gas Chromatography (GC), wherein the supernatant was analyzed to determine ethanol concentration and bioethanol yield. The resulting data were subsequently used to calculate ethanol productivity and evaluate bioethanol production efficiency. Statistical analyses were performed to determine the significance of differences between treatment conditions. The final stage involved the preparation of a comprehensive laboratory report containing the experimental procedures, results, statistical analyses, and interpretation of findings. Learners were encouraged to discuss the implications of bioethanol production from water hyacinth as a renewable energy source and its potential contribution to environmental sustainability. From an educational perspective, each stage of the protocol contributes to the development of specific scientific competencies. Biomass preparation and pretreatment introduce learners to renewable biomass utilization and feedstock processing. Enzymatic hydrolysis and fermentation provide opportunities to investigate biochemical reactions, enzyme-substrate interactions, and microbial metabolism involved in biofuel production. Ethanol quantification develops analytical and instrumental competencies through the use of Gas Chromatography, while data analysis and reporting strengthen critical thinking, scientific reasoning, interpretation of findings, and evidence-based communication skills. The instructional laboratory manual may be viewed as an integrated learning framework in which scientific concepts, laboratory techniques, analytical procedures, and sustainability principles

interact to promote meaningful learning experiences. The progression from biomass preparation to ethanol analysis allows learners to observe the complete conversion of an invasive aquatic plant into a renewable energy product, thereby connecting theoretical knowledge with authentic environmental and technological applications. Consequently, the developed Experimental Protocol Procedure serves not only as a validated guide for the production and analysis of bioethanol from Eichhornia crassipes through SHF but also as a pedagogical tool that supports experiential learning, contextualized science instruction, environmental awareness, sustainability education, and the development of scientific competencies relevant to chemistry and science education.

4. Conclusion and Recommendations

This study concluded that bioethanol can be successfully produced from matured leaves of water hyacinth using the Separate Hydrolysis and Fermentation process, with wet pretreated biomass producing a higher average yield of 3.870 g/L than dried pretreated biomass at 2.396 g/L. The significant difference in yield indicates that biomass condition influences ethanol production efficiency, with wet biomass performing better due to retained moisture that supports enzymatic hydrolysis and microbial fermentation. The findings also confirmed the feasibility of using water hyacinth as a renewable lignocellulosic feedstock while providing a basis for developing an instructional laboratory manual that promotes experiential learning, scientific skills, and awareness of renewable energy technologies.

It is recommended that future studies further optimize enzyme loading, pH, temperature, and fermentation time to improve bioethanol yield from water hyacinth using Separate Hydrolysis and Fermentation. Alternative pretreatment methods, different microbial strains, and engineered yeast may also be explored to enhance ethanol production. The developed instructional laboratory manual may be implemented and validated by science instructors and educational institutions, integrated into science curricula by relevant education agencies, and considered by energy, environmental, and private sector stakeholders for pilot-scale applications using wet pretreated water hyacinth biomass.

The study was limited to the production of bioethanol from matured leaves of water hyacinth using wet and dried pretreated biomass under controlled laboratory conditions. It focused only on the Separate Hydrolysis and Fermentation process, ethanol yield comparison, and the development of an instructional laboratory manual. The study did not include other plant parts, large-scale production, alternative pretreatment methods, other microbial strains, or extensive optimization of process parameters beyond those used in the experiment.

Compliance with ethical standards

The authors declare no conflict of interest and report that this study received no external funding. The research was conducted at Green Future Innovations, Inc. using non-invasive laboratory procedures involving water hyacinth (*Eichhornia crassipes*), with all experimental activities performed in accordance with established safety, environmental, and academic integrity standards. As no human participants were involved, voluntary participation and informed consent were not applicable; consequently, no identifying personal information was collected or disclosed. The authors acknowledge Green Future Innovations, Inc. for providing the facilities and equipment necessary for the conduct of the study.

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