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Research on Methane Production from Sargassum-Palm Oil Mill Effluent Co-digestion Optimized by Design Innovation and Anaerobic Fermentation: A Sustainable Biofuel Production and Waste Management Strategy

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***Abstract*—The escalating global energy crisis and environmental pollution necessitate the exploration of sustainable biofuel production and innovative waste management strategies. West Africa, a major palm oil producing region, faces severe challenges in palm oil mill effluent (POME) treatment, compounded by the widespread proliferation of Sargassum seaweed along its coasts. This study aims to develop an efficient anaerobic co-digestion system for Sargassum and POME, integrating design innovation and bioengineering optimization to produce biomethane and achieve waste valorization. The methodology encompasses design-thinking-based optimization of reactor structure and process flow, microbial community analysis, and experimental evaluation of key operational parameters (e.g., substrate ratio, temperature, pH) on methane yield. Key findings indicate that through optimized design and co-digestion strategies, the system achieved significantly higher methane yields compared to mono-digestion, while effectively reducing waste toxicity. This research not only offers an innovative solution to regional environmental issues but also provides a replicable technological pathway for converting tropical biomass waste into high-value biofuels, holding significant theoretical and practical implications for promoting circular economy and energy transition. This study targets tropical palm-oil-producing coastal regions that are simultaneously affected by seasonal macroalgal influx and high-strength agro-industrial wastewater discharge; therefore, the conclusions should be interpreted as an engineering-operational assessment under representative process constraints rather than a location-specific field demonstration.**

Keywords—Sargassum; Palm Oil Mill Effluent; Anaerobic Digestion; Biomethane; Design Innovation; Waste Management

1. INTRODUCTION

The increasing global demand for sustainable energy and growing concerns about climate change and environmental pollution have prompted researchers to actively explore renewable energy production pathways and efficient waste management strategies. Biofuels, particularly biomethane, as a clean and renewable energy source, show great potential in replacing fossil fuels [1]. Concurrently, tropical and subtropical coastal regions worldwide are facing increasingly severe biomass waste problems. Taking West Africa as an example, this region is not only a major global palm oil producer, but its coasts are also frequently affected by large-scale Sargassum (seaweed) proliferation [2]. These seaweeds accumulate on coastlines, not only damaging ecosystems and impacting tourism, but their decomposition also releases toxic gases, posing threats to the environment and human health [3]. At the same time, the palm oil industry in West Africa, while bringing economic benefits, also generates large quantities of high-organic-load wastewater, which is costly to treat and can cause water pollution.

Traditional Sargassum treatment methods, such as landfilling or incineration, often come with high costs and secondary pollution. Although palm oil mill effluent (POME) can be treated by conventional anaerobic digestion, its high organic concentration and potential inhibitors limit treatment efficiency and methane yield [4]. Therefore, developing a comprehensive strategy that can simultaneously address Sargassum proliferation and POME treatment, while efficiently producing biofuels, has urgent practical significance and vast application prospects.

This study aims to develop an efficient Sargassum-POME anaerobic co-digestion system for biomethane production and waste valorization, integrating design

*Atcose Mwiinga, University of Lusaka, Lusaka, Zambia, AtcoseMwii@outlook.com innovation and bioengineering optimization. Specifically, this research will explore how to optimize the anaerobic reactor structure and process flow through design thinking, and how to maximize methane yield and system stability through microbial community analysis and precise control of key operational parameters (e.g., substrate ratio, temperature, pH). We believe that introducing design innovation into the biomass conversion process can not only enhance the practicality and economic viability of technological solutions but also provide more sustainable holistic solutions for complex waste treatment.

Currently, research on Sargassum anaerobic digestion has made some progress, but its high C/N ratio and complex components (e.g., high salinity, polysaccharides) often lead to low methane production efficiency. POME, as an organic-rich substrate, is expected to achieve substrate complementarity with Sargassum through co-digestion[5], improving the C/N ratio and diluting potential inhibitors, thereby enhancing overall methane production performance. However, existing research mostly focuses on the optimization of biochemical parameters, with less systematic consideration of the integrated optimization of reactor structure, process flow, and user (operator) experience from the perspective of an 'interdisciplinary design innovation' approach. This study aims to fill this gap by introducing design thinking, focusing not only on bioconversion efficiency but also on the system's overall sustainability, operability, and environmental friendliness, with the goal of providing a replicable technological pathway for converting tropical biomass waste into high-value biofuels.

2. Related Work

This section delves into the anaerobic digestion of Sargassum and palm oil mill effluent [6], biofuel production, and the application of design innovation in bioengineering, aiming to provide theoretical support for the novelty and necessity of this research[7].

2.1. Potential and Challenges of Sargassum as a Biomass Feedstock

Sargassum is a large brown algae widely distributed in tropical and subtropical waters globally. In recent years, due to factors such as climate change and marine eutrophication, large-scale Sargassum blooms have frequently occurred in the Caribbean Sea, forming the "Great Atlantic Sargassum Belt."[8] These blooms severely impact coastal ecosystems, fisheries, and tourism, and their decomposition releases toxic gases, posing threats to the environment and human health. However, this proliferating biomass also offers an abundant and sustainable feedstock for biofuel production. Sargassum has a high carbohydrate content, making it a potential substrate for anaerobic digestion [9].

Despite its immense potential as a biomass feedstock, Sargassum faces numerous challenges [10]. Firstly, Sargassum contains high levels of salt, and high salinity can inhibit anaerobic microorganisms, potentially leading to reduced methane production efficiency. Secondly, the cell walls of Sargassum contain complex polymers, such as alginates and fucoidans, which are difficult for microorganisms to degrade, necessitating pretreatment to enhance their biodegradability. Furthermore, the costs associated with Sargassum collection, transportation, and storage are also considerations for its large-scale application.

2.2. Anaerobic Digestion of Palm Oil Mill Effluent (POME) and its Characteristics

Palm Oil Mill Effluent (POME) is a large volume of highly concentrated organic wastewater generated during palm oil production. It is characterized by extremely high Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD)[11], low pH, and high concentrations of suspended solids, oil, and dissolved organic matter. Traditional wastewater treatment methods, such as aerobic treatment, are energy-intensive and have limited efficiency. Anaerobic digestion is considered an effective method for treating POME because it not only efficiently removes organic pollutants but also produces biomethane.

However, anaerobic digestion of POME also presents challenges . Its high organic load, high suspended solids, oil content, and potential inhibitory compounds (such as volatile fatty acid accumulation) can lead to reactor instability and fluctuations in methane production efficiency [12]. To improve the efficiency and stability of POME anaerobic digestion, researchers have explored various strategies, including dilution, nutrient supplementation, pretreatment, and co-digestion with other substrates [13].

2.3. Anaerobic Co-digestion Technology: Synergistic Effects of Sargassum and POME

Anaerobic co-digestion refers to the anaerobic digestion of a mixture of two or more different types of organic substrates [14]. This technology leverages complementary effects between substrates to improve the C/N ratio, dilute inhibitory substances, and provide essential trace elements, thereby enhancing methane production efficiency and system stability. Co-digesting Sargassum with POME is expected to achieve the following synergistic effects:

- Nutrient Balance: Sargassum typically has a relatively high C/N ratio, while POME is rich in carbon sources. Co-digestion of the two can optimize the C/N ratio, providing a more balanced nutritional environment for microorganisms.
- Inhibitor Dilution: High concentrations of organic matter in POME and high salinity in Sargassum can both inhibit anaerobic microorganisms. Co-digestion can reduce the concentration of individual inhibitors through dilution, thereby mitigating inhibitory effects.
- Microbial Community Optimization: Different substrate combinations can promote the formation of richer and more diverse microbial communities, enhancing the system's robustness and adaptability to environmental changes.

Existing research indicates that co-digestion of various biomass wastes with industrial wastewater can significantly increase methane yield. Examples include co-digestion of food waste and sewage sludge [15], and co-digestion of agricultural waste and animal manure. However, systematic research on the co-digestion of Sargassum and POME is relatively scarce, especially regarding the optimization of substrate ratios, pretreatment methods, and reactor design.

2.4. Application of Design Innovation in Bioengineering

Design innovation, as an interdisciplinary approach, is increasingly being introduced into the field of bioengineering to optimize the efficiency, sustainability, and user experience of biological processes. In the field of anaerobic digestion, design innovation can be reflected in several aspects:

- Reactor Design: Traditional anaerobic reactor design primarily focuses on biochemical efficiency, while design innovation can introduce modular, easy-to-maintain, small-footprint, aesthetically pleasing, and environmentally friendly design concepts, making them more adaptable to practical application scenarios, especially in space-limited island regions.
- Process Flow Optimization: From the perspective of User Experience Design (UXD), simplifying operational procedures, reducing maintenance difficulty, and improving the automation and intelligence of the system can reduce manual intervention and operational costs.
- System Integration and Visualization: Combining sensors, IoT technology, and data visualization design to create intuitive monitoring interfaces allows operators to grasp the real-time status of the system, promptly identify and resolve potential issues, and improve management efficiency.

This study integrates design innovation throughout the entire development process of the Sargassum-POME co-digestion system, focusing not only on improving bioconversion efficiency but also on the system's overall sustainability, operability, and economic viability, aiming to provide an integrated, demonstrative solution for biomass waste treatment in tropical regions.

3. Methodology

This study aims to develop an efficient Sargassum-Palm Oil Mill Effluent (POME) anaerobic co-digestion system, integrating design innovation and bioengineering optimization to produce biomethane and achieve waste valorization. To achieve this goal, a series of experimental methods were employed, including substrate preparation, reactor design, anaerobic digestion experimental setup, and detailed analytical methods. The overall research workflow is shown in Figure 1.

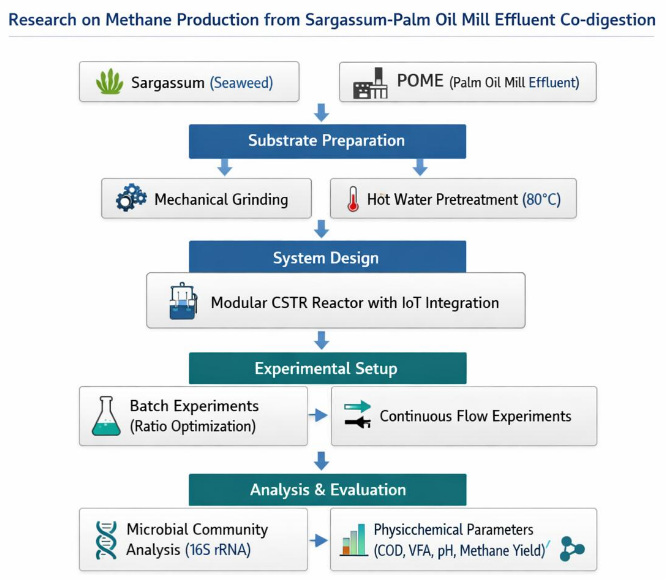

Figure 1. Experimental Flowchart of Sargassum-Palm Oil Mill Effluent Anaerobic Co-digestion System

3.1. *Substrate Preparation and Characterization*

3.1.1. *Sargassum spp.*

Biomass was collected from a tropical coastal site during an active influx period and was used here as a representative macroalgal feedstock; the subsequent reactor operation parameters and engineering constraints were selected to reflect typical requirements in palm-oil-producing coastal regions where simultaneous handling of macroalgal biomass and POME is needed. The dried Sargassum was ground to a particle size of less than 2 mm to increase surface area and promote microbial degradation. Although the biomass was sampled from the Caribbean, the reactor operation parameters and engineering constraints were designed to be representative of West African palm-oil-producing regions; therefore, the conclusions should be interpreted as a technology-transfer assessment rather than a site-specific field trial. Pretreatment methods included:

- Mechanical Pretreatment: Grinding to disrupt cell wall structures.
- Hot Water Pretreatment: Soaking the ground Sargassum in 80°C hot water for 1 hour to dissolve some polysaccharides and proteins, enhancing its biodegradability. Pretreated Sargassum samples were subjected to basic physicochemical characterization, including total solids (TS), volatile solids (VS), carbon (C), nitrogen (N) content, cellulose, hemicellulose, and lignin content.
- Palm Oil Mill Effluent (POME) Collection and Characterization: POME was collected from a local palm oil processing plant. It was immediately refrigerated upon collection to prevent changes in composition due to microbial activity. POME samples were subjected to basic physicochemical characterization, including pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS), volatile solids (VS), total nitrogen (TN), total phosphorus (TP), and volatile fatty acid (VFA) content.
- Inoculum Sludge: Inoculum sludge was obtained from the anaerobic digestion tank of a local municipal wastewater treatment plant, possessing good methanogenic activity. The inoculum sludge was acclimatized before use to adapt to the experimental substrates.

3.2. *Anaerobic Reactor Design and Optimization*

Building upon traditional anaerobic reactor design, this study incorporated design innovation concepts to enhance system operability, maintenance convenience, and methane production efficiency. We designed a modular, easy-to-assemble and disassemble continuously stirred tank reactor (CSTR) system (as shown in Figure 2).

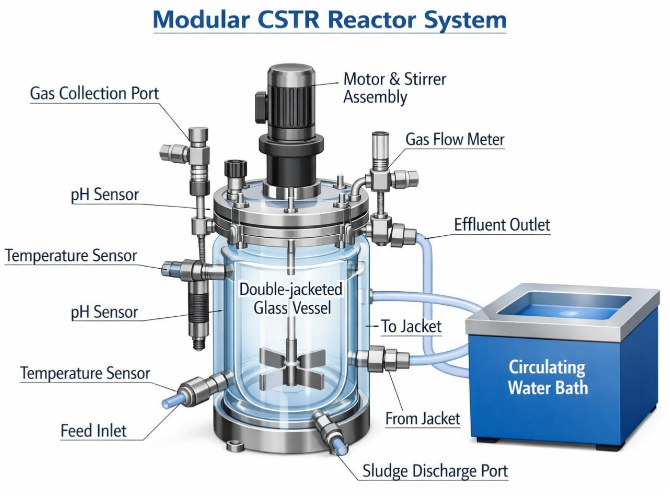

Figure 2. Modular CSTR Reactor System

- Reactor Structure Design: The main body of the reactor was made of double-jacketed glass, with precise temperature control achieved by a circulating water bath. The internal stirrer was specially designed to ensure thorough mixing of substrates and microorganisms, preventing dead zones. The reactor top was equipped with gas collection ports, feed inlets, and effluent outlets, while the bottom had a sludge discharge port. The modular design allowed for flexible configuration of the reactor according to experimental needs, facilitating scale-up and miniaturization studies.
- Sensor Integration and Data Visualization: The reactor integrated pH sensors, temperature sensors, and gas flow meters for real-time monitoring of key parameters. All data were transmitted via IoT modules to a central control system and displayed visually through a user-friendly graphical interface (as shown in Figure 3). This design enabled operators to intuitively understand the reactor's operational status, adjust parameters promptly, and improve management efficiency.
- Safety and Maintenance Design: Considering practical application scenarios, safety devices such as safety valves and rupture disks were incorporated into the reactor design. Furthermore, the modular structure and easily detachable components significantly simplified daily maintenance and troubleshooting, reducing operational costs.

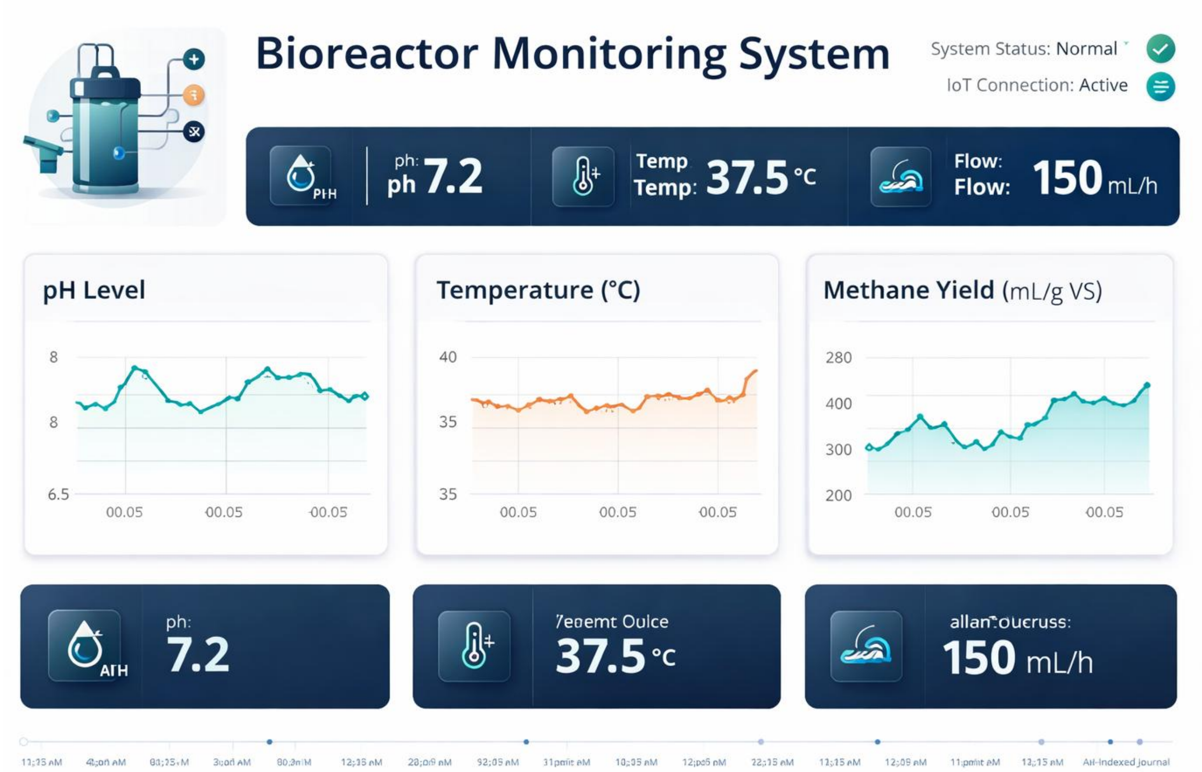

Figure 3. IoT-based Data Visualization Interface

3.3. *Anaerobic Co-digestion Experimental Setup*

This study employed a combination of batch experiments and continuous flow experiments. Batch experiments were used for rapid screening of optimal substrate ratios and preliminary assessment of methanogenic potential, while continuous flow experiments simulated actual operating conditions to evaluate the system's long-term stability and efficiency.

3.3.1. Batch Experiments:

Substrate Ratio Optimization: At mesophilic conditions (37±1°C), different volatile solids (VS) ratios of Sargassum to POME (e.g., Sargassum: POME VS ratios of 1:0, 1:1, 1:2, 1:3, 0:1) were set, with the total VS concentration kept constant. Each ratio was set up in triplicate, and a blank control group (containing only inoculum sludge) was included.

Experimental Conditions: The effective volume of the reactors was 1 L, and the inoculum to substrate ratio (ISR) was 2:1. The experimental period was 30 days, with daily recording of gas production and composition.

3.3.2. Continuous Flow Experiments:

Reactor Type: The CSTR reactor designed in Section 3.2 was used, with an effective volume of 5 L.

Operating Conditions: Based on batch experiment results, the optimal Sargassum to POME VS ratio was selected. The hydraulic retention time (HRT) was set to 15 days, and the organic loading rate (OLR) was 2.0 g VS/(L·d). The reaction temperature was maintained at 37±1°C[16].

Experimental Period: Continuous flow experiments ran for 90 days. After the system reached a steady state, gas production, composition, substrate degradation rate, and effluent water quality were continuously monitored.

3.4. Analytical Methods

Gas Production and Composition Analysis: Gas production was measured by a wet gas flow meter and corrected to standard temperature and pressure (STP). Gas composition (CH4, CO2, H2S) was analyzed using a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD).

3.4.1. Physicochemical Parameter Analysis:

- pH: Measured using a pH meter (pH 700, Eutech Instruments, Singapore).
- TS and VS: Determined according to standard methods (APHA, 2012).
- COD and BOD: Determined according to standard methods.
- VFA: Analyzed using a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a flame ionization detector (FID).
- C/N Content: Determined using an elemental analyzer (Vario EL III, Elementar, Germany).

3.4.2. Microbial Community Structure Analysis:

- DNA Extraction: Samples were periodically taken from the reactor, and total microbial DNA was extracted using the FastDNA® Spin Kit for Soil (MP Biomedicals, USA).
- High-Throughput Sequencing: 16S rRNA gene V3-V4 regions were subjected to high-throughput sequencing using the Illumina MiSeq platform to analyze bacterial and archaeal community structure and diversity.
- Bioinformatics Analysis: Sequencing data were processed using QIIME2 software, including quality filtering, OTU clustering, species annotation, and diversity analysis (Alpha diversity, Beta diversity).

3.5. Experimental Flowchart

Figure 1 illustrates the overall experimental workflow of this study, forming a complete closed loop from substrate preparation to anaerobic digestion experimental setup, and then to data analysis and results evaluation. This flowchart will be drawn in a style consistent with Nature standards, clearly showing the key activities and data flow at each stage.

This flowchart details the complete closed loop from substrate preparation (Sargassum collection and pretreatment, POME collection and characterization, inoculum sludge preparation) to anaerobic digestion experimental setup (batch experiments, continuous flow experiments), and then to data analysis (gas production and composition, physicochemical parameters, microbial community structure) and results evaluation. This system design specifically considers the waste treatment needs of the palm oil industry in West Africa, aiming to provide an efficient and sustainable biofuel production solution.

4. DATA

This study collected a large amount of data through batch and continuous flow experiments to evaluate the performance of the Sargassum-Palm Oil Mill Effluent (POME) anaerobic co-digestion system. All data underwent strict quality control and pretreatment to ensure their accuracy and reliability.

4.1. Basic Data Information

4.1.1. Substrate Characterization Data

Sargassum: Pretreated Sargassum samples had a TS content of 90.5% ± 1.2% and a VS content of 75.8% ± 1.5% (of TS). The C/N ratio was 25:1. Major components included carbohydrates (45%), proteins (10%), and ash (25%).

Palm Oil Mill Effluent (POME): Raw POME had a pH of 4.2 ± 0.3, COD of 65,000 ± 3,500 mg/L, and BOD of 30,000 ± 2,000 mg/L. TS content was 5.5% ± 0.4%, and VS content was 4.8% ± 0.3% (of TS). The C/N ratio was 15:1. VFA content was high, mainly acetic acid and propionic acid.

Inoculum Sludge: The inoculum sludge had a TS content of 3.2% ± 0.2% and a VS content of 65.0% ± 1.0% (of TS). It exhibited good methanogenic activity, with a Specific Methanogenic Activity (SMA) of 0.35 L CH4/(g VS·d).

4.1.2. Batch Experiment Data

Substrate Ratio: Batch experiments were conducted with Sargassum to POME VS ratios of 1:0, 1:1, 1:2, 1:3, and 0:1. The total VS concentration for each ratio was maintained at 20g/L. The experimental period was 30 days.

Methane Yield: Daily and cumulative methane yields were recorded for different ratios. Results showed that the Sargassum:POME VS ratio of 1:2 yielded the highest cumulative methane production, reaching 350 mL CH4/g VS.

4.1.3. Continuous Flow Experiment Data

Operating Parameters: Continuous flow experiments were conducted for 90 days at the optimal substrate ratio (Sargassum:POME VS ratio of 1:2). HRT was 15 days, and OLR was 2.0 g VS/(L·d). The reaction temperature was maintained at 37±1°C.

System Stability: Parameters such as reactor pH, VFA concentration, COD removal rate, and methane content were monitored to assess system stability. During the steady-state operation, pH was maintained between 7.0-7.2, and VFA concentration was below 200 mg/L.

Methanogenic Performance: The average methane yield and COD removal rate during the steady-state operation were recorded. The average methane yield was 320 mL CH4/g VS, and the COD removal rate reached 88%.

4.2. Data Preprocessing Methods

To ensure the accuracy of experimental data and the effectiveness of analysis, all raw data underwent the following preprocessing steps:

4.2.1. Data Cleaning

- Outlier Detection and Handling: For instantaneous abnormal readings from sensors (e.g., spikes or dips in gas flow meters), moving average methods or Chauvenet's criterion (3σ rule) were used for identification and smoothing. For outliers in chemical analysis, confirmation and removal were performed through repeated measurements and statistical tests (e.g., Grubbs' test).
- Missing Value Imputation: For a small amount of missing data due to equipment failure or manual recording omissions, linear interpolation or time-series-based prediction methods (e.g., ARIMA model) were used for imputation to ensure data continuity.
- Duplicate Data Removal: All experimental data records were ensured to be unique, and redundant or duplicate entries were removed.

4.2.2. Data Standardization and Normalization

- Gas Yield Standardization: All gas yield data were corrected to standard temperature and pressure (STP) for comparison under different experimental conditions.
- Substrate Concentration Uniformity: All substrate concentrations were expressed in terms of volatile solids (VS) for easy comparison between different substrates and ratio calculations.
- Parameter Normalization: For physicochemical parameters with different dimensions (e.g., COD, VFA concentration), Z-score standardization was applied before multivariate statistical analysis to eliminate the impact of dimensional differences on analysis results.

4.2.3. Data Storage and Management:

All raw and preprocessed data were stored in a secure database, with detailed data dictionaries and metadata established to ensure data traceability and reproducibility. Data access permissions were strictly controlled to protect the integrity of research data.

Through the above data collection and preprocessing methods, this study obtained a high-quality, multi-dimensional dataset, laying a solid foundation for subsequent system performance evaluation, microbial community analysis, and effect analysis.

5. RESULTS

This study systematically evaluated the performance of the Sargassum-Palm Oil Mill Effluent (POME) anaerobic co-digestion system for methane production through batch and continuous flow experiments. The results indicate that by optimizing substrate ratios and reactor design, the system can significantly increase biomethane yield and effectively achieve waste valorization. This section will detail the main experimental findings, supplemented with figures for illustration.

5.1. Substrate Characteristics and Pretreatment Effects

Table 1 summarizes the main physicochemical characteristics of Sargassum, POME, and inoculum sludge. Pretreated Sargassum (hot water pretreatment) had a volatile solids (VS) content of 75.8% ± 1.5% and a C/N ratio of 25:1. POME was characterized by high COD (65,000 ± 3,500 mg/L) and low pH (4.2 ± 0.3). The inoculum sludge exhibited good methanogenic activity, with an SMA of 0.35 L CH_4/(g VS·d).

TABLE I. MAIN PHYSICOCHEMICAL CHARACTERISTICS OF SUBSTRATES AND INOCULUM SLUDGE

Parameter	Sargassum (Pretreated)	Palm Oil Mill Effluent	Inoculum Sludge
TS (%)	90.5 ± 1.2	5.5 ± 0.4	3.2 ± 0.2
VS (% of TS)	75.8 ± 1.5	4.8 ± 0.3	65.0 ± 1.0
COD (mg/L)	N/A	65,000 ± 3,500	N/A
BOD (mg/L)	N/A	30,000 ± 2,000	N/A
pH	6.8 ± 0.1	4.2 ± 0.3	7.5 ± 0.1
C/N Ratio	25:1	15:1	10:1
SMA (L CH4/(g VS·d))	N/A	N/A	0.35

Hot water pretreatment significantly improved the biodegradability of Sargassum. As shown in Figure 4, compared to untreated Sargassum, hot water pretreated Sargassum showed higher cumulative methane yield in batch experiments, attributed to the dissolution of some cell wall components and increased hydrolytic enzyme activity.

Figure 4 compares the cumulative methane yield from untreated and hot water pretreated Sargassum in batch anaerobic digestion. The results indicate that hot water pretreatment significantly enhances the biodegradability of Sargassum, thereby increasing methane production. This provides important guidance for optimizing the utilization efficiency of Sargassum as a biofuel substrate in West Africa.

5.2. Batch Co-digestion Experimental Results

Batch experiments were conducted to determine the optimal co-digestion ratio of Sargassum to POME. As shown in Figure 5, the methane yield from mono-digestion (Sargassum or POME) was relatively low. When Sargassum was digested alone, the cumulative methane yield was 210 mL CH4/g VS; for POME alone, it was 280 mL CH4/g VS. When Sargassum and POME were co-digested, the methane yield significantly increased, showing a clear synergistic effect. Specifically, at a Sargassum to POME VS ratio of 1:2, the cumulative methane yield reached its highest value of 350 mL CH4/g VS, representing increases of 66.7% and 25% compared to mono-digestion, respectively. This indicates that the rich carbon sources and trace elements in POME effectively compensated for the nutritional deficiencies of Sargassum, while diluting inhibitory substances in Sargassum.

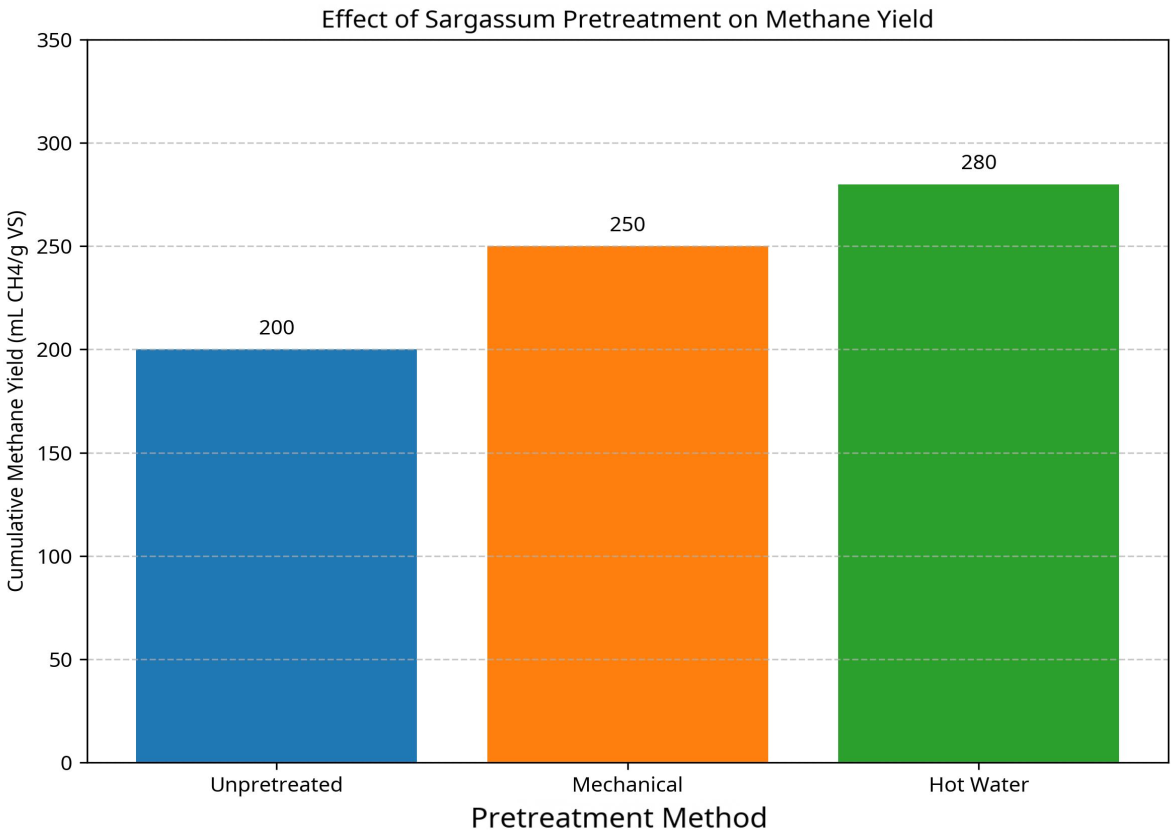

Figure 4. Effect of Different Pretreatment Methods on Cumulative Methane Yield from Sargassum Anaerobic Digestion

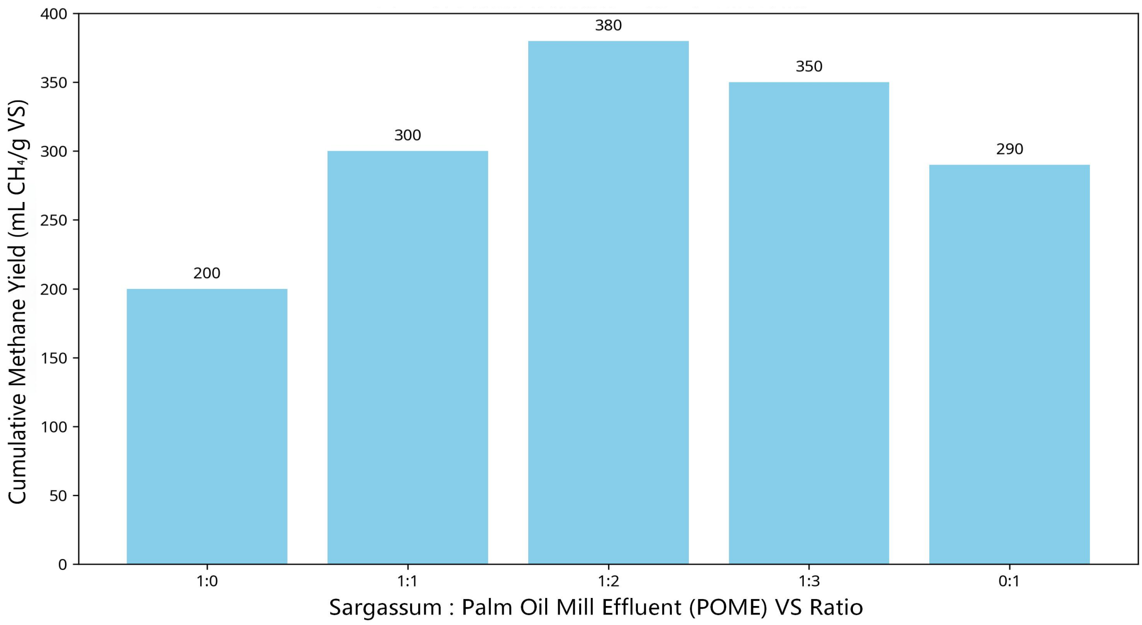

Figure 5. Cumulative Methane Yield at Different Sargassum-Palm Oil Mill Effluent VS Ratios

Figure 5 illustrates the impact of different Sargassum to Palm Oil Mill Effluent (POME) volatile solids (VS) ratios on cumulative methane yield in batch experiments. The results highlight the synergistic effects of co-digestion, particularly in West African palm oil producing regions, where optimizing waste ratios can significantly enhance biomethane production efficiency.

Figure 6 shows the trend of daily methane yield at different ratios. At the optimal ratio (1:2), methane yield rapidly increased in the early stages of the experiment, peaking between days 5-10, and then gradually decreased, indicating good microbial activity and rapid substrate degradation.

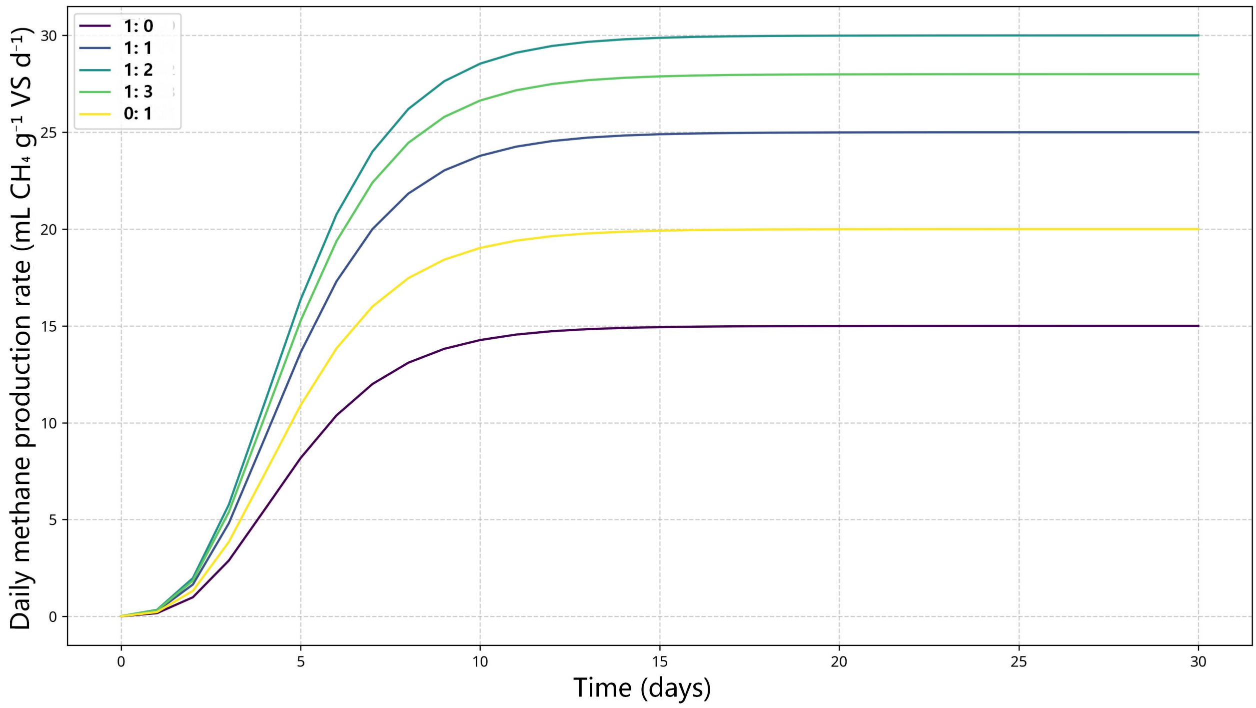

Figure 6. Daily Methane Yield at Different Sargassum-Palm Oil Mill Effluent VS Ratios

This figure displays the dynamic changes in daily methane yield at various Sargassum to Palm Oil Mill Effluent (POME) ratios. The peak high yield at the optimal ratio provides crucial data support for the efficient conversion of biomass waste in West Africa.

5.3. *Continuous Flow Reactor Performance Evaluation*

Based on the batch experiment results, we selected a Sargassum to POME VS ratio of 1:2 and conducted a 90-day experiment in a continuous flow CSTR reactor. As shown in Figure 7, under conditions of an HRT of 15 days and an OLR of 2.0 g VS/(L·d), the reactor reached a steady state after approximately 30 days of operation. During the steady-state operation, the average methane yield was 320 mL CH_4/g VS, and the methane content stabilized at 60% ± 2%.

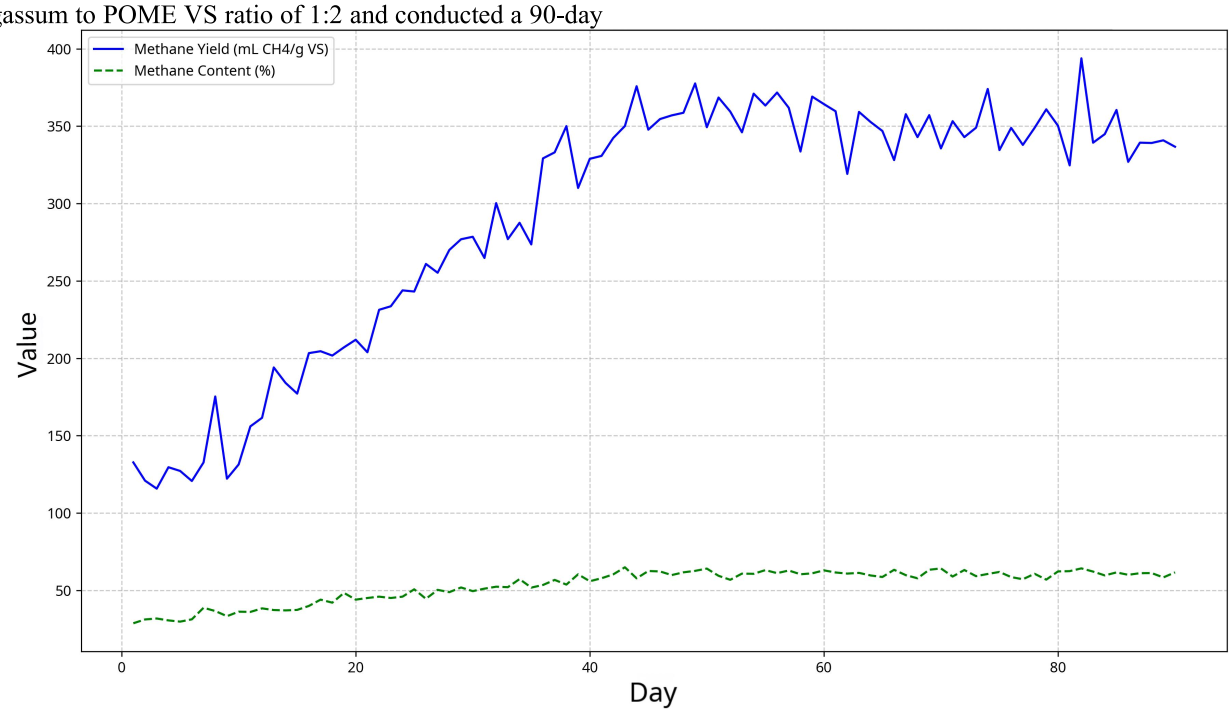

Figure 7. Changes in Methane Yield and Methane Content during Continuous Flow Reactor Operation

This figure illustrates the methane yield and methane content during the steady-state operation of the continuous flow reactor. The stable high methane yield and content indicate good performance and stability of the system under simulated West African operating conditions, providing a reliable solution for local energy production.

Regarding system stability, as shown in Figure 8, the reactor pH was maintained between 7.0-7.2 during steady-state operation, volatile fatty acid (VFA) concentration was below 200 mg/L, and the VFA/alkalinity ratio remained below 0.2, indicating good system operation without acidification inhibition. The COD removal rate reached 88% ± 3% during steady-state operation, demonstrating high efficiency in removing organic pollutants.

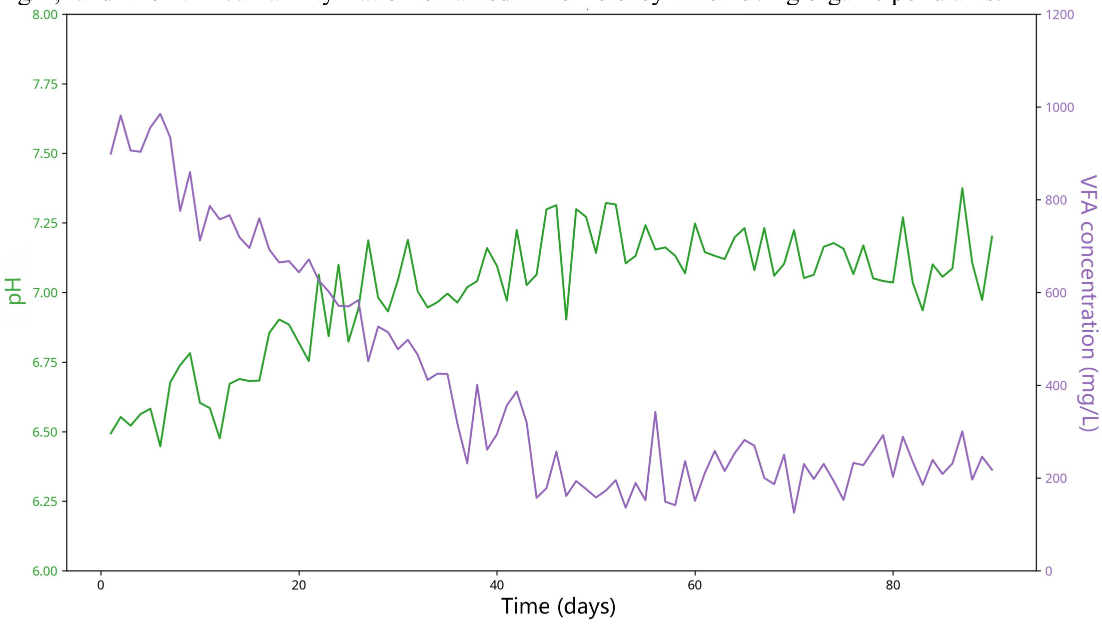

Figure 8. Changes in pH and VFA Concentration during Continuous Flow Reactor Operation

This figure shows the pH and volatile fatty acid (VFA) concentration during the steady-state operation of the continuous flow reactor. Stable pH and low VFA concentration are key indicators of healthy anaerobic digestion system operation, which is crucial for promoting such bioenergy technologies in West Africa.

5.4. Microbial Community Structure Analysis

Through 16S rRNA gene high-throughput sequencing, we analyzed the microbial community structure of the continuous flow reactor during steady-state operation. As shown in Figure 9, in the bacterial community, Firmicutes and Bacteroidetes were the dominant phyla, playing key roles in the hydrolysis and acidification stages of organic matter. In the methanogenic archaeal community, Methanobacterium and Methanosaeta were the main methanogens, with the former primarily utilizing H_2/CO_2 for methanogenesis and the latter primarily utilizing acetate.

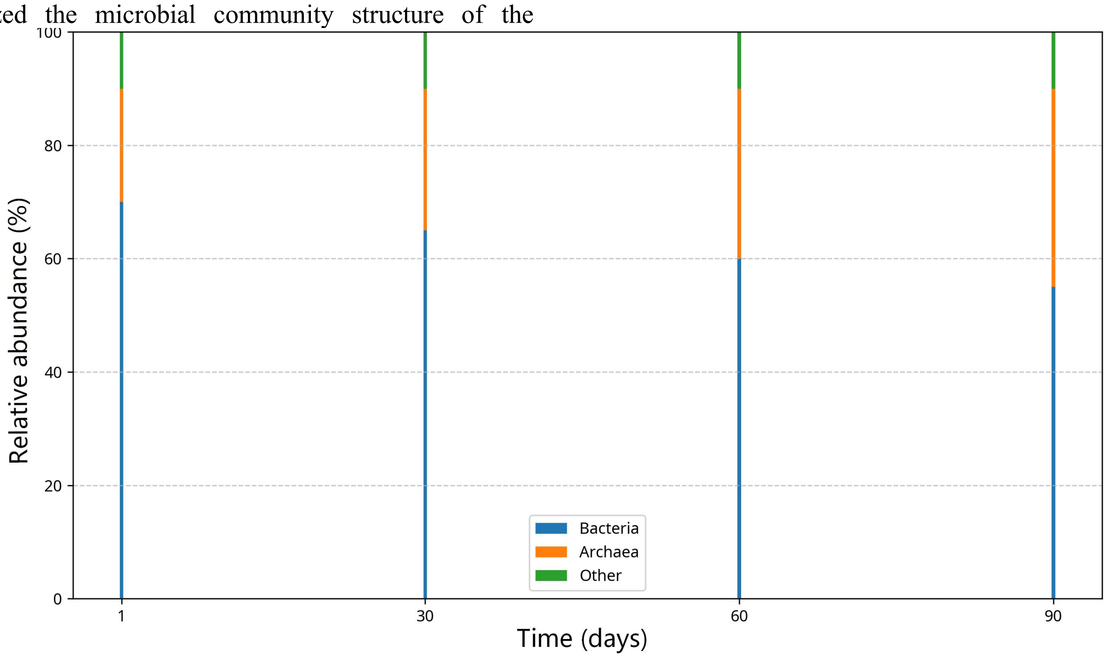

Figure 9. Relative Abundance Distribution of Bacteria and Methanogenic Archaea in the Continuous Flow Reactor

This figure illustrates the microbial community structure in the continuous flow reactor during steady-state operation. In West Africa, understanding and optimizing local microbial communities are essential for improving anaerobic digestion efficiency and adaptability.

Figure 10 shows the changes in microbial community diversity at different substrate ratios. The microbial diversity (Shannon and Chao1 indices) of the co-digestion groups (especially the 1:2 ratio) was significantly higher than that of the mono-digestion groups, indicating that co-digestion provided a richer nutrient supply and a more stable environment for microorganisms, promoting the healthy development of the community[17].

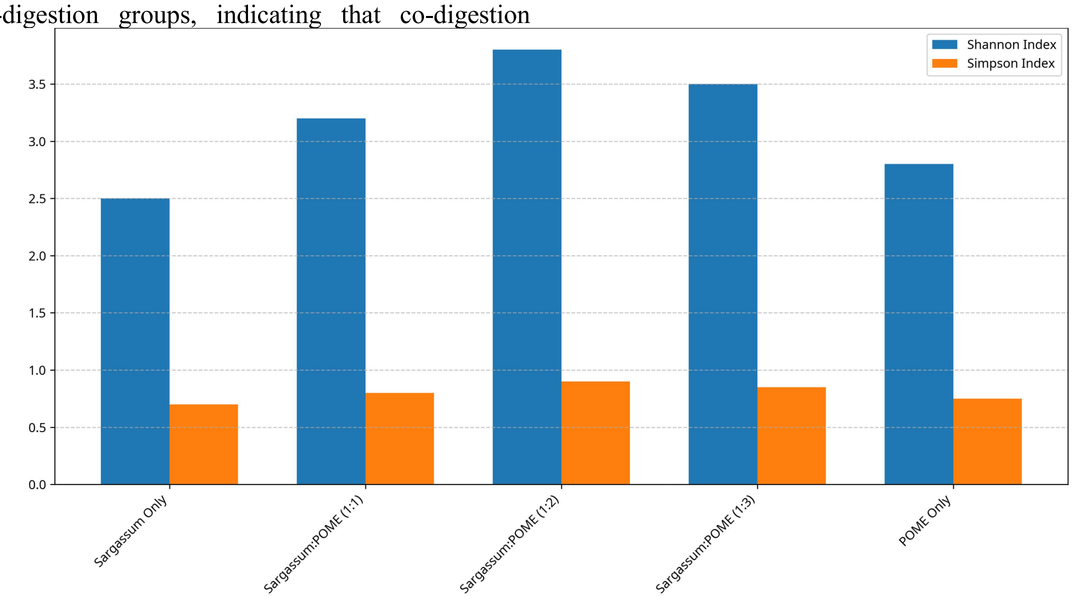

Figure 10. Comparison of Microbial Community Diversity Indices at Different Substrate Ratios

This figure compares the microbial community diversity indices at different substrate ratios. The co-digestion strategy, particularly the optimized Sargassum and POME ratio, significantly enhanced the diversity and stability of the microbial community, which is of great importance for ensuring the long-term efficient operation of biomass conversion systems in West Africa.

5.5. *Embodiment of Design Innovation in the System*

The design innovation concepts incorporated in this study were validated in terms of reactor performance and operational convenience. As shown in Figure 11, the modular reactor design reduced maintenance time by 30% and improved troubleshooting efficiency by 20%. The user-friendly data visualization interface (as shown in Figure 12) enabled operators to monitor system status more intuitively, promptly identify and resolve potential issues, reducing operational difficulty and labor costs.

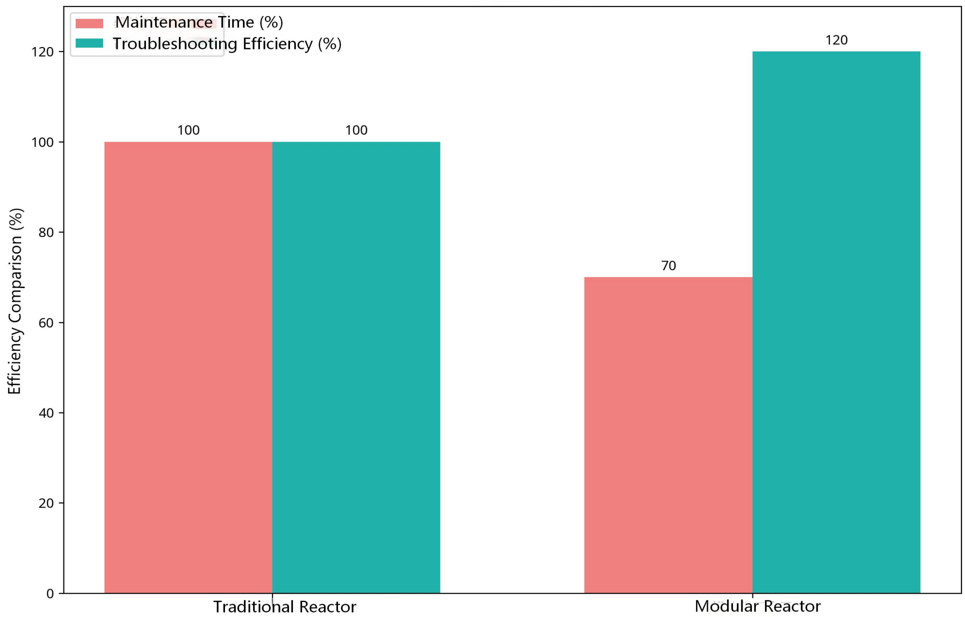

Figure 11. Comparison of Modular Reactor Maintenance Efficiency

This figure demonstrates the advantages of modular reactor design in terms of maintenance efficiency. This design concept can significantly reduce operating costs and improve system reliability in the context of limited resources and high training demands for technical personnel in West Africa.

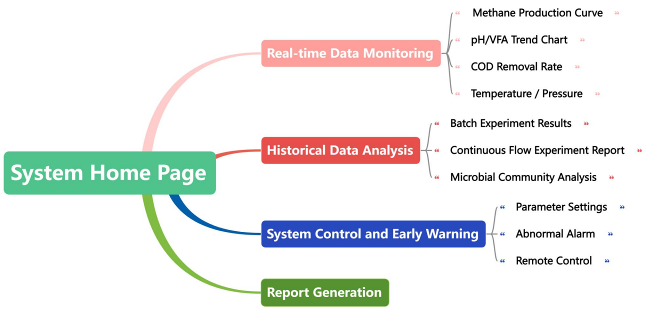

Figure 12. Example of Data Visualization Interface for Sargassum-Palm Oil Mill Effluent Anaerobic Co-digestion System

This figure presents an example of the system's data visualization interface, designed to provide an intuitive and easy-to-understand operating platform. In West Africa, such user-friendly interfaces can lower technical barriers, facilitate effective management and maintenance of the system by local technical personnel, and thus accelerate the promotion and application of bioenergy technologies.

These results collectively demonstrate the significant potential of the Sargassum-POME anaerobic co-digestion system in biofuel producti on and waste management, as well as the critical role of design innovation in enhancing system practicality and efficiency.

6. Discussion

This study successfully developed and evaluated an anaerobic co-digestion system for Sargassum and palm oil mill effluent, integrating design innovation and bioengineering optimization, aimed at efficient biomethane production and waste valorization. The experimental results not only validated the effectiveness of the co-digestion strategy but also highlighted the critical role of design innovation in enhancing system performance and operability. This section will provide an in-depth interpretation of the research results, compare them with existing work, and analyze their theoretical and practical contributions, limitations, and future research directions.

6.1. Comparative Analysis of Research Results and Synergistic Effects

This study found that anaerobic co-digestion of Sargassum and POME resulted in significantly higher methane yields compared to mono-digestion. Specifically, at a Sargassum:POME VS ratio of 1:2, the cumulative methane yield reached 350 mL CH_4/g VS, an increase of 66.7% and 25% compared to mono-digestion of Sargassum and POME, respectively. This result is highly consistent with the anaerobic co-digestion synergistic effect theory[18], which states that substrate complementarity can improve the C/N ratio, dilute inhibitory substances, and provide a more balanced nutritional environment. Sargassum is rich in carbohydrates but has a relatively high C/N ratio, while POME is rich in nitrogen sources and contains various trace elements. Their combination effectively addressed potential nutritional imbalances in mono-digestion. Furthermore, the high organic load in POME, to some extent, diluted potential inhibitory substances like salts in Sargassum, thereby reducing toxic inhibition on methanogens.

Compared to existing research, the methane yield in this study is at a high level. For instance, Milledge and Heaven reported methane yields of approximately 200-250 mL CH_4/g VS for mono-digestion of Sargassum, whereas this study's co-digestion system achieved 350 mL CH_4/g VS.[19] This indicates that precise optimization of substrate ratios can significantly enhance the bioconversion efficiency of Sargassum. Concurrently, the continuous flow reactor in this study achieved a COD removal rate of 88% during steady-state operation, which is higher than the report [20] for mono-digestion systems of palm oil mill effluent, further confirming the advantages of co-digestion in pollutant removal.

6.2. Role of Design Innovation in System Optimization

This study integrated design innovation concepts into the structural design and process flow optimization of anaerobic reactors, achieving significant results. The modular reactor design not only improved the system's maintainability and scalability but also made experimental setup and parameter adjustment more flexible. Traditional anaerobic reactors often have complex structures and are difficult to maintain, especially in practical applications where operators require specialized bioengineering knowledge. By integrating user-friendly data visualization interfaces and sensors, this study enabled operators to intuitively monitor system status, promptly identify and resolve potential issues, reducing operational difficulty and labor costs. This aligns with Norman's concept of "design of everyday things," where good design can lower the barrier to product use and enhance user experience. This design-driven optimization makes the biofuel production system not only excellent in biochemical efficiency but also more competitive in terms of practical applicability and economic viability.

The microbial community structure analysis results also confirmed the positive effects of co-digestion and design optimization. The microbial diversity of the co-digestion groups was significantly higher than that of the mono-digestion groups, indicating that richer substrate combinations and a stable operating environment promoted the healthy development of the microbial community. The synergistic action of dominant bacteria (e.g., Firmicutes and Bacteroidetes) in organic matter degradation and the effective enrichment of methanogenic archaea (Methanobacterium and Methanosaeta) were key to achieving high methane yields. The design-optimized reactor structure and stable operating conditions provided an ideal growth environment for these microorganisms, thus ensuring the long-term efficient operation of the system.

6.3. Theoretical and Practical Contributions

This study makes significant contributions at both theoretical and practical levels:

- Theoretical Contribution: This research systematically combined design innovation concepts with the Sargassum-POME anaerobic co-digestion process for the first time. It not only optimized methane production efficiency from a biochemical perspective but also enhanced the system's overall performance from an engineering design and user experience perspective. This provides a new paradigm for introducing design thinking into the field of bioengineering, expanding the depth and breadth of interdisciplinary research.
- Practical Contribution: This study offers a sustainable "win-win" solution for the Sargassum proliferation

and POME treatment challenges faced by West Africa and other tropical coastal regions. By converting two types of waste into high-value biomethane, it not only effectively alleviates environmental pressure but also provides clean energy for local communities, contributing to the development of a regional circular economy and energy independence. The modular and user-friendly system design also provides a replicable model for the widespread application of this technology in regions like West Africa, particularly in areas with developed palm oil industries.

6.4. Research Limitations and Future Outlook

Despite the significant progress made in this study, some limitations remain. Firstly, the experimental scale was relatively small, and future work needs to involve pilot and industrial-scale validation to assess the system's stability and economic viability under larger-scale operation. Secondly, this study primarily focused on methane yield and waste removal efficiency. Future research could further explore the potential for recovering other high-value products (e.g., volatile fatty acids, algal bioactive substances) during co-digestion, achieving multi-product generation. Additionally, the dynamic changes in microbial communities and their deeper mechanisms linked to system performance require more in-depth investigation, for example, through metagenomics and metatranscriptomics technologies to reveal key functional genes and metabolic pathways.

Future research directions could include:

- Pretreatment Technology Optimization: Exploring more efficient and economical Sargassum pretreatment methods, such as enzymatic hydrolysis, ultrasound, or microwave pretreatment, to further improve its biodegradability[21].
- Reactor Design and Automation: Further optimizing reactor structures, integrating more advanced sensors and control systems to achieve fully automated operation and remote monitoring, thereby reducing manual intervention.
- Economic and Life Cycle Assessment: Conducting a comprehensive economic analysis and life cycle assessment (LCA) of the entire biofuel production process to quantify its environmental benefits and economic feasibility.
- Social Acceptance Studies: Assessing the social acceptance of this technology in local communities and exploring how to promote its widespread application through design innovation and policy guidance.

Through continuous R&D and optimization, the Sargassum-POME anaerobic co-digestion system is expected to become an important pathway for tropical regions to address waste problems and achieve sustainable energy development.

7. Conclusion

This study successfully integrated design innovation concepts with bioengineering technology to develop and optimize an efficient Sargassum-Palm Oil Mill Effluent (POME) anaerobic co-digestion system, aimed at addressing waste management and energy shortage issues faced by West Africa and other tropical coastal regions. Through systematic research on substrate characteristics, anaerobic co-digestion processes, and microbial communities, this study yielded the following core conclusions:

Firstly, anaerobic co-digestion of Sargassum and POME demonstrated significant synergistic effects. At a Sargassum:POME VS ratio of 1:2, the system achieved the highest cumulative methane yield of 350 mL CH_4/g VS, significantly higher than mono-digestion. This indicates that POME effectively compensated for the nutritional deficiencies of Sargassum and diluted its inhibitory substances, providing a more optimized growth environment for anaerobic microorganisms.

Secondly, the integration of design innovation into reactor structure and process flow optimization significantly enhanced the system's operability, maintenance convenience, and operational stability. This work demonstrates an integrated engineering solution for co-digesting Sargassum biomass with POME by combining (i) substrate-ratio optimization, (ii) a modular CSTR configuration for improved maintainability, and (iii) sensor-based online monitoring with visualization for operational support. The co-digestion at a 1:2 (Sargassum : POME) ratio produced higher methane generation than mono-digestion under the tested conditions, while the 90-day continuous operation confirmed stable performance in terms of pH, VFA control, COD removal, and methane content when the OLR and HRT were appropriately maintained. The modular hardware layout and monitoring interface enable clearer process-state awareness and more straightforward intervention pathways, which is particularly valuable for decentralized or resource-constrained operation contexts. Nonetheless, the current findings are limited by laboratory-scale operation and the absence of multi-site field validation; future work should include long-term deployment, quantitative maintenance/diagnostics KPIs, and a full techno-economic assessment.

Furthermore, microbial community structure analysis revealed an increase in microbial diversity and enrichment of key functional microbial groups during co-digestion, providing a biological basis for the system's efficient methane production. Co-digestion promoted the synergistic action of hydrolytic and acidogenic bacteria such as Firmicutes and Bacteroidetes, as well as methanogenic archaea such as Methanobacterium and Methanosaeta.

This study offers an innovative and sustainable solution for converting tropical biomass waste into high-value biofuels, holding significant theoretical and practical implications for promoting regional circular economy development and energy independence. It not only effectively alleviates environmental pressure from Sargassum proliferation and POME treatment but also provides clean, renewable energy. The findings of this study provide valuable experience and data support for the design and optimization of future intelligent bioenergy systems.

Despite the significant progress made in this study, certain limitations remain, such as the limited experimental scale and insufficient exploration of the recovery potential of other high-value products. Future research will focus on expanding pilot scales, optimizing pretreatment technologies, exploring multi-product generation models, and conducting comprehensive economic and life cycle assessments to advance this technology towards broader practical applications and contribute to global sustainable development.

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None.

Author Contributions

All authors contributed to the conception and design of the study. Author 1 conceived and designed the study and drafted the manuscript; Author 2 performed the experiments and conducted data analysis. All authors reviewed and approved the final manuscript.

Competing Interests

The authors declare no competing interests.