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Biorefinery-Based Energy Recovery from Algae: Comparative Evaluation of Liquid and Gaseous Biofuels

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Abstract

In recent years, biofuels and bioenergy derived from algae have gained increasing attention, fueled by the growing demand for renewable energy sources and the urgent need to lower CO_2 emissions. This research examines the generation of bioethanol and biomethane using freshly harvested and sedimented algal biomass. Employing a factorial experimental design, various trials were conducted, with ethanol yield as the primary optimization target. The findings indicated that the sodium hydroxide concentration during pretreatment and the amylase dosage in enzymatic hydrolysis were key parameters influencing the ethanol production efficiency. Under optimized conditions—using 0.3 M NaOH, 25 μL/g starch, and 250 μL/g cellulose—fermentation yielded ethanol concentrations as high as 2.75 ± 0.18 g/L (45.13 ± 2.90%), underscoring the significance of both enzyme loading and alkali treatment. Biomethane potential tests on the residues of fermentation revealed reduced methane yields in comparison with the raw algal feedstock, with a peak value of 198.50 ± 25.57 mL/g volatile solids. The integrated process resulted in a total energy recovery of up to 809.58 kWh per tonne of algal biomass, with biomethane accounting for 87.16% of the total energy output. However, the energy recovered from unprocessed biomass alone was nearly double, indicating a trade-off between sequential valorization steps. A comparison between fresh and dried feedstocks also demonstrated marked differences, largely due to variations in moisture content and biomass composition. Overall, this study highlights the promise of integrated algal biomass utilization as a viable and energy-efficient route for sustainable biofuel production.

Keywords: bioethanol; biomethane; enzymatic hydrolysis; microalgae

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1. Introduction

The continuous increase in anthropogenic greenhouse gas (GHG) emissions, particularly carbon dioxide (CO_2), remains one of the greatest challenges for climate stability [1]. CO_2 is the most abundant GHG released by human activities and contributes significantly to global warming due to its long atmospheric lifetime. According to the Intergovernmental Panel on Climate Change, atmospheric CO_2 levels have reached unprecedented concentrations, exceeding 420 ppm in 2023 [2]. This rise is mainly driven by the combustion of fossil fuels for energy and transportation, along with industrial activities and deforestation [3].

To address this, global policy frameworks and research efforts have focused on decarbonizing key sectors, particularly transport, which accounts for nearly 25% of the total CO_2

emissions in the EU. One of the strategies adopted involves the replacement of fossil fuels with renewable, low-carbon alternatives. Within this context, biofuels have emerged as viable transitional energy carriers.

The EU has implemented successive directives—namely RED I, RED II, and most recently RED III (2023)—that promote the use of renewable energy in transport. These policies prioritize biofuels derived from non-food and non-feed biomass, such as wastes, residues, and algae. RED III introduces a higher binding target of 29% renewable energy use in transport by 2030, while limiting the contribution of first-generation biofuels and supporting advanced biofuels and renewable fuels of non-biological origin [4,5].

Microalgae have gained particular interest as a third-generation biofuel feedstock due to their rapid growth, high photosynthetic efficiency, and ability to fix CO_2 from both atmospheric and industrial sources [6,7]. Unlike terrestrial crops, they do not compete with food production or require arable land and can be cultivated using nutrient-rich wastewater or saline water, further enhancing their sustainability profile. Beyond their carbon capture potential, microalgae produce biomass rich in carbohydrates, proteins, and lipids, making them suitable for a wide range of applications, from biofuels and bioplastics to animal feed and pharmaceuticals [8–12].

In the context of biofuel production, algal biomass offers potential for both first- and second-generation conversion pathways. Lipids can be extracted and processed into biodiesel, while the carbohydrate-rich fractions are suitable for bioethanol production via fermentation. Furthermore, the residual biomass—including fermentation byproducts—can be valorized through anaerobic digestion to generate biogas, primarily methane [13]. This approach aligns with the biorefinery concept, which aims to maximize resource efficiency by integrating multiple conversion processes and minimizing waste.

Within this framework, the sequential integration of bioethanol fermentation and biomethane recovery presents a particularly promising dual-output valorization strategy for microalgal biomass. Bioethanol is a well-established liquid biofuel, fully compatible with existing engine infrastructure and commonly blended with gasoline. Biomethane, meanwhile, serves as a renewable alternative to natural gas, with applications in both heat and transport sectors. Crucially, the cascading use of biomass through these coupled processes enhances the overall energy efficiency, supports environmental sustainability, and contributes to the development of circular bioeconomy models.

This study aims to assess such an integrated biorefinery concept using fresh microalgal biomass. A series of factorial experiments was designed to optimize bioethanol production through enzymatic hydrolysis and fermentation. The fermentation residues were then subjected to anaerobic digestion for biomethane recovery. Through mass and energy balance calculations, the study evaluates the total energy yield and efficiency of the process. The findings contribute to the ongoing effort to establish scalable, circular, and sustainable biofuel production systems that make use of CO_2-rich biomass such as microalgae.

In recent years, several studies have explored integrated valorization strategies for microalgae and lignocellulosic biomass, focusing on combined pathways such as lipid extraction followed by anaerobic digestion [14,15] or hydrothermal pretreatment coupled with gasification or biohydrogen production [13,16–18]. Other works have proposed the co-production of bioethanol and biogas, but most rely on pre-dried or pre-extracted biomass, increasing energy and cost requirements [19–21]. In contrast, the present study investigates a low-energy, enzymatic route for bioethanol production from fresh algal biomass, coupled with biomethane recovery from fermentation residues, thus representing a cascading biorefinery model that emphasizes simplicity, process integration, and circularity. The novelty lies in the combined experimental optimization of both stages, the use of undried microalgal feedstock, and the quantitative assessment of total energy output and conversion

efficiency, which are rarely addressed together in the literature. Although microalgae have long been promoted as a promising feedstock for third-generation biofuels due to their high productivity and CO_2 fixation capacity, numerous studies have shown that large-scale, standalone energy production from algae remains economically unviable under current technological and market conditions [22,23]. In practice, microalgae are primarily cultivated today for high-value applications, such as nutraceuticals, pigments, and cosmetic ingredients, where the product value offsets the high cultivation and processing costs [24].

Nonetheless, there is growing interest in developing integrated biorefinery models to improve overall process economics, increase resource efficiency, and align with circular bioeconomy goals [25,26]. The present study contributes to this direction by evaluating a dual-output system using fresh algal biomass in a cascading valorization scheme.

Through this framework, the study aims to advance the development of algae-based biofuel systems, offering practical insights into circular economy principles by promoting efficient resource use, energy recovery, and waste minimization. This integrated approach supports the overarching objectives of biorefinery design and advances the shift toward more sustainable energy solutions.

2. Materials and Methods

In the following section of this manuscript, all the necessary steps for pretreatment of the feedstock, ethanolic fermentation, and the biochemical methane potential assay are presented, along with the analytical methods and materials employed to carry out the current research.

2.1. Materials

The algal biomass used in this study consisted of a mixed microalgal culture derived from open pond cultivation at ALGEN facilities (Ljubljana, Slovenia). The culture was non-axenic and was maintained under outdoor conditions, but microscopic examination confirmed the predominance of *Scenedesmus* spp., with other green microalgae present in minor proportions. The sedimented algal biomass was collected, and immediately after harvesting, it was transferred into sterile, airtight containers and stored on dry ice to prevent microbial activity and enzymatic degradation. Upon arrival at the Environmental Science and Technology Unit at the School of Chemical Engineering, National Technical University of Athens (NTUA), the samples were stored at −20 °C until use. To prevent unintended fermentation during transport, the biomass was sealed in airtight containers. Upon arrival, it was stored under controlled conditions to preserve its quality for subsequent analyses and processing. A total of approximately 5 L of this feedstock was received and analyzed to determine its physicochemical characteristics, as well as its potential for ethanol and biomethane production. Analytical-grade reagents were used throughout the study. These included sodium hydroxide pellets (NaOH, ≥98.0% purity, CAS No. 1310-73-2, Penta Chemicals Unlimited, Prague, Czech Republic) and sulfuric acid (H_2SO_4, ≥96.0% purity, CAS No. 7664-93-9, Fisher Chemicals, Pittsburgh, PA, USA). Two enzymatic products, Spirizyme Excel XHS (2337 U/mL) and Cellic® CTec3 (171.7 FPU/mL), were provided by Novozymes® (Bagsværd, Denmark) and used for enzymatic hydrolysis and fermentation steps. The enzyme Spirizyme Excel XHS, an amylolytic product, was used to hydrolyze intracellular storage polysaccharides, particularly starch-like compounds present in the algal biomass. In parallel, Cellic® CTec3, a cellulolytic and hemicellulolytic enzyme cocktail, was applied to disrupt cell wall components and release structural sugars. The combined use of these enzymes was selected based on the biochemical profile of Scenedesmus-dominated biomass, which contains both digestible carbohydrates and recalcitrant cell wall polymers. Their application aimed to maximize the yield of fermentable sugars for

bioethanol production. The enzyme dosages were varied in accordance with a factorial design, as described below. The yeast strain *Saccharomyces cerevisiae* (brewer's yeast) was used for fermentation at a constant concentration of 2% (*w*/*w*, dry weight).

2.2. Analytical and Statistical Methods

The algal biomass composition—specifically lignin, structural carbohydrates, total starch, and lipids—was analyzed following standardized protocols, as described by Chatzimaliakas et al. [27]. Glucose and ethanol concentrations in the liquid phase were measured photometrically using commercial assay kits, as presented in Chatzimaliakas et al. [27]. All experiments and analyses were conducted in triplicate, and data are reported as mean ± standard deviation. One-way analysis of variance (ANOVA) was applied to compare the effect of different experimental conditions (e.g., enzyme load, biomass concentration), with significance thresholds set at $p < 0.05$. Statistical analysis was performed using OriginPro 2022 v. 0.0.1.7 (OriginLab Corporation, Northampton, MA, USA) or equivalent statistical software.

2.3. Experimental Methods

2.3.1. Alcoholic Fermentation

Simultaneous saccharification and fermentation (SSF) was used for bioethanol production, as this approach had demonstrated effectiveness in preliminary trials and previous studies [27]. The experiments were performed in autoclavable bottles (250 mL) placed in a shaking incubator (FS-70B, Hinotek, Hangzhou, China) to maintain controlled agitation and temperature conditions. The optimization trials were designed using a factorial design approach, where three sodium hydroxide concentrations (0.1, 0.2, and 0.3 M) and multiple enzyme loadings (Cellic CTec3: 250, 500, 750 $\mu L/g_{cellulose}$; Spirizyme Excel XHS: 25, 45, 65 $\mu L/g_{starch}$) were tested. The solid loading was set at 7% for all experiments. This value corresponded to the moisture content of the algae as they were harvested. The pretreatment step involved heating the algal biomass to 90 °C in a water bath after the chemical addition. The fermentation was performed for 24 h at 35 °C by adding 2% *S. cerevisiae* (*w*/*w*).

The effectiveness of fermentation was monitored by measuring ethanol and glucose levels. Ethanol yield was calculated as presented in Chatzimaliakas et al. [27].

2.3.2. Biomethane Potential (BMP) Assay

To assess the methane production potential of the fermentation residues (stillage), BMP tests were conducted in a batch reactor equipped with a gas scrubbing unit and an integrated data logger, following the protocol outlined by Angelidaki et al. [28]. Each assay run included 16 batch experiments, with controls and blanks to validate the inoculum's activity and account for background methane production. The BMP assays were conducted in triplicate for each condition using 500 mL batch reactors with a working volume of 400 mL. The results are expressed as mean ± standard deviation.

The inoculum, sourced from a municipal anaerobic digestion facility in Athens treating sewage sludge, had a volatile solids (VS) content of 15.58 g/L. The substrate-to-inoculum VS ratio was maintained at 1:4. Methane production was monitored for up to 35 days, ensuring full digestion, and corrected for standard temperature and pressure (STP) conditions. The methane yield was calculated as presented in Chatzimaliakas et al. [27]. The statistical analysis was carried out using OriginPro 2022 and IBM SPSS Statistics 26, applying one-way ANOVA to assess differences among conditions, with significance set at $p < 0.05$.

3. Results and Discussion

3.1. Composition

To initiate the investigation, the biomass received was physico-chemically characterized. Once the components of the feedstock were identified, valuable information was extracted. All of the measured values are presented in Table 1.

Table 1. Composition of algal species (mainly *Scenedesmus* spp.), cultivated in open ponds under nutrient-limited conditions, without any pretreatment prior to compositional analysis.

Category	Parameter	Value (% d.w.)
Moisture and Solids	Total Solids	7.00 ± 0.52
	Moisture	93.00 ± 0.52
	Volatile Solids	66.40 ± 0.25
	Ash	33.60 ± 0.25
Biochemical Components	Lipids and Photosynthetic Pigments	7.31 ± 0.01
	Water Soluble Solids	5.62 ± 0.11
	Free Glucose	0.05 ± 0.01
	Hemicellulose	12.53 ± 0.66
	Cellulose	14.42 ± 0.33
	Starch	0.87 ± 0.10
	Acid-Insoluble Residue	27.58 ± 0.74
Nitrogen Content	Total Nitrogen (Kjeldahl)	3.95 ± 0.20

The compositional analysis showed that the algal biomass had a relatively elevated acid-insoluble residue content alongside lower levels of carbohydrates, specifically starch and cellulose. These values slightly diverge from commonly reported ranges in the literature. Typically, depending on the species, algal biomass may contain lipid concentrations between 8% and 77% on a dry weight basis [29]. In this study, the lipid content—combined with photosynthetic pigments that are also soluble in lipids—remained below 8% (d.w.), suggesting that the actual proportion of pure lipids was likely even lower. The measured carbohydrate content fell within the expected range found in prior studies, where values usually span from 11% to 50% (d.w.), though certain strains cultivated under stress conditions can accumulate up to 70% [30,31]. In this case, the carbohydrate content was 15.29 ± 0.43% (d.w.). The nitrogen content of the biomass was approximately 4% (d.w.), which, when corrected for the volatile solids content (~66% of d.w.) and estimated carbon content of 45–55% of VS, yields an approximate C/N ratio of 7.4–9.0. This is slightly higher than the Redfield ratio (~6.6–7.2) but still within the realistic range for microalgal biomass, particularly under nutrient-limited or open cultivation conditions. Although open ponds are generally considered prone to contamination, species dominance can be sustained under optimized environmental and operational conditions. In this case, *Scenedesmus* spp. were the prevailing strain, likely due to their robustness and competitive fitness in the cultivation system. This may account for deviations in composition compared to biomass derived from controlled monocultures. These analytical results served as the foundation for planning a series of experiments aimed at evaluating the conversion efficiency of the fresh algal biomass into bioethanol and biomethane.

3.2. Factorial Design for Bioethanol Production

Building on insights from earlier research on pretreatment techniques to improve the algal biomass saccharification efficiency [27], and supported by preliminary trial data (not presented), a factorial experimental design was implemented to evaluate the bioethanol production potential of freshly sedimented microalgae. The primary objective was to optimize the ethanol yield. After 24 h of simultaneous saccharification and fermentation

(SSF), the liquid phase from each sample was analyzed to determine both ethanol content and any remaining glucose. The corresponding data are summarized in Table 2. To assess variability, the mean values and standard deviations were estimated, with a 95% confidence interval used to identify any random deviations. The Cochran test was applied to evaluate the consistency and uniformity of the results.

Table 2. Experimental conditions and results of ethanolic fermentation and biomethane production from fresh *Scenedesmus* spp. biomass, based on the 2^3 factorial design. The tested variables were NaOH concentration and enzyme loading (Cellic® CTec3 + Spirizyme Excel XHS). The table includes the corresponding ethanol yields, the biomethane yields from stillages, and the fractional contribution of each to the total energy output. The condition labeled "center" represents the central point of the experimental design, where all variables were set at intermediate levels. All values are based on triplicate measurements; standard deviations are provided where applicable.

Experiment No.	1	2	3	4	5	6	7	8	Center of Factorial Design
NaOH (M)	0.1	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.2
CelicTec3 ($\mu L/g_{cellulose}$)	250	750	250	750	250	750	250	750	500
Spirizyme ($\mu L/g_{starch}$)	25	25	65	65	25	25	65	65	45
Ethanol (g/L)	1.06 ± 0.27	1.19 ± 0.09	0.58 ± 0.28	0.69 ± 0.09	2.75 ± 0.18	2.44 ± 0.09	1.81 ± 0.44	2.38 ± 0.02	2.48 ± 0.10
Glucose (g/L)	0.10 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.12 ± 0.03	0.14 ± 0.02	0.14 ± 0.04	0.09 ± 0.05	0.07 ± 0.01
Ethanol Yield (%)	17.43 ± 4.35	19.49 ± 1.45	9.44 ± 4.64	11.28 ± 1.45	45.13 ± 2.90	40.00 ± 1.45	29.74 ± 7.25	38.97 ± 1.95	40.61 ± 1.57
BMP (mL CH_4/g VS)	171.67 ± 36.27	190.14 ± 42.83	198.50 ± 33.57	164.46 ± 36.18	102.36 ± 24.74	116.74 ± 11.46	153.39 ± 27.78	116.98 ± 31.43	80.31 ± 20.67
Ethanol (kWh/t algae)	286.14	304.60	280.70	323.22	258.39	359.98	341.47	406.13	290.94
Methane (kWh/t algae)	722.68	698.54	604.46	574.20	786.08	492.43	607.90	585.89	678.70
Energy (kWh)	1008.82	1003.14	885.16	897.42	1044.48	851.41	949.36	991.02	969.64
Bioethanol (%)	28.36	30.36	31.71	36.02	24.74	42.23	35.97	40.94	30.01
Biomethane (%)	71.64	69.64	68.29	63.98	75.26	57.77	64.03	59.06	69.99

The highest ethanol yield from freshly sedimented algal biomass was observed under specific experimental conditions: treatment with 0.3 M NaOH, supplemented by 25 µL of Spirizyme Excel XHS per gram of starch and 250 µL of CellicTec3 per gram of cellulose. Under these conditions, the ethanol concentration reached 2.75 ± 0.18 g/L, corresponding to a yield of 45.13 ± 2.90%. Notably, the post-fermentation analysis indicated that glucose levels were nearly undetectable in all cases, suggesting that *Saccharomyces cerevisiae* had effectively fermented all available sugars into ethanol, as confirmed by the data presented in Table 2.

To better understand the influence of process variables on ethanol yield, a mathematical model was constructed. Ethanol yield served as the primary optimization parameter. The model's validity and predictive power were evaluated using the Fisher criterion [32,33]. Leveraging the results obtained from the factorial design (Table 2) and applying statistical tools such as ANOVA, the study derived equations in both coded and actual units to illustrate the contribution and significance of each operational parameter in optimizing ethanol production.

Coded values:

$$Y_{etOH} = 0.2643 + 0.1202X1 - 0.0408X2 \quad (1)$$

Physical values:

$$Y_{etOH} = 0.09734 + 1.202NaOH - 0.001632Spirizyme \quad (2)$$

The factorial design analysis indicated that the most favorable ethanol yields were obtained when the pretreatment involved higher concentrations of sodium hydroxide in combination with lower dosages of both amylase and cellulase enzymes. Furthermore, the findings emphasized that among the variables tested, the sodium hydroxide concentration had the most significant positive effect on ethanol production from fresh algal biomass. This suggests that enhancing the alkali level during pretreatment substantially boosts the saccharification efficiency and, consequently, ethanol output. These results reinforce the role of alkaline pretreatment as a key strategy for improving the fermentability of algae-

derived substrates. Consistent with these observations, Harun et al. [34] also reported high bioethanol yields using a similar approach that combined elevated temperatures and alkaline treatment when processing *C. infusionum*.

The breakdown of solids and key polysaccharides across the factorial experiments using fresh algal biomass is illustrated in Figure 1. The post-fermentation analysis of the solid fractions revealed notably high degradation efficiencies for major carbohydrate components, particularly cellulose and starch—exceeding 95% in certain instances. The overall solid degradation varied between 16.71 ± 0.01% in experiment 1 and 37.92 ± 0.05% in experiment 7. Despite the relatively modest total solid reductions in some cases, the cellulose degradation remained high, ranging from 79.18 ± 0.04% (experiment 3) to a peak of 95.54 ± 0.01% (experiment 7). A similar trend was observed in starch degradation, with values spanning from 64.05 ± 0.05% (experiment 4) to 85.34 ± 0.01% (experiment 5).

Figure 1 also includes data on hemicellulose degradation. The substantial reduction of hemicellulose observed is likely attributed to the action of CellicTec 3, an enzyme formulation capable of hydrolyzing not only cellulose but also hemicellulose. According to Huang et al. [35], hemicellulose is typically converted into xylose—a fermentable monosaccharide. However, it is acknowledged that *Saccharomyces cerevisiae*, the strain employed in this study, does not naturally ferment xylose into ethanol. As such, the xylose released from hemicellulose hydrolysis was not expected to contribute to ethanol production. The use of Cellic® CTec3, which also targets hemicellulose, aimed to maximize the degradation of polysaccharides and generate a more digestible residue for subsequent anaerobic digestion rather than increase ethanol yield. While ethanol production was not optimized with respect to pentose fermentation, the enzymatic hydrolysis step was designed to support a two-stage valorization strategy where both ethanol and biomethane yields are collectively considered. This approach aligns with biorefinery principles, wherein ethanol is recovered from hexose sugars, and the xylose-rich residue is valorized via anaerobic digestion.

3.3. Biomethane Potential

Following the completion of the fermentation process in the proposed valorization pathway, the resulting stillages from fresh feedstock were subjected to a BMP test to quantify the biomethane produced. After the completion of the test, according to the definition of biomethane yield, the factorial experiment was calculated, and the results are presented in Table 2. As a baseline control, an additional anaerobic digestion experiment was conducted using untreated fresh algal biomass, without any pretreatment or fermentation, to assess its standalone biomethane potential. This result is reported as "untreated."

The variation in biomethane yields across the different experimental conditions cannot be attributed solely to enzymatic degradation of cellulose or hemicellulose, as shown in Figure 1. While carbohydrate degradation is an important factor, it is not the sole determinant of methane potential. Other influential factors include the composition and concentration of fermentation inhibitors, pH shifts from alkaline pretreatment, and differences in residual protein and lipid content in the stillages. For example, in experiments with higher enzyme loading, despite greater cellulose and hemicellulose breakdown, inhibitory byproducts or imbalanced C/N ratios may have suppressed microbial activity during anaerobic digestion. Moreover, as ethanol production preferentially consumes the carbohydrate-rich fraction, the residual biomass is enriched in non-carbohydrate components—and those components' digestibility varies with pretreatment severity and enzymatic activity. These compositional shifts likely contributed to the observed differences in methane yields across the experiments.

The highest yield of 198.50 ± 25.57 mL/g VS was recorded in experiment 3, when 0.1 M NaOH, 65 µL Spirizyme Excel XHS/g_{starch} and 250 µL CellicTec3/$g_{cellulose}$ were added, whereas the untreated fresh algae presented a BMP value of 273.76 ± 22.64 mL/g VS.

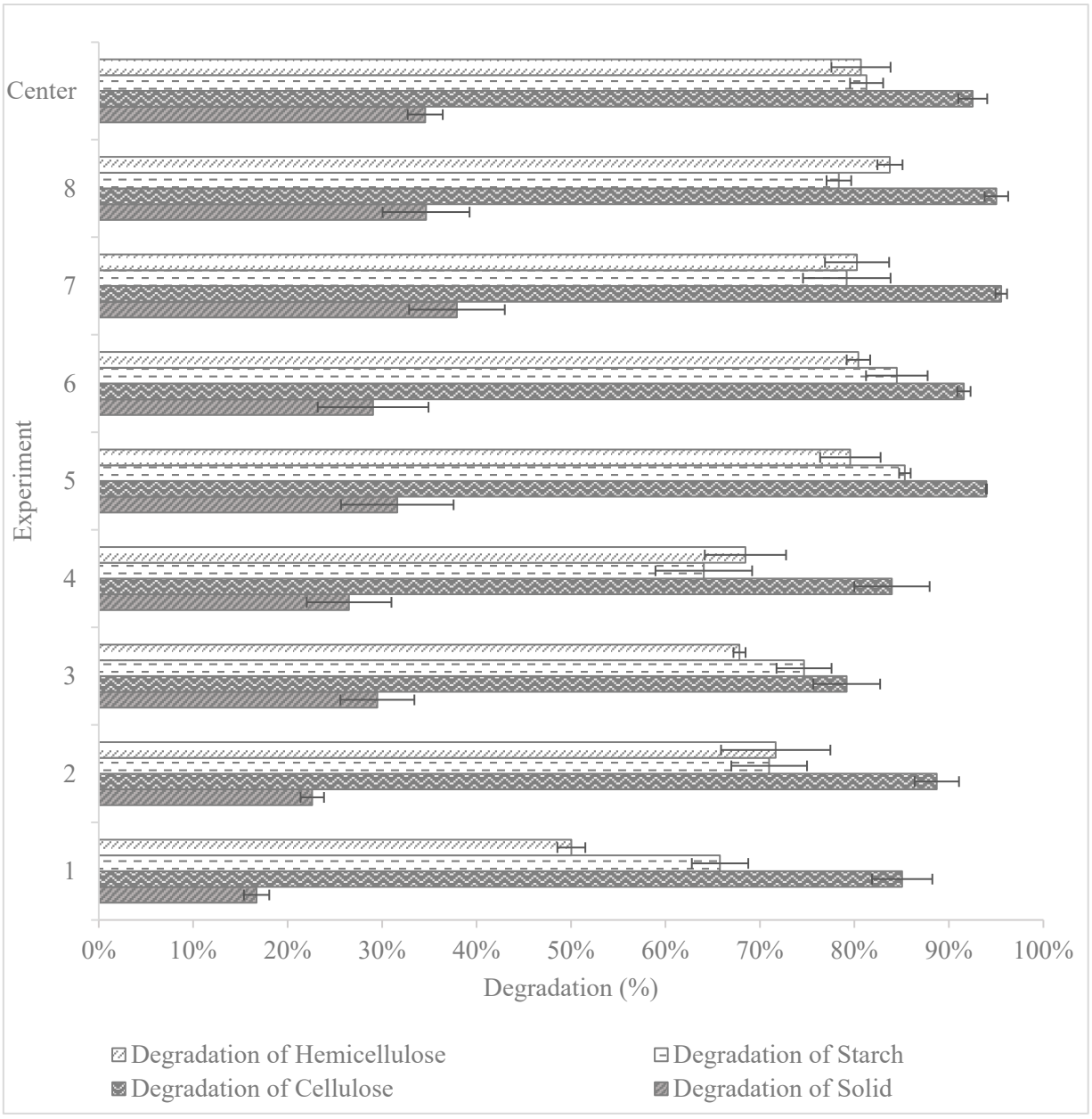

Figure 1. The degradation of solid, major polysaccharides, and hemicellulose obtained under different experimental conditions, as defined by the 2^3 factorial design (Table 2). The three variables were the NaOH concentration and the enzyme loading (Cellic® CTec3 + Spirizyme Excel XHS). The point labeled "center" corresponds to the central experimental condition, where both variables were set at intermediate levels. The error bars represent the standard deviations from the triplicate measurements.

3.4. Energy Production Routes

Based on the experimental findings outlined above, two energy production scenarios were developed using algal biomass. These scenarios aimed to assess the potential of converting algal-derived products—bioethanol and biogas—into usable bioenergy. To compare the efficiency of each valorization route, the total energy output was determined based on the energy content of the corresponding biofuels.

Scenario 1:

In the first scenario, algal biomass was utilized directly for biogas production without any form of pretreatment. As described in Section 3.3, the raw biomass yielded 273.76 ± 22.64 mL CH_4/g VS in the BMP test. Considering this figure and adopting a lower heating value (LHV) of 10 kWh per cubic meter of biomethane [36], the theoretical energy potential could be determined. Thus, the total energy yield from biogas was estimated to be 1804.90 ± 63.54 kWh per ton of dried algal biomass.

Scenario 2:

The second scenario combined bioethanol fermentation with anaerobic digestion of the residual biomass. The ethanol energy output was estimated based on concentrations obtained in factorial design experiments—such as 2.75 ± 0.18 g/L in experiment 5—along with ethanol's density and its LHV of 7.44 kWh/L. The calculation was as follows:

For the biogas, the BMP assay data from the fermentation residues were used. For example, in experiment 3, the stillage produced 198.50 ± 33.57 mL/g VS of biomethane. The same energy conversion method as in Scenario 1 was applied using the LHV of biomethane.

Summing up the energy contributions from both bioethanol and biomethane, the total bioenergy yield for each experimental condition was determined. The results for all experiments are illustrated in Table 2. Among these, experiment 2 produced the highest total energy output (809.58 kWh/ton of algae), while the lowest was observed in the center experiment (514 kWh/ton of algae). These estimations assume the complete recovery and conversion of both ethanol and biomethane into usable energy.

Table 2 also presents a thorough summary of the bioethanol and biomethane energy yields across the different experimental trials.

It is clear from Table 2 that the contribution of bioethanol to the total energy producible from algae biomass is lower than that of biomethane. To be more specific, the maximum energy that could be produced was calculated to be 1044.48 kWh/tn of algae. The contributions of biomethane and bioethanol were calculated to be 75.26% and 24.74%, respectively.

To enable a robust comparison across valorization routes, all energy yields were normalized to kWh per ton of dry algae. In addition to the experimental results of this study (Scenarios 1 and 2: anaerobic digestion and integrated ethanol–methane production from fresh algae), comparative data from Chatzimaliakas et al. [27] (Scenario 3: anaerobic digestion of dried algae; Scenario 4: ethanol–methane production from dried algae) were included.

Table 3 summarizes the bioethanol yields, methane potentials, and total energy recovery. Although the ethanol yields in this study (2.75 g/L; 45.13% theoretical) are lower than those from dried or pretreated algae, the process benefits from avoiding energy-intensive drying. The methane yields (198.50 mL/g VS) were consistent with the literature values. The total energy output of the ethanol–methane route (~810 kWh/t algae) was competitive, approaching values from standalone anaerobic digestion.

Scenario 1 (anaerobic digestion of fresh algae) achieved the highest total energy recovery (Figure 2), which is attributed to the nutrient richness of fresh biomass and the absence of energy loss from drying. In all cases, biomethane contributed more significantly to total energy than bioethanol.

Resource efficiency (RE), calculated as the mass of products (ethanol, methane, digestate) relative to the initial algal biomass, was highest for Scenarios 1 (7.00%) and 3 (7.56%) and lowest for Scenario 4 (5.09%). Digestate represented ~90% of the total RE, highlighting its dominant role in mass recovery. The low RE values across scenarios are primarily due to the high moisture content of algae. Thus, anaerobic digestion emerges as the most sustainable pathway for both energy and mass recovery from algal biomass.

Table 3. Comparison of bioethanol yields, methane potentials, and total energy recovery from various studies utilizing algal biomass.

Study	Feedstock	Bioethanol Yield (g/L or % Theo.)	Methane Potential (mL CH_4/g VS)	Total Energy Recovery (kWh/t algae)	Notes
This study	Fresh *Scenedesmus*-dominated microalgae	2.75 g/L (45.13%)	198.50	809.58	Enzymatic hydrolysis + AD of stillage
Harun et al. [34]	*C. infusionum* (alkali-treated)	~4.0 g/L	Not reported	Not reported	High ethanol via alkaline pretreatment
Passos et al. [26]	Mixed microalgae	Not applicable	220–280	~1000	Anaerobic digestion only
Liu et al. [20]	Dried *Chlorella* biomass	~3.2 g/L	~210	~700	Dry biomass, two-step valorization
Chatzimaliakas et al. [27]	Dried *Scenedesmus*	3.1 g/L (41%)	180–220	640–790	Integrated biorefinery, dried algae

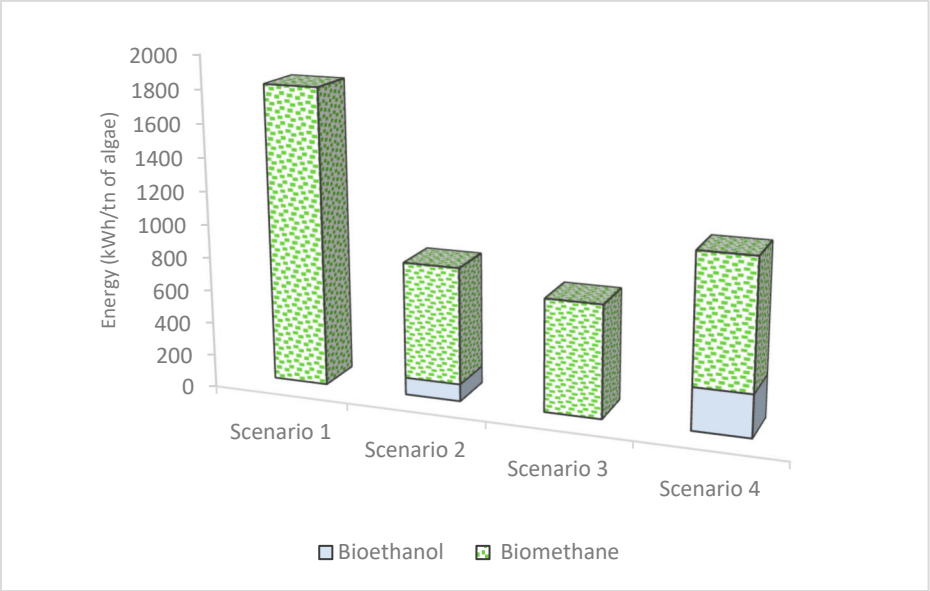

Figure 2. Total energy production of all valorization scenarios.

4. Conclusions

This work demonstrated the potential of freshly sedimented algae as a versatile substrate for the integrated bioethanol and biomethane production within a biorefinery framework. Through a factorial experimental design, key parameters influencing bioethanol production—such as sodium hydroxide concentration and enzymatic loadings—were systematically optimized. Under the most favorable conditions, ethanol concentrations reached 2.75 ± 0.18 g/L, with a corresponding yield of 45.13 ± 2.90% and minimal residual glucose, indicating efficient fermentation.

The anaerobic digestion of fermentation residues provided additional energy recovery, although with lower biomethane yields compared to untreated feedstock (198.50 ± 25.57 mL/g VS vs. 273.76 ± 22.64 mL/g VS, respectively). This outcome highlights a critical trade-off between extracting fermentable sugars and retaining digestible organics for methane production.

The energy output analysis showed that direct anaerobic digestion of fresh algae offered the highest yield (1804.90 ± 63.54 kWh/tn). However, the integrated bioethanol–biomethane route produced up to 809.58 kWh/tn, demonstrating the viability of multi-output strategies that align with circular economy principles. The resource efficiency

estimates also confirmed the superior performance of anaerobic digestion routes, particularly when high moisture content is maintained.

Although biomethane contributed the larger share of energy recovery, the inclusion of bioethanol offers complementary advantages. As a liquid fuel, ethanol is more easily stored, transported, and integrated into an existing fuel infrastructure, especially in transport applications. This functional flexibility makes it a valuable component in diversified bioenergy systems.

Overall, the findings support the technical feasibility of dual-stage algae valorization and emphasize the importance of selecting the most appropriate conversion pathway depending on energy priorities, infrastructure, and resource constraints. This study contributes valuable insights toward the advancement of sustainable, algae-based systems and their integration into future biorefineries.

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Abbreviations

The abbreviations used in this manuscript are as follows:

AD	Anaerobic Digestion
BMP	Biomethane Potential
d.w.	Dry Weight
LHV	Lower Heating Value
RED	Renewable Energy Directive
SSF	Simultaneous Saccharification and Fermentation

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