

## Article

# Balancing Biomass Yield and Lignocellulosic Recalcitrance for Methane and Energy–Economic Optimization of *Sida hermaphrodita*

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## Abstract

The aim of this study was to evaluate the effect of *Sida hermaphrodita* harvest timing on biomass composition, properties, and methane fermentation performance. In addition, an energy–economic assessment was performed for biomass obtained at different stages of the growing season. The economic assessment assumed CHP electrical and thermal efficiencies of 38% and 47%, electricity and heat prices of 0.18 and 0.05 EUR/kWh, respectively, month-specific agrotechnical costs, and OPEX equal to 30% of total energy revenue. The biomass exhibited clear seasonal changes, transitioning from a material with high bioavailability during the summer period to a structurally more recalcitrant substrate in the autumn and winter months, as indicated by increasing lignification and fibrous fraction contents. The highest CH<sub>4</sub> production yields, ranging from 300 to 320 mL/g VS, and maximum production rates of up to 33.5 mL/g VS·d were obtained between June and August. In December, the CH<sub>4</sub> yield decreased to 180 ± 9 mL/g VS, accompanied by a substantial deterioration in kinetic performance. Despite the relatively stable theoretical methane potential, which ranged from 405 to 430 mL/g VS, its conversion efficiency declined from 77.1% in the summer period to 41.9% in the winter period. Regression analysis confirmed the key influence of the C/N ratio and total solids content, with model fits reaching R<sup>2</sup> values of 0.74–0.80, while the structure of lignocellulosic complexes had a less pronounced but still relevant effect. The maximum CH<sub>4</sub> production per unit cultivation area, approaching 3380 m<sup>3</sup>/ha, was achieved in July–August, reflecting a balance between high specific methane yield and biomass productivity. At the same time, the results demonstrated that the maximum biomass yield did not translate into the highest energy and economic performance. The highest net economic return, 1789 ± 330 EUR/ha, was obtained in July, despite biomass yield being 13.6% higher in September. These findings indicate a seasonal decoupling between biomass yield and energy performance, highlight biomass quality as a critical determinant of anaerobic digestion efficiency, and support harvest-date optimization as a low-cost strategy for the practical use of *S. hermaphrodita* in agricultural biogas plants. Further long-term continuous and semi-continuous studies are required to validate process stability and performance under industrial operating conditions.

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## 1. Introduction

The accelerating energy transition in the European Union (EU), driven by increasingly stringent requirements for the decarbonisation of transport and industry, is increasing the importance of renewable fuels produced from non-food feedstocks [1]. The development of renewable energy sources is driven not only by climate protection considerations and the principles of sustainable development, but also by geopolitical factors, including the unstable international situation and armed conflicts, which have caused disruptions in global energy commodity markets [2]. The revised RED III Directive established a binding EU target of 42.5% renewable energy in gross final energy consumption by 2030, with an additional indicative increase to 45%. In the transport sector, it introduced the obligation to achieve either a 29% share of renewable energy or a 14.5% reduction in greenhouse gas emission intensity [3]. At the same time, a combined sub-target of 5.5% was set for advanced biofuels and renewable fuels of non-biological origin, which in practice strengthens the role of biomass that does not directly compete with food production [3]. Under these conditions, there is growing demand for perennial energy crops capable of maintaining stable biomass yields under limited agronomic inputs and suitable for cultivation on poor-quality and marginal soils [4].

Among the species that meet these criteria is *Sida hermaphrodita* Rusby (*S. hermaphrodita*), commonly known as Virginia fanpetals, a perennial crop characterised by high plantation persistence, a deep root system, and good adaptation to diverse environmental conditions that are less favourable for conventional crops [5]. After reaching full productive maturity, usually from the 3rd–4th year of plantation use, *S. hermaphrodita* can produce approximately 10–20 Mg total solids (TS) per hectare annually, although yields exceeding 20 Mg TS/ha-year have also been reported under more intensive cultivation conditions [6,7]. The importance of this species results not only from its yield potential but also from the quality and biochemical composition of its biomass. Depending on harvest timing and site conditions, the TS of *S. hermaphrodita* typically contains more than 85% volatile solids (VS). Cellulose, hemicellulose, and lignin generally account for approximately 30–45%, 20–30%, and 10–18% of TS, respectively [8]. This composition indicates that the biomass may represent a promising feedstock for bioenergy conversion processes, including anaerobic digestion (AD).

From the perspective of AD, the critical factor is not only the total VS content but primarily the biodegradability and biological accessibility of the organic fraction. In *S. hermaphrodita* biomass, as in other lignocellulosic feedstocks, the course of anaerobic digestion is also determined by the C/N ratio and the proportion of fibrous fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) [9]. The C/N ratio in *S. hermaphrodita* is highly variable, depending on site conditions, harvest timing, and developmental stage [10]. As the biomass matures, the proportion of structural carbohydrates and lignified cell-wall components increases, as reflected in higher NDF, ADF, and ADL contents, while protein content decreases and TS increases as a result of dehydration and cell-wall restructuring [11]. These changes may favour thermochemical conversion processes but limit susceptibility to enzymatic hydrolysis and reduce the efficiency of anaerobic methane fermentation [12].

Methane production during AD depends on the coordinated activity of hydrolytic, fermentative, syntrophic, and methanogenic microorganisms. In lignocellulosic sub-

strates, hydrolysis is frequently the rate-limiting stage because lignin and the structural organization of cellulose and hemicellulose restrict the release of soluble compounds required for subsequent acidogenesis and methanogenesis. Recent research on the co-digestion of lignocellulosic biomass with cow manure demonstrated that methane yield and production rate were closely related to biomass hydrolyzability and negatively associated with lignin content, confirming the importance of substrate structure for methane conversion [13]. Methanogenesis is also influenced by feeding conditions and the adaptive response of the microbial community. Feeding regime may affect the distribution of acetoclastic methanogens and their metabolic interactions, with recent evidence showing that feeding strategy played a major role in shaping methanogenic community structure and enriching *Methanothrix* populations [14]. Similarly, acclimated bacterial and methanogenic communities were able to maintain stable biogas production and methane concentration under fluctuations in substrate concentration [15]. These findings indicate that methane production is determined by the combined effects of substrate composition and hydrolyzability, the availability of degradation intermediates, feeding conditions, and the adaptive capacity of the methanogenic community.

Previous studies on the use of *S. hermaphrodita* for biogas production have confirmed its considerable methane potential, while also showing that high methane yields often require additional substrate pretreatment [16]. For biomass subjected to microwave treatment and thermohydrolysis, methane yields of 575–590 L CH<sub>4</sub>/kg VS were reported, i.e., approximately 39% higher than those obtained from untreated samples [17]. Similarly, the application of hydrodynamic cavitation increased lignocellulose solubilisation and methane yields to approximately 439 L CH<sub>4</sub>/kg TS [18]. These findings indicate that the main limitation is not the intrinsic energy potential of the biomass, but rather its restricted bioavailability resulting from lignocellulosic recalcitrance [19]. In practical terms, this necessitates capital-intensive pretreatment, which may substantially limit the scalability and economic viability of the technology under real operating conditions [20].

In this context, it is reasonable to ask whether the selection of an appropriate harvest timing can improve the overall energy and economic efficiency of the process and whether this strategy may represent either a simpler alternative or a complementary approach to pretreatment [21]. Harvest timing and pretreatment act at different stages of biomass utilization: earlier harvesting limits the development of lignocellulosic recalcitrance, whereas pretreatment disrupts the already established cell-wall structure after harvest. Despite these mechanistic differences, both approaches aim to increase the accessibility of biodegradable fractions and improve methane conversion efficiency. Earlier harvesting is associated with lower TS content, reduced lignification, and lower ADF and NDF values, which results in greater accessibility of biodegradable fractions during AD [22]. Consequently, the suitability of *S. hermaphrodita* as a feedstock depends not solely on its biochemical methane potential (BMP), but on the trade-off between biomass yield, biomass structure, actual CH<sub>4</sub> production performance, and the energy–economic outcome per hectare [8]. This approach enables a system-level assessment of biomass efficiency, extending beyond the conventional evaluation based exclusively on methane potential (BMP).

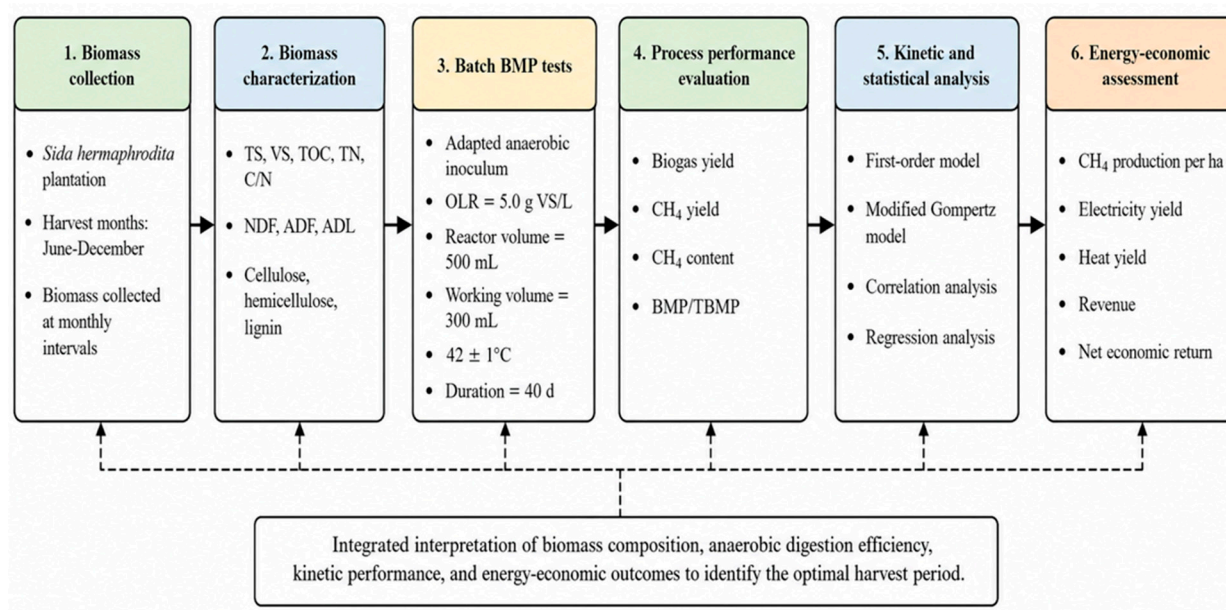
Despite the growing number of publications on *S. hermaphrodita*, there remains a lack of studies integrating the seasonal variability of biomass composition, including lignocellulosic fractions, with biomass bioavailability, anaerobic digestion performance, and energy–economic outcomes. Addressing this knowledge gap may enable the development of harvest management strategies that improve biomass utilization efficiency without the need for additional technological treatments. The aim of this study was to evaluate the effect of *S. hermaphrodita* harvest timing on biomass composition, properties, methane fermentation performance, and the resulting energy–economic outcome. The study included

physicochemical and lignocellulosic characterization of biomass harvested from June to December, batch biochemical methane potential tests, kinetic modelling, correlation and regression analyses, and an energy–economic assessment per unit cultivation area.

## 2. Materials and Methods

### 2.1. Study Concept and Experimental Design

The study was conducted using biochemical methane potential (BMP) tests operated in batch mode. Seven experimental variants (V) were established, corresponding to successive harvest months of *S. hermaphrodita* biomass: June (V1), July (V2), August (V3), September (V4), October (V5), November (V6), and December (V7). The experimental design was intended to evaluate the effect of seasonal changes in biomass properties on anaerobic digestion performance and CH<sub>4</sub> production kinetics. Based on the obtained results, relationships between biomass characteristics and CH<sub>4</sub> production were determined, empirical models were developed, and methane and energy yields per unit cultivation area were estimated. The analysis also included an assessment of economic performance, taking into account both the energy value of the biomass and agronomic production costs. The overall experimental design and analytical workflow applied in the study are presented in Figure 1.



**Figure 1.** Schematic overview of the experimental design and analytical workflow.

### 2.2. Materials

#### 2.2.1. *S. hermaphrodita* Biomass

The organic feedstock used in the study was *S. hermaphrodita* biomass obtained from a plantation located in Baldy, at the Teaching and Research Station of the University of Warmia and Mazury in Olsztyn, north-eastern Poland; 53°36.026' N, 20°36.625' E. The plantation was in a stabilised productive phase during the 3rd–6th year of use, thereby reducing the influence of plant-age-related variability and allowing the analysis to focus on seasonal changes in biomass properties. Biomass was sampled regularly from June (V1) to December (V7), three times per month, on the 10th, 20th, and 30th day. Immediately after harvesting, the material was mechanically comminuted to a particle size of 2–5 mm using a Retsch SM100 cutting mill (RETSCH GmbH, Haan, Germany) to standardise particle-size distribution and minimise its influence on the AD process. When immediate analysis was not possible, we stored the comminuted biomass at 4 ± 1 °C for no longer

than 72 h before performing physicochemical analyses and initiating the fermentation tests. These conditions were adopted to limit secondary biodegradation processes and changes in the chemical composition of the material. The characteristics of the biomass in the individual seasonal variants are presented in Section 3.

### 2.2.2. Anaerobic Inoculum

The inoculum consisted of anaerobic sludge obtained from a 1-MW electrical-capacity NAWARO-type agricultural biogas plant located in Łęguty, warmińsko-mazurskie Voivodeship, Poland; approximately 53°35' N, 20°24' E. The installation operates under upper-mesophilic conditions at  $42 \pm 1$  °C, with an organic loading rate of approximately 3.2 kg VS/m<sup>3</sup>·d and a hydraulic retention time of approximately 40 days. The feedstock consists of a mixture of substrates, including pig slurry, maize silage, distillery stillage, poultry manure with straw, glycerine, and plant biomass, which supports high microbial activity and functional diversity. The microbial community comprised bacteria detected with the EUB338 probe at approximately 68–72% and archaea detected with the ARC915 probe at approximately 23–27%, including mainly Methanosarcinaceae MSMX860 at approximately 11–15% and *Methanosaeta* MX825 at approximately 8–11%. Before use, the inoculum was homogenised and adapted to the lignocellulosic substrate in a reactor with a working volume of 5 L for 120 days, corresponding to approximately three hydraulic volume turnovers. During this period, the reactor was fed with *S. hermaphrodita* silage harvested in August, at a loading rate of approximately 2.5 g VS/L·d and a moisture content of  $90 \pm 1$ %. This procedure ensured stabilisation of microbial activity and reduced the risk of inhibition effects in the batch tests. The physicochemical characteristics of the anaerobic inoculum before and after adaptation to *S. hermaphrodita* biomass are presented in Table 1.

**Table 1.** Physicochemical characteristics of the anaerobic inoculum before and after adaptation to *S. hermaphrodita* biomass.

| Parameter                               | Unit                   | Before Adaptation | After Adaptation |
|-----------------------------------------|------------------------|-------------------|------------------|
| Total solids (TS)                       | g/L                    | 30.85 ± 1.60      | 33.80 ± 1.75     |
| Volatile solids (VSs)                   | g/L                    | 23.80 ± 1.45      | 26.50 ± 1.55     |
| Mineral solids (MSs)                    | g/L                    | 7.05 ± 1.05       | 7.30 ± 1.02      |
| Total nitrogen (TN)                     | g/L                    | 1.05 ± 0.10       | 0.95 ± 0.08      |
| Total phosphorus (TP)                   | g/L                    | 0.44 ± 0.05       | 0.41 ± 0.05      |
| Chemical oxygen demand, COD             | g/L                    | 40.50 ± 4.70      | 45.20 ± 5.10     |
| Total carbon (TC)                       | g/L                    | 18.50 ± 1.85      | 20.80 ± 2.10     |
| Total organic carbon (TOC)              | g/L                    | 12.40 ± 1.55      | 14.20 ± 1.65     |
| Inorganic carbon (IC)                   | g/L                    | 6.10 ± 0.80       | 6.60 ± 0.85      |
| Dissolved chemical oxygen demand (CODD) | g/L                    | 5.60 ± 0.90       | 7.10 ± 1.00      |
| Dissolved total organic carbon (TOCD)   | g/L                    | 1.40 ± 0.30       | 2.00 ± 0.40      |
| COD/TN ratio                            | –                      | 38.57 ± 5.79      | 47.58 ± 6.70     |
| TOC/TN ratio                            | –                      | 11.81 ± 1.86      | 14.95 ± 2.14     |
| pH                                      | –                      | 7.40 ± 0.07       | 7.55 ± 0.06      |
| Volatile fatty acids (VFAs)             | g/L                    | 1.70 ± 0.30       | 2.30 ± 0.35      |
| Total alkalinity capacity (TAC)         | g CaCO <sub>3</sub> /L | 5.60 ± 0.60       | 6.20 ± 0.65      |
| FOS/TAC ratio                           | –                      | 0.30 ± 0.05       | 0.37 ± 0.05      |

### 2.3. Biochemical Methane Potential (BMP) Tests

AD tests were conducted in batch mode using sealed OxiTop® Control respirometric reactors (WTW, Troisdorf, Germany) with a total volume of 500 mL and a working volume of 300 mL. The reactors were filled with a mixture of inoculum and substrate at an

initial organic load of 5.0 g VS/L. Control assays containing inoculum without substrate addition were prepared to determine the endogenous biogas production of the inoculum. Before incubation, the reactor headspace was deoxygenated by flushing with nitrogen for approximately 2–3 min to ensure anaerobic conditions. The reactors were then tightly sealed with OxiTop® pressure-measuring heads.

The reactors were placed in a TS 608/2-i thermostatic cabinet (WTW, Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany), designed for respirometric measurements using the OxiTop® system. The cabinet was equipped with forced-air circulation and an integrated temperature-control system. The temperature was maintained at  $40 \pm 1$  °C throughout the entire BMP test and was regularly monitored to ensure that it remained within the specified range. The tests were performed according to the standard operating procedure recommended by the manufacturer for monitoring anaerobic biodegradation and biogas production using the OxiTop® system.

Total biogas production was monitored based on pressure changes recorded in the reactor headspace. The measured pressure increase represented the production of total biogas, including primarily CH<sub>4</sub> and CO<sub>2</sub>, rather than methane alone. The recorded pressure changes were converted into dry gas volumes using the ideal gas law, taking into account the incubation temperature, reactor headspace volume, atmospheric pressure, and water-vapour pressure. Gas-volume calculations and corrections for experimental conditions were performed according to the standardized BMP procedures described by Holliger et al. [23] and Strömberg et al. [24]. Biogas composition, including CH<sub>4</sub> and CO<sub>2</sub>, was analysed chromatographically using a gas chromatograph equipped with a thermal conductivity detector (GC-TCD; Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA). Methane production was calculated by multiplying the corrected total biogas volume by the chromatographically determined CH<sub>4</sub> fraction. Accordingly, CO<sub>2</sub> and the remaining gaseous components were excluded from the calculated methane yield based on the measured biogas composition, whereas water vapour was accounted for during gas-volume correction. All biogas and methane volumes were converted to normal conditions, defined as a temperature of 0 °C (273.15 K) and an absolute pressure of 101.325 kPa, before calculating the specific gas yields. The process was continued until a biogas-production plateau was reached. All tests were performed in at least triplicate for each seasonal variant, and the results were corrected by subtracting the corresponding biogas and methane production measured in control assays containing inoculum without substrate addition. Methane yield was expressed as the normalized volume of CH<sub>4</sub> produced per unit of volatile solids of the substrate.

#### 2.4. AD Kinetic Modelling

Two kinetic models were applied to quantitatively describe the AD process and CH<sub>4</sub> production kinetics. The first-order model [25] was used to determine the rate constant (*k*). The modified Gompertz model [26] was applied to estimate the maximum CH<sub>4</sub> production rate (*r*) and the remaining kinetic parameters. The use of the Gompertz model was justified by the sigmoidal pattern of cumulative CH<sub>4</sub> production curves, which included a lag phase, a phase of intensive methane production, and a plateau. Model parameters were estimated using nonlinear least-squares regression based on the experimental data, while the goodness of fit was evaluated using the coefficient of determination (*R*<sup>2</sup>) and the root mean square error (RMSE). The theoretical biochemical methane potential (TBMP) was determined from biomass composition using the Buswell–Mueller equation, assuming complete mineralisation of organic matter to CH<sub>4</sub> and CO<sub>2</sub> [27]. The uncertainty of TBMP was estimated by error propagation, taking into account both the variability in biomass composition and the uncertainty of analytical determinations [28].

### 2.5. Calculation Methods

Calculations of the energy potential and energy–economic efficiency of the AD process for *S. hermaphrodita* biomass were performed for all seasonal variants based on BMP test results, agronomic costs, and the adopted technological and economic assumptions, both per unit mass and per unit cultivation area (ha). A combined heat and power (CHP) system, typical of agricultural biogas plants, was assumed. Biomass procurement costs included both fixed and variable costs. Month-specific harvesting and agrotechnical cost estimates were provided by the technical staff responsible for managing the experimental plots at the Teaching and Research Station in Bałdy. These estimates were based on actual machinery use, fuel consumption, labour input, biomass handling, and transport requirements. Fixed costs covered mineral fertilisation and basic crop-management operations for plantations in the 3rd–6th year of use, excluding establishment costs. The detailed technological and economic assumptions adopted in the analysis are summarised in Table 2.

**Table 2.** Key assumptions for energy and economic analysis.

| Parameter                      | Symbol                | Unit                      | Value    | Notes                                                                                                                                                                               |
|--------------------------------|-----------------------|---------------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Lower heating value of methane | LHV_CH <sub>4</sub>   | kWh/m <sup>3</sup>        | 9.97     | Standard value [29]                                                                                                                                                                 |
| Electrical efficiency of CHP   | η <sub>el</sub>       | –                         | 0.38     | Gas engines, 0.5–2 MW [30]                                                                                                                                                          |
| Thermal efficiency of CHP      | η <sub>th</sub>       | –                         | 0.47     | Heat recovery [30]                                                                                                                                                                  |
| Total efficiency of CHP        | η <sub>tot</sub>      | –                         | 0.85     | η <sub>el</sub> + η <sub>th</sub>                                                                                                                                                   |
| Electricity price              | P <sub>el</sub>       | EUR/kWh                   | 0.18     | EU average [31]                                                                                                                                                                     |
| Heat price                     | P <sub>th</sub>       | EUR/kWh                   | 0.05     | Local utilization [32]                                                                                                                                                              |
| Installation scale             | –                     | MW <sub>el</sub>          | 0.5–2.0  | Typical range                                                                                                                                                                       |
| Biomass procurement costs      |                       |                           |          |                                                                                                                                                                                     |
| June                           | C <sub>biom_Jun</sub> | EUR/ha                    | 360 ± 35 | Fertilization (NPK), low-resistance mowing, shredding, and transport of high-moisture biomass                                                                                       |
| July                           | C <sub>biom_Jul</sub> | EUR/ha                    | 380 ± 39 | Higher fresh biomass mass, increased fuel and transport costs                                                                                                                       |
| August                         | C <sub>biom_Aug</sub> | EUR/ha                    | 400 ± 39 | Increased fibre content and higher energy demand                                                                                                                                    |
| September                      | C <sub>biom_Sep</sub> | EUR/ha                    | 430 ± 43 | Higher mechanical resistance and increased fuel consumption                                                                                                                         |
| October                        | C <sub>biom_Oct</sub> | EUR/ha                    | 460 ± 47 | Worsening field conditions                                                                                                                                                          |
| November                       | C <sub>biom_Nov</sub> | EUR/ha                    | 490 ± 51 | High soil moisture, downtime, and increased fuel consumption                                                                                                                        |
| December                       | C <sub>biom_Dec</sub> | EUR/ha                    | 520 ± 56 | Winter conditions, downtime, and high logistical inputs                                                                                                                             |
| Operating expenditure          | OPEX                  | % of total energy revenue | 30       | Aggregated estimate of routine plant operating expenditure, including maintenance, labour, consumables, and auxiliary energy demand; applied uniformly to all harvest variants [33] |

The specific chemical energy potential of the produced CH<sub>4</sub> per unit of volatile solids (VSs) was calculated from CH<sub>4</sub> yield according to Equation (1):

$$E_{VS} = \text{BMP}_{\text{CH}_4} \cdot \text{LHV}_{\text{CH}_4} \quad (1)$$

where  $E_{VS}$  denotes energy [kWh/Mg VS], and  $BMP_{CH_4}$  is the methane yield [ $m^3 CH_4/Mg VS$ ].

The specific energy yield based on fresh matter (FM) was determined according to Equation (2):

$$E_{FM} = E_{VS} \cdot VS_{FM} \quad (2)$$

where  $VS_{FM}$  denotes the volatile solids content in fresh biomass [Mg VS/Mg FM].

Total  $CH_4$  production per unit cultivation area was calculated according to Equation (3):

$$V_{CH_4,ha} = M_{VS,ha} \cdot BMP_{CH_4} \quad (3)$$

where  $M_{VS,ha}$  denotes the volatile solids yield [Mg VS/ha].

The corresponding chemical energy yield per hectare was determined according to Equation (4):

$$E_{ha} = V_{CH_4,ha} \cdot LHV_{CH_4} \quad (4)$$

Electricity and heat production were calculated according to Equations (5) and (6):

$$E_{el,ha} = E_{ha} \cdot \eta_{el} \quad (5)$$

$$E_{th,ha} = E_{ha} \cdot \eta_{th} \quad (6)$$

The economic value of the electricity and heat produced was determined according to Equations (7) and (8):

$$Val_{el} = E_{el,ha} \cdot P_{el} \quad (7)$$

$$Val_{th} = E_{th,ha} \cdot P_{th} \quad (8)$$

where  $P_{el}$  and  $P_{th}$  denote the prices of electricity and heat, respectively.

The total energy value per hectare was calculated according to Equation (9):

$$Val_{tot} = Val_{el} + Val_{th} \quad (9)$$

Agronomic costs ( $C_{agro}$ ) included expenditures related to plantation management, biomass harvesting, and biomass transport, excluding plantation establishment costs. The operating costs of the biogas plant were estimated using an aggregated OPEX ratio equal to 30% of the total energy revenue ( $Val_{tot}$ ). The OPEX estimate represented routine plant operating expenses, including maintenance, consumables, labour, and auxiliary energy demand. Capital expenditure, depreciation, financing costs, taxes, and plantation establishment costs were not included. Therefore, the calculated indicator represents a comparative net operating return per unit cultivation area rather than the full investment profitability of a biogas installation. The assumed OPEX ratio was used as an aggregated estimate of routine plant operating expenditure, including reactor heating, mixing, pumping, maintenance, labour, consumables, and auxiliary energy demand. These components were not calculated separately because the study was not based on a specific full-scale installation. Costs associated with digestate separation, composting, external treatment, treatment or recirculation of the liquid fraction, land purchase or lease, and site-specific transport or disposal requirements were not explicitly modelled. Consequently, the analysis should be interpreted as a comparative assessment of harvest variants under common operational assumptions rather than as a complete plant-level net-energy or investment-feasibility analysis. The net operating return was determined according to Equation (10):

$$B_{net} = Val_{tot} - OPEX - C_{agro} \quad (10)$$

where  $B_{net}$  represents the net operating return of the analysed variant before capital charges, depreciation, financing costs, and taxes.

## 2.6. Analytical Methods and Measurements

The physicochemical characterisation of biomass, inoculum, and digestate was performed using standard analytical methods. Total solids (TS) in fresh mass (FM) were determined gravimetrically by drying at 105 °C (PN-EN 12880:2004; UF55 drying oven, Memmert GmbH & Co. KG, Schwabach, Germany), whereas volatile solids (VSs) were determined by ignition at 550 °C (PN-EN 12879:2004; L9/11 muffle furnace, Nabertherm GmbH, Lilienthal, Germany), and mineral solids (MSs) were calculated as the difference between TS and VS. The pH was measured potentiometrically using a pH meter Model 1000 L (VWR International, Radnor, PA, USA). Total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) were quantified by catalytic combustion using a TOC-L CPH/CPN analyser equipped with a TNM-L module (Shimadzu, Kyoto, Japan), while inorganic carbon (IC) was calculated as the difference between TC and TOC. Alternatively, TN was analysed using the Kjeldahl method with a K-435 digestion unit and a K-350 distillation unit (Büchi Labortechnik AG, Flawil, Switzerland). Protein content was then calculated as  $TN \times 6.25$ . Chemical oxygen demand (COD), total phosphorus (TP), and nitrogen in the liquid phase were determined spectrophotometrically using a DR 6000 spectrophotometer with an HT200S thermostat (Hach, Düsseldorf, Germany). Dissolved fractions, including CODD and TOCD, were determined after centrifugation and filtration through 1.2 µm filters using a ROTINA 380 centrifuge (Andreas Hettich, Tuttlingen, Germany). The structural composition of biomass, including cellulose, hemicellulose, lignin, NDF, ADF, and ADL, was determined according to the Van Soest method (PN-EN ISO 13906 [34]; PN-EN 16472 [35]) using an ANKOM 2000 analyser and the DaisyII system (ANKOM Technology, Macedon, NY, USA), whereas crude fibre was determined by the gravimetric method (PN-EN ISO 6865 [36]; Fibertec 8000, Foss Analytical A/S, Hillerød, Denmark). Reducing sugars were determined by the anthrone method using a DR 2800 spectrophotometer (Hach-Lange, Düsseldorf, Germany), glucose was determined enzymatically using a YSI 2700 Select analyser (YSI Inc., Yellow Springs, OH, USA), and lipids were determined by the Soxhlet method using a Büchi B-811 extraction system (Büchi AG, Flawil, Switzerland). Volatile fatty acids (VFA) and total alkalinity capacity (TAC) were determined by titration using a TitraLab AT1000 system (Hach, Mississauga, Canada), and the FOS/TAC ratio was calculated. Biogas volume was determined during BMP assays using the OxiTop® Control system (WTW, Troisdorf, Germany) or, in the continuous system, using a mass flow meter (Aalborg Instruments, Orangeburg, NY, USA). Biogas composition, including CH<sub>4</sub> and CO<sub>2</sub>, was analysed by gas chromatography with a thermal conductivity detector (GC-TCD; Agilent 7890A, Agilent, Santa Clara, CA, USA), using Haysep Q, Porapak Q, and Molecular Sieve columns, with Ar/He as the carrier gas at a flow rate of 15 mL/min. Control analyses were performed using a multicomponent gas analyser (GMF 430, Gas Data, Coventry, UK).

## 2.7. Statistical Methods

Statistical analysis was performed using STATISTICA 13.1 PL software (StatSoft, Tulsa, OK, USA). The normality of data distribution was verified using the Shapiro–Wilk test, while the homogeneity of variance was assessed using Levene’s test. The significance of differences among variants was evaluated by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test at  $p = 0.05$ . The uncertainty of derived parameters was determined by error propagation based on the standard deviations of the input variables. Regression analyses were performed using 21 observations, comprising seven harvest months from each of the three growing seasons. Three-year monthly averages were used

only for presenting seasonal trends in the tables and figures. Relationships between biomass properties and CH<sub>4</sub> production yield, biogas production, and kinetic parameters were evaluated using multiple regression with stepwise variable selection, while predictor collinearity was assessed before model interpretation. Model fit was evaluated based on the coefficient of determination (R<sup>2</sup>), and statistical significance was tested using the F-test. Model adequacy was verified by residual analysis, including assessment of residual normality and identification of trends relative to predicted values.

### 3. Results and Discussion

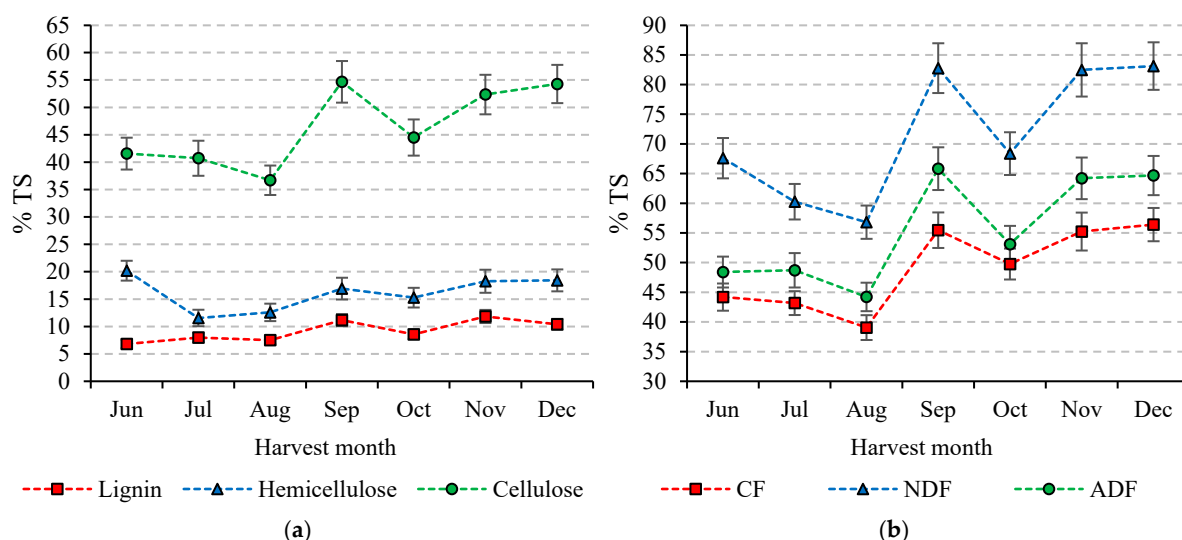
#### 3.1. Seasonal Variability of Biomass Properties

The properties of *S. hermaphrodita* biomass differed significantly among harvest dates ( $p < 0.05$ ). These seasonal changes reflected the transition from a substrate with high biological availability to a material with a well-developed lignocellulosic structure and reduced susceptibility to AD (Table 3, Figure 2). The results indicate a clear seasonal distinction between the June–August period, during which the biomass was characterized by a favourable physicochemical profile and limited lignification, and the September–December period, in which structural development and an increasing proportion of lignocellulosic fractions predominated. This pattern is typical of perennial lignocellulosic crops and results from the physiological transition from intensive vegetative growth to the stage of maturation and cell-wall lignification. This process leads to an increased proportion of secondary structural components and restricted enzymatic accessibility of the substrate. This was confirmed in the authors' previous study [37] on the seasonal dynamics of *Miscanthus × giganteus* biomass composition. A statistically significant increase in NDF content, reaching up to 83% TS, as well as increases in ADF and ADL, was observed with the progression of the growing season from June to January. This resulted in a systematic decrease in CH<sub>4</sub> production, from 281 ± 32 mL/g VS in June to 170–172 mL/g VS for winter harvest dates [37]. Similar trends were reported by Jablonowski et al. [7] in studies on seasonal harvests of *S. hermaphrodita*, where CH<sub>4</sub> production decreased from 204.2 m<sup>3</sup>/t VS in June to 124.1–131.1 m<sup>3</sup>/t in October harvest dates.

**Table 3.** Seasonal variability in the basic physicochemical properties of *S. hermaphrodita* biomass.

| Parameter | Unit      | June         | July        | August      | September    | October     | November    | December    |
|-----------|-----------|--------------|-------------|-------------|--------------|-------------|-------------|-------------|
| FM        | [Mg/ha]   | 32.7 ± 4.6   | 37.3 ± 5.1  | 42.6 ± 5.8  | 31.6 ± 3.9   | 31.2 ± 3.7  | 27.8 ± 3.2  | 25.1 ± 2.9  |
| TS        | [% FM]    | 27.5 ± 1.3   | 29.5 ± 1.0  | 28.2 ± 1.1  | 39.6 ± 0.9   | 35.9 ± 0.2  | 42.5 ± 1.0  | 45.0 ± 1.5  |
| VS        | [% TS]    | 95.8 ± 0.5   | 95.9 ± 0.3  | 94.3 ± 0.2  | 95.0 ± 0.3   | 95.7 ± 0.2  | 94.5 ± 0.4  | 93.8 ± 0.5  |
| TOC       | [% TS]    | 45.4 ± 1.9   | 50.2 ± 1.2  | 51.6 ± 2.4  | 48.7 ± 3.4   | 44.7 ± 1.3  | 44.0 ± 2.0  | 43.0 ± 2.0  |
| TN        | [% TS]    | 2.44 ± 0.27  | 2.24 ± 0.15 | 2.68 ± 0.05 | 1.79 ± 0.17  | 1.78 ± 0.06 | 1.44 ± 0.10 | 1.42 ± 0.08 |
| C/N ratio | [–]       | 18.6 ± 2.9   | 22.4 ± 2.1  | 19.2 ± 0.9  | 27.3 ± 4.2   | 25.1 ± 1.1  | 30.6 ± 3.5  | 30.3 ± 3.2  |
| TP        | [mg/g TS] | 19.1 ± 2.0   | 16.5 ± 1.6  | 21.4 ± 0.9  | 28.7 ± 2.5   | 38.1 ± 0.6  | 43.5 ± 2.0  | 46.1 ± 3.2  |
| Proteins  | [mg/g TS] | 152.5 ± 16.9 | 140.0 ± 9.4 | 167.5 ± 3.1 | 111.9 ± 10.6 | 111.3 ± 3.8 | 90.0 ± 6.3  | 88.8 ± 5.0  |
| Lipids    | [mg/g TS] | 35.4 ± 1.6   | 34.7 ± 5.2  | 25.9 ± 5.2  | 35.2 ± 2.3   | 25.6 ± 3.0  | 30.6 ± 0.5  | 27.6 ± 2.8  |
| Sugars    | [mg/g TS] | 25.9 ± 0.6   | 21.5 ± 1.2  | 18.3 ± 0.4  | 21.3 ± 2.3   | 19.0 ± 0.4  | 27.3 ± 2.0  | 27.3 ± 1.2  |

From June to August, the biomass retained the characteristics of a favourable substrate for AD (Table 3). The TS content was low, amounting to 27.5 ± 1.3% FM in June, 29.5 ± 1.0% FM in July, and 28.2 ± 1.1% FM in August, while the VS fraction remained high (94.3–95.9% TS). TN content was highest during this period, ranging from 2.24 ± 0.15 to 2.68 ± 0.05% TS, whereas protein content ranged from 140.0 ± 9.4 to 167.5 ± 3.1 mg/g TS. This corresponded to a C/N ratio of 18.6 ± 2.9 to 22.4 ± 2.1, which was significantly lower than in the later part of the season ( $p < 0.05$ ).



**Figure 2.** Seasonal changes in structural and fiber composition of *S. hermaphrodita* biomass as a function of harvest month: (a) lignocellulosic fractions (lignin, hemicellulose and cellulose); (b) fiber fractions (CF, NDF and ADF).

The relatively low C/N ratio and high nitrogen content observed during the summer period indicated a more favourable nutrient balance and a lower risk of nitrogen limitation during AD. However, the improved digestion performance cannot be attributed to the C/N ratio alone, because these changes coincided with lower lignification and reduced NDF, ADF, and ADL contents. The C/N ratio primarily influenced nutrient availability, whereas lignification and fibrous fractions restricted the physical and enzymatic accessibility of structural carbohydrates. Therefore, the favourable AD performance of the summer-harvested biomass resulted from the combined effects of nutrient balance and reduced lignocellulosic recalcitrance. These findings are consistent with previous studies showing that a C/N ratio of approximately 15–25 supports stable AD performance [38,39], while increasing lignification limits the hydrolysis and methane conversion of *S. hermaphrodita* biomass [7].

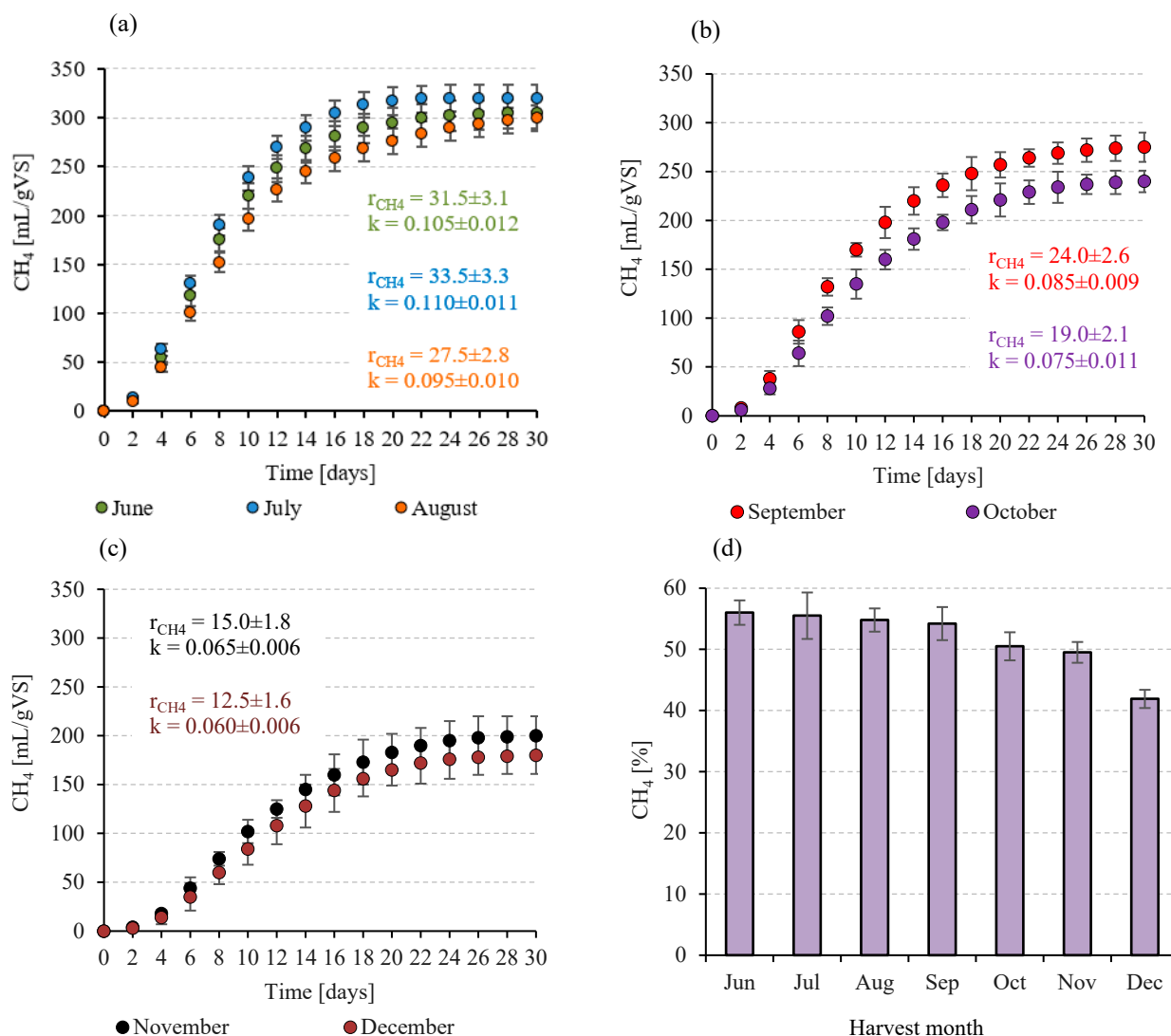
At the same time, biomass harvested during this period was characterized by a limited degree of lignification and a lower proportion of fibrous fractions (Figure 2a,b). NDF content ranged from  $56.82 \pm 2.8$  to  $67.61 \pm 3.4\%$  TS, ADF from  $44.23 \pm 2.4$  to  $48.71 \pm 2.9\%$  TS, and lignin from  $6.82 \pm 0.8$  to  $7.98 \pm 0.9\%$  TS (Figure 2b), which was significantly lower than from September onwards ( $p < 0.05$ ). The lower proportion of lignin and ADF fractions indicates reduced cross-linking of the cell-wall structure [8], thereby increasing the accessibility of cellulose and hemicellulose to hydrolytic enzymes [40]. This relationship was confirmed by Donkor et al. [20] in a study on the AD of lignocellulosic biomass subjected to alkaline pretreatment, in which a strong negative correlation ( $r = -0.87$ ) was demonstrated between lignin content and both hydrolysis efficiency and overall AD performance. Similar results were reported by Pokój et al. [41] in a study on *S. hermaphrodita* silage, where an increase in lignin content was found to limit the removal of fibrous fractions to 0–46%, thereby reducing the cumulative  $\text{CH}_4$  yield. Within this group, August represented the most favourable combination of substrate characteristics, as supported by previous studies showing that maximum biogas conversion efficiency occurs in the period preceding intensive tissue lignification [42]. In the study by Jablonowski et al. [7], *S. hermaphrodita* harvested before completion of stem lignification, i.e., in week 16, achieved the highest biogas production rate, whereas the sample with the highest lignin content, harvested in week 32, showed a clear inhibition of degradation. It was demonstrated that, at comparable VS content, the difference in biogas production rate was statistically signifi-

cant. TN content reached a maximum of  $2.68 \pm 0.05\%$  TS, protein content reached  $167.5 \pm 3.1$  mg/g TS, while NDF content decreased to  $56.82 \pm 2.8\%$  TS and ADF to  $44.23 \pm 2.4\%$  TS (Figure 2b), representing the lowest values recorded over the entire study period. Together with a C/N ratio of  $19.2 \pm 0.9$ , these parameters indicate optimal conditions in terms of both nutrient availability and the structural accessibility of organic matter.

A clear and statistically significant shift in biomass characteristics occurred from September onwards ( $p < 0.05$ ), when TS content increased to  $39.6 \pm 0.9\%$  FM (Table 3), while TN content decreased to  $1.79 \pm 0.17\%$  TS, resulting in an increase in the C/N ratio to  $27.3 \pm 4.2$ . The increase in the C/N ratio and the decrease in nitrogen content during this period are consistent with the findings of Bužinskienė [22], who, in a study on the optimization of AD of perennial grasses, found that delaying the harvest beyond the period of intensive growth led to an increase in C/N ratio, reduced nitrogen availability for methanogenic microorganisms, and decreased stability and efficiency of the AD process. Nitrogen deficiency at high C/N values may lead to fatty acid accumulation and pH reduction, which is consistent with the general mechanism of AD inhibition described by Wang et al. [12]. At the same time, a sharp increase in the proportion of fibrous fractions was observed (Figure 2b). NDF content reached  $82.77 \pm 4.2\%$  TS, ADF  $65.84 \pm 3.6\%$  TS, and lignin  $11.17 \pm 1.1\%$  TS, with NDF values remaining higher than ADF values throughout the entire study period. From September to December, the biomass exhibited features of pronounced structural development (Table 3; Figure 3). TS content remained high, ranging from  $35.9 \pm 0.2$  to  $45.0 \pm 1.5\%$  FM, whereas TN content remained low, ranging from  $1.42 \pm 0.08$  to  $1.79 \pm 0.17\%$  TS, resulting in a C/N ratio of  $25.1 \pm 1.1$  to  $30.6 \pm 3.5$ . Simultaneously, NDF content remained high at 68–83% TS, ADF at 53–65% TS, and lignin at  $8.56 \pm 0.9$ – $11.85 \pm 1.2\%$  TS (Figure 2b). This phenomenon was described by Schrey et al. [8] in studies on the maturation of *S. hermaphrodita* biomass, where an intensification of lignification processes and an increase in the proportion of structural fractions were observed in the late stages of vegetation. Donkor et al. [20] further reported the formation of lignin–polysaccharide complexes, which create spatially cross-linked structures that restrict microbial and enzymatic access to cellulose and hemicellulose. This phenomenon is particularly pronounced in the late vegetation stages of energy crops [20].

Despite the increase in cellulose content from  $36.71 \pm 2.7$ – $41.58 \pm 2.9\%$  TS during the summer period to  $52.36 \pm 3.6$ – $54.67 \pm 3.8\%$  TS in the later months, with a distinct maximum in September (Figure 2a), the concurrent increase in lignin and ADF suggests reduced accessibility of structural polysaccharides during anaerobic digestion. This interpretation is consistent with the established role of lignin–carbohydrate associations and cellulose crystallinity in limiting the microbial and enzymatic degradation of lignocellulosic biomass [20]. The increase in crude fibre content from  $39.06 \pm 2.1$ – $44.20 \pm 2.3\%$  TS to  $55.24 \pm 3.2$ – $56.42 \pm 2.8\%$  TS (Figure 2b) further confirms the progressive structural stabilization of the biomass and indicates the predominance of poorly biodegradable fractions. This phenomenon results in a reduced hydrolysis rate and an extended lag phase, as confirmed by kinetic studies on the anaerobic digestion of late-harvested *S. hermaphrodita* biomass reported by Zieliński et al. [43] and Kisiełewska et al. [44]. In all these cases, an increase in the ADL fraction above 10% TS was associated with a 30–60% extension of the lag phase and a decrease in the maximum  $\text{CH}_4$  production rate. Additionally, TOC content reached its highest values in July and August ( $50.2 \pm 1.2$ – $51.6 \pm 2.4\%$  TS; Table 3), and then decreased to  $43.0 \pm 2.0$ – $44.7 \pm 1.3\%$  TS in the autumn–winter months ( $p < 0.05$ ). TP content increased from  $16.5 \pm 1.6$ – $21.4 \pm 0.9$  mg/g TS to  $43.5 \pm 2.0$ – $46.1 \pm 3.2$  mg/g TS (Table 3), reflecting the concentration of mineral constituents with increasing TS content. This phenomenon is typical of above-ground biomass in late vegetation stages and has been widely described in the literature as a mineral concentration effect in plant dry matter, resulting from water loss and progressive tissue lignification [11]. Bilandžija et al. [45]

confirmed that delaying the harvest of *S. hermaphrodita* until spring resulted in an increase in lignin content to 25.4% TS and a decrease in N content to 0.65% TS, which reflects the seasonal trends described in the present study. Reducing sugar content remained low, at  $18.3 \pm 0.4$ – $27.3 \pm 2.0$  mg/g TS, throughout the entire study period and did not compensate for the increase in structural fractions.



**Figure 3.** Seasonal variation in methane production kinetics and biogas composition during anaerobic digestion of *S. hermaphrodita* biomass: (a–c) cumulative  $\text{CH}_4$  production curves with corresponding kinetic parameters ( $r_{\text{CH}_4}$  and  $k_{\text{CH}_4}$ ) for individual harvest periods: June–August (a), September–October (b), and November–December (c); (d) methane content in biogas (%). Kinetic parameters are expressed as  $r_{\text{CH}_4}$  [mL/g VS-d] and  $k_{\text{CH}_4}$  [1/d]. Experimental data and model fits are presented.

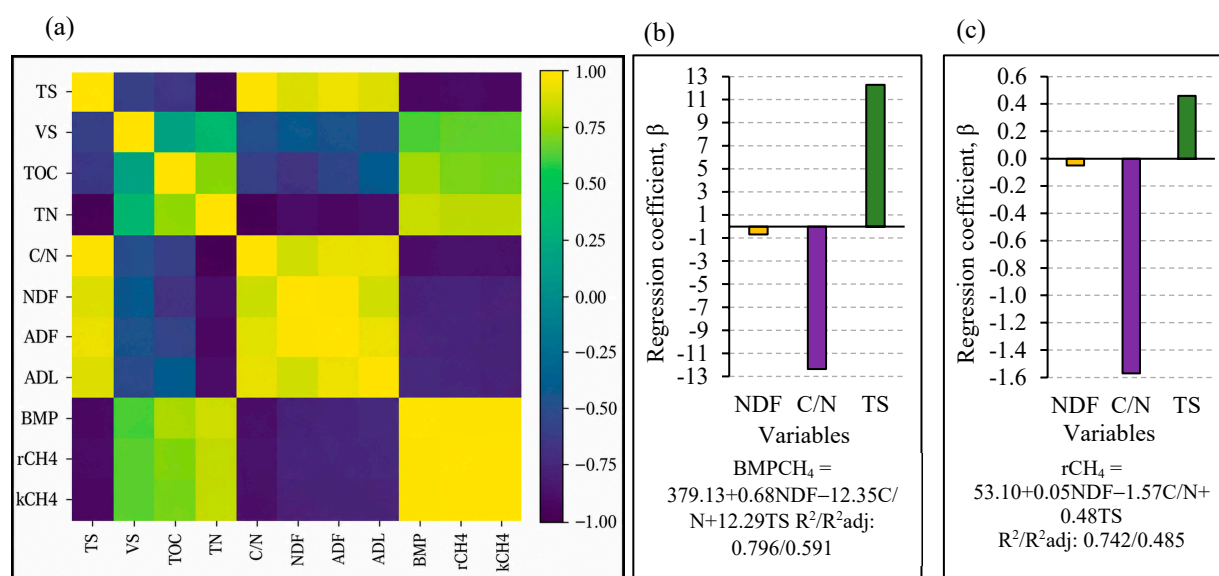
### 3.2. Seasonal Variation in AD Performance and Kinetics

AD process parameters for *S. hermaphrodita* biomass differed significantly among harvest dates ( $p < 0.05$ ). The observed changes were associated with seasonal modifications in biomass composition, particularly with increasing NDF and ADL contents (Table 4; Figure 4a–c). During the June–August period, the highest process efficiency was achieved (Table 4; Figure 3a), with  $\text{CH}_4$  yield ranging from  $300 \pm 13$  to  $320 \pm 15$  mL/g VS and biogas production from  $543 \pm 26$  to  $577 \pm 28$  mL/g VS. The  $\text{CH}_4$  content in biogas remained between  $54.8 \pm 1.9$  and  $56.0 \pm 2.0\%$  (Figure 3d). At the same time, the highest process rates

were observed, with  $rCH_4$  ranging from  $27.5 \pm 2.8$  to  $33.5 \pm 3.3$  mL/g VS-d and the kinetic constant  $kCH_4$  from  $0.095 \pm 0.010$  to  $0.110 \pm 0.011$  1/d (Figure 3a). The Gompertz model parameters were consistent with the experimental results (Table 4), indicating maximum  $V_{max}$  values of up to 322 mL/g VS and  $R_{max}$  values of up to 34.5 mL/g VS-d, together with the shortest lag phase duration, ranging from 2.20 to 2.80 d. Within this group, July showed the highest kinetic parameter values and some of the highest  $BMPCH_4$  values, whereas August maintained high  $BMPCH_4$  values while showing a simultaneous reduction in kinetic parameters.

**Table 4.** Biodegradability indicators and Gompertz model parameters of methane production from *S. hermaphrodita* biomass.

| Harvest Month | TBMP_VS [mL/g VS] | BMP/TBMP [%]   | Gompertz Model Parameters ( $CH_4$ ) |                       |               |       |                |
|---------------|-------------------|----------------|--------------------------------------|-----------------------|---------------|-------|----------------|
|               |                   |                | $V_{max}$ [mL/g VS]                  | $R_{max}$ [mL/g VS-d] | $\lambda$ [d] | $R^2$ | RMSE [mL/g VS] |
| June          | 410 $\pm$ 12      | 74.1 $\pm$ 3.2 | 305                                  | 32.0                  | 2.5           | 0.995 | 6.2            |
| July          | 415 $\pm$ 11      | 77.1 $\pm$ 3.0 | 322                                  | 34.5                  | 2.2           | 0.996 | 5.8            |
| August        | 405 $\pm$ 10      | 74.1 $\pm$ 2.8 | 302                                  | 28.5                  | 2.8           | 0.995 | 6.5            |
| September     | 420 $\pm$ 13      | 65.5 $\pm$ 3.4 | 277                                  | 25.0                  | 3.2           | 0.994 | 7.2            |
| October       | 415 $\pm$ 12      | 57.8 $\pm$ 2.9 | 242                                  | 20.0                  | 3.8           | 0.993 | 7.9            |
| November      | 425 $\pm$ 14      | 47.1 $\pm$ 3.1 | 202                                  | 16.0                  | 4.3           | 0.992 | 8.5            |
| December      | 430 $\pm$ 15      | 41.9 $\pm$ 2.7 | 182                                  | 13.5                  | 4.8           | 0.991 | 9.1            |



**Figure 4.** Pearson correlation heatmap between biomass composition parameters and methane production yield and kinetics (a). Regression coefficients ( $\beta$ ) for models describing methane potential ( $BMPCH_4$ ) (b) and methane production rate ( $rCH_4$ ) (c) as a function of NDF, C/N, and TS. Note: the positive coefficient for NDF results from partial collinearity among explanatory variables and does not reflect a direct biological effect.

The high process efficiency observed during the June–August period resulted from the favourable combination of physicochemical and structural characteristics of *S. hermaphrodita* biomass, including relatively low TS content, a favourable C/N ratio, limited lignification, and a lower proportion of NDF, ADF, and ADL fractions. This substrate composition increased the availability of fractions susceptible to hydrolysis, which translated into both high  $CH_4$  yield and favourable process kinetics. This relationship is consistent with the findings of Jablonowski et al. [7,46], who demonstrated a decline in the

methane production yield of *S. hermaphrodita* with delayed harvesting and progressive structural development of the biomass. Mechanistic confirmation of this relationship was provided by Pokój et al. [41], who indicated that during AD of *S. hermaphrodita* silage, non-structural fractions were removed much more efficiently than lignin and fibres. This indicates that the actual process efficiency was determined not only by the total organic potential of the biomass, but primarily by the biological availability of fermentable fractions.

From September onwards, a systematic and statistically significant deterioration of all analysed parameters was observed ( $p < 0.05$ ; Table 4; Figure 3b,c). CH<sub>4</sub> yield decreased from  $275 \pm 12$  mL/g VS to  $180 \pm 9$  mL/g VS in December, while biogas production declined from  $507 \pm 24$  to  $375 \pm 18$  mL/g VS. At the same time, the CH<sub>4</sub> content decreased from  $54.2 \pm 2.1$  to  $48.0 \pm 1.5\%$  (Figure 3d). The decline in yield was correlated with a marked reduction in process kinetics, with rCH<sub>4</sub> decreasing from  $24.0 \pm 2.6$  to  $12.5 \pm 1.6$  mL/g VS·d and kCH<sub>4</sub> from  $0.085 \pm 0.009$  to  $0.060 \pm 0.006$  1/d (Figure 3b,c), accompanied by an extension of the lag phase from 3.20 to 4.80 d (Table 4). The Gompertz model parameters reflected this trend, indicating a decrease in Vmax to 182 mL/g VS and Rmax to 13.5 mL/g VS·d. The deterioration in AD performance observed from September onwards was associated with the progressive increase in lignin, NDF, and ADF contents, which restricted microbial and enzymatic access to biodegradable organic matter. Lignin forms a structurally resistant matrix and lignin–carbohydrate complexes that limit the hydrolysis of cellulose and hemicellulose and consequently reduce their conversion into methane. However, the seasonal decline in methane yield should not be attributed exclusively to lignification. It was accompanied by an increase in TS content and a corresponding reduction in biomass moisture, both of which reflected progressive tissue maturation and structural stabilization. Although moisture content was not a direct determinant of methane yield under the standardized BMP conditions, because all reactors were operated at the same initial VS loading, it may be regarded as an indirect indicator of biomass maturity and reduced physical accessibility of biodegradable fractions. In addition, the content of reducing sugars remained relatively low and variable throughout the growing season and was therefore insufficient to compensate for the increasing proportion of lignified and fibrous fractions. The observed deterioration in methane production consequently resulted from the combined effects of structural recalcitrance, biomass maturation, and limited availability of readily biodegradable compounds.

Previous studies have demonstrated that disruption of the lignocellulosic structure can substantially improve the conversion of *S. hermaphrodita* biomass. Microwave thermohydrolysis performed at 150 °C for 15 min increased methane yield to 590 NL CH<sub>4</sub>/kg VS, corresponding to a 39.1% increase relative to untreated biomass [17]. Hydrodynamic cavitation applied for 20 min resulted in a methane yield of 439.1 L CH<sub>4</sub>/kg TS [18]. In semi-continuous AD of *S. hermaphrodita* silage, Pokój et al. reported that the removal efficiency of non-structural fractions reached 65–70%, whereas the corresponding removal of lignin and fibrous fractions ranged from 0 to 46% [41]. This difference confirms that the accessibility of organic matter strongly determines its effective conversion into methane. In addition, the absence of structural disintegration was associated with a 38% reduction in AD yield and a 57% extension of the lag phase compared with hydrothermally pretreated biomass [47].

Further evidence is provided by studies comparing theoretical and experimentally achieved conversion. Microwave thermohydrolysis increased TBMP utilization from approximately 67% in untreated samples to 93% after pretreatment [17]. Hydrodynamic cavitation improved the conversion degree by 25–32% [18]. Moreover, the enzymatic saccharification efficiency of untreated *S. hermaphrodita* biomass was only 17–34% of glucan, depending on the genotype, whereas OrganoCat fractionation substantially improved cellu-

lose accessibility [8]. These findings confirm that lignocellulosic recalcitrance is a major limitation to the effective utilization of the intrinsic methane potential of the biomass.

However, pretreatment requires additional equipment, operating expenditure, and energy inputs. This is particularly important for mechanical, microwave, thermal, and other processes using electricity or heat, because the energy consumed during pretreatment must be deducted from the gross energy recovered in the form of methane. Therefore, the technological effectiveness of pretreatment should be evaluated on the basis of the net energy balance rather than methane yield alone.

In contrast to energy-consuming post-harvest pretreatment, the present study demonstrates that substrate bioavailability can be improved through appropriate harvest-date selection. Biomass harvested during the summer period exhibited lower lignocellulosic recalcitrance, higher methane yield, and more favourable process kinetics, whereas harvesting during autumn and winter was associated with progressive lignification and reduced substrate conversion. Despite these differences, TBMP remained relatively stable at  $405 \pm 10$ – $430 \pm 15$  mL/g VS, while TBMP utilization decreased from  $77.1 \pm 3.0\%$  in July to  $41.9 \pm 2.7\%$  in December,  $p < 0.05$ . This indicates that the seasonal decline in methane production resulted primarily from reduced bioavailability rather than from a reduction in the intrinsic energy potential of the biomass.

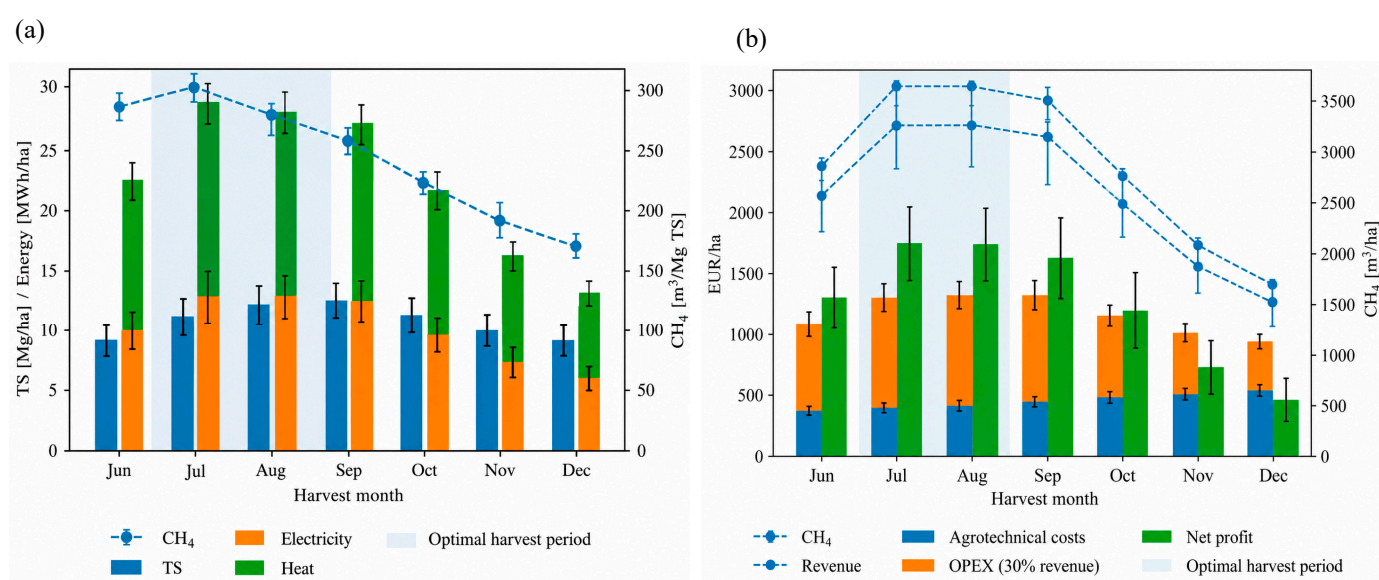
The relationships between biomass properties and AD process parameters were first visualized using correlation analysis (Figure 4a), which indicated that an increase in the proportion of structural biomass fractions, including NDF and ADL, as well as an increase in the C/N ratio, were associated with reduced CH<sub>4</sub> yield and production kinetics. At the same time, higher process parameter values were observed for biomass with a more favourable physicochemical composition, confirming the important role of the availability of biodegradable fractions [48]. To quantify these associations and evaluate the relative contribution of individual biomass characteristics, regression analysis was subsequently performed (Figure 4b,c). The regression results showed that CH<sub>4</sub> yield was most strongly associated with the C/N ratio, and an increase in this parameter resulted in a decrease in BMPCH<sub>4</sub> and the kinetic parameters of the process. TS content also exerted a significant effect, confirming the complex nature of the influence of biomass physicochemical properties on AD performance. Multiple regression models confirmed the significant effects of the C/N ratio and TS content on the course of the process, while also accounting for the contribution of fibrous fractions represented by NDF (Figure 4b,c). The obtained models achieved coefficients of determination of  $R^2 = 0.796$  for BMPCH<sub>4</sub> and  $R^2 = 0.742$  for rCH<sub>4</sub>, indicating a good fit while maintaining biological interpretability. However, NDF had a positive regression coefficient, contrary to the observed seasonal trend and the correlation analysis (Figure 4a). This sign reversal likely resulted from collinearity among the predictors and the limited number of observations. Therefore, it should not be interpreted as evidence that increasing NDF content improves AD performance. An analogous situation was reported by Liu et al. [49] in a study on BMP prediction based on lignocellulosic composition data from 36 substrates, where multiple regression using NDF, ADF, cellulose, and lignin as predictors generated apparent directional effects resulting from variable collinearity, which did not correspond to the biological relationships observed in batch experiments. Similar discrepancies were described by Monlau et al. [50], who, when modelling biogas production from lignocellulosic composition, indicated that without eliminating collinearity, for example using PLS, regression models may provide biologically misleading interpretations of coefficients. Therefore, these findings are partially consistent with literature reports and confirm that fibrous fractions, despite showing a positive coefficient in the model, actually limit process efficiency by restricting substrate accessibility.

The results demonstrate a clear seasonal shift in the bioavailability of *S. hermaphrodita* biomass. The June–August period was associated with the highest methane yields and the

most favourable process kinetics, whereas progressive lignification and accumulation of fibrous fractions during autumn and winter reduced substrate conversion. CH<sub>4</sub> yield decreased from 320 to 180 mL/g VS between the summer maximum and the December harvest, corresponding to an approximately 44% reduction. Importantly, selecting the optimal harvest period made it possible to avoid this decline without additional mechanical, thermal, chemical, or physicochemical pretreatment and therefore without the associated energy demand. Harvest timing should thus be regarded not only as an agronomic decision, but also as an energy-efficiency strategy that limits lignocellulosic recalcitrance before digestion and improves the net energy recovery from biomass.

### 3.3. Energy and Economic Analysis

The energy and economic outcomes differed significantly among the harvest dates of *S. hermaphrodita* biomass ( $p < 0.05$ ). These differences resulted from the opposing seasonal effects of specific methane yield and biomass yield. The observed changes were consistent with the seasonal dynamics of the AD process (Figure 3a–c) and with changes in the structural composition of the biomass, including an increase in the proportion of lignocellulosic fractions (Figure 2a,b; Figure 4a–c). The integrated effect of these relationships is presented in Figure 5a,b, where the optimal harvest period is additionally indicated. The obtained results indicate a seasonal trade-off between biomass yield and energy–economic efficiency, which is characteristic of lignocellulosic biomass and results from simultaneous quantitative and qualitative changes in the substrate [22]. An example is provided by the study of Von Cossel et al. [11], who demonstrated in a multi-year investigation of *S. hermaphrodita* (2014–2018) that, despite a 51% decrease in dry matter yield under a summer harvest strategy, the specific methane yield remained stable at  $279 \pm 8$  LN/kg VS, which was higher than that recorded for biomass harvested in winter. Jablonowski et al. [7] confirmed that double harvesting of *S. hermaphrodita* (June and October) resulted in a higher total biogas yield per hectare than a single autumn harvest, indicating that harvest-date management is a key tool for optimizing the economics of the biogas system.



**Figure 5.** Seasonal variability in biomass yield (TS), methane productivity, and energy production, including electricity and heat (a); seasonal variability in methane yield, revenue, costs, and net operating return depending on harvest date (b). In panel (a), TS yield, electricity production, and heat production are presented on the left y-axis, whereas CH<sub>4</sub> productivity is presented on the right y-axis. In panel (b), revenue, agrotechnical costs, OPEX, and net operating return are presented on the left y-axis, whereas CH<sub>4</sub> yield per unit cultivation area is presented on the right y-axis.

During the July–August period, the highest values of energy production and revenues were obtained (Figure 5a,b), while the differences between these months were not statistically significant ( $p > 0.05$ ). Area-based  $\text{CH}_4$  production (Figure 5b) ranged from  $2620 \pm 390$  to  $3384 \pm 460 \text{ m}^3/\text{ha}$ , corresponding to electricity production of  $9.9 \pm 1.5$  to  $12.8 \pm 1.8 \text{ MWh/ha}$  and heat production of  $12.3 \pm 1.8$  to  $15.9 \pm 2.2 \text{ MWh/ha}$  (Figure 5a). The maximum economic values were recorded in July, reaching  $3099 \pm 430 \text{ EUR/ha}$  in revenue and  $1789 \pm 330 \text{ EUR/ha}$  in net operating return. In August, however, the higher biomass yield compensated for the lower specific  $\text{CH}_4$  yield, resulting in a comparable area-based  $\text{CH}_4$  output. No statistically significant differences were observed between July and August ( $p > 0.05$ ). Similar relationships were reported by Von Cossel et al. [11] in studies on AD of *S. hermaphrodita*, indicating that maximum biomass yield does not directly translate into the highest  $\text{CH}_4$  production, and that the availability of biodegradable fractions is of key importance. Zieliński et al. [43] showed that, among nine *S. hermaphrodita* silages with different compositions, cellulose and ammonium nitrogen were the key predictors of methane yield, exceeding both VS content and other lignocellulosic fractions in predictive strength.

The values obtained in September did not differ significantly from those recorded in July and August ( $p > 0.05$ ). However, depending on the parameter analysed, they were on average approximately 4–8% lower. From October onwards, the energy and economic parameters deteriorated significantly ( $p < 0.05$ ).  $\text{CH}_4$  production decreased from  $3250 \pm 520 \text{ m}^3/\text{ha}$  in September to  $1521 \pm 240 \text{ m}^3/\text{ha}$  in December, accompanied by corresponding reductions in energy output and revenue (Figure 5a,b). At the same time, agrotechnical costs increased from  $360 \pm 35 \text{ EUR/ha}$  in June to  $520 \pm 56 \text{ EUR/ha}$  in December (Table 2). This increase was associated with the greater fibre content of mature biomass and less favourable harvesting conditions. These trends were consistent with the observed changes in lignocellulosic structure (Figure 4a–c). Previous studies have similarly reported higher energy demand and more difficult mechanical processing of mature biomass containing larger proportions of structural fractions [11,51,52].

When operating costs of the installation were assumed at 30% of total revenue, net operating return showed a clear downward trend with delayed harvesting (Figure 5b). Although the values obtained in July and August were on average higher than those recorded in September, these differences were not statistically significant ( $p > 0.05$ ), whereas from October onwards a significant decline in net operating return was observed ( $p < 0.05$ ). Although the highest biomass yield was recorded in September at  $12.5 \pm 1.5 \text{ t TS/ha}$ , which was approximately 13.6% higher than in July, this did not translate into improved system performance. In July, despite the lower yield of  $11.0 \pm 1.5 \text{ t TS/ha}$ , higher total revenue ( $3099 \pm 430 \text{ EUR/ha}$ ) and higher net operating return ( $1789 \pm 330 \text{ EUR/ha}$ ) were achieved, representing increases of approximately 4.2% and 8.3%, respectively, compared with September (Figure 5b). This clearly indicates that biomass quality during the summer period determined system performance to a greater extent than maximum biomass yield. A sensitivity analysis was performed for OPEX ratios ranging from 20% to 40% of total energy revenue, with 30% retained as the baseline scenario. The results are presented in Table 5.

**Table 5.** Sensitivity analysis of net economic return depending on the assumed OPEX ratio.

| Harvest Month | OPEX 20%<br>[EUR/ha] | OPEX 25%<br>[EUR/ha] | OPEX 30%<br>[EUR/ha] | OPEX 35%<br>[EUR/ha] | OPEX 40%<br>[EUR/ha] |
|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| June          | 1558 ± 297           | 1438 ± 279           | 1319 ± 260           | 1199 ± 241           | 1079 ± 223           |
| July          | 2099 ± 377           | 1944 ± 354           | 1789 ± 330           | 1634 ± 306           | 1479 ± 283           |
| August        | 2079 ± 366           | 1924 ± 343           | 1769 ± 320           | 1614 ± 297           | 1459 ± 274           |
| September     | 1949 ± 423           | 1800 ± 396           | 1652 ± 370           | 1503 ± 344           | 1354 ± 317           |
| October       | 1398 ± 331           | 1282 ± 311           | 1166 ± 290           | 1050 ± 269           | 934 ± 249            |
| November      | 888 ± 263            | 802 ± 246            | 716 ± 230            | 630 ± 214            | 544 ± 197            |
| December      | 599 ± 206            | 529 ± 193            | 459 ± 180            | 389 ± 167            | 319 ± 154            |

Net economic return was calculated as  $B_{\text{net}} = Val_{\text{tot}}(1 - s) - C_{\text{agro}}$ , where  $s$  denotes the assumed OPEX ratio. The 30% OPEX scenario corresponds to the baseline values used in the main economic analysis.

To assess the robustness of the economic results to changes in the assumed operating-cost ratio, a sensitivity analysis was performed for OPEX levels ranging from 20% to 40% of total energy revenue, with 30% retained as the baseline scenario. The resulting net economic returns for individual harvest months are presented in Table 5. Changes in the assumed OPEX ratio affected the absolute net economic return but did not alter the ranking of the harvest variants. July remained the most economically favourable harvest month across the entire analysed range, with the net economic return decreasing from 2099 ± 377 EUR/ha at an OPEX ratio of 20% to 1479 ± 283 EUR/ha at 40%. August consistently ranked second, with corresponding values ranging from 2079 ± 366 to 1459 ± 274 EUR/ha, while September remained the third most favourable variant. All harvest dates retained a positive net economic balance even at the highest OPEX level considered. However, the relative economic performance of late-season harvests declined markedly, particularly in November and December.

These findings confirm that July remained the most favourable harvest period across the analysed OPEX range. However, the results should be interpreted as a comparative, area-based assessment of harvest variants under common operational assumptions rather than as a complete determination of the net-energy sustainability or investment relative economic performance of an anaerobic digestion plant. Actual plant performance depends on the specific energy demand for reactor heating, mixing, pumping, feedstock handling, digestate separation, and treatment or recirculation of the liquid fraction. It also depends on the selected digestate-management pathway, including direct agricultural use, composting, external treatment, transport requirements, and compliance with local environmental regulations. Land ownership or leasing conditions may further substantially affect the economic outcome. These factors were not modelled separately in the present study and were represented only partially through the aggregated OPEX assumption. As emphasized by Gomez-Camacho et al. [53], a reliable assessment of AD sustainability requires the energy consumed by the process itself to be deducted from the gross energy recovered from biogas. Therefore, a complete plant-specific analysis should include measured auxiliary electricity and heat consumption, digestate- and liquid-management costs, CAPEX, depreciation, financing, taxation, land-access conditions, and local utilization options before industrial implementation.

Nevertheless, the assumptions adopted in the present study are consistent with those commonly used in preliminary technical, technological, and conceptual design studies for biogas installations. Such assumptions are routinely applied when comparing technological variants, assessing substrate suitability, estimating expected energy output, and identifying the most favourable operating scenario before a specific plant configuration is selected. Therefore, although the analysis does not constitute a complete investment-feasi-

bility study, it retains practical and application-oriented value as a comparative decision-support tool for harvest-strategy optimization and preliminary technological planning.

#### 4. Conclusions

This study was designed to identify the harvest period that provides the most favourable balance between biomass quality, methane production performance, and energy–economic return for *S. hermaphrodita*. The results demonstrated that seasonal changes in biomass composition strongly affected substrate biodegradability, methane production kinetics, and the final economic outcome.

Progressive structural recalcitrance was reflected by increasing lignification and fibrous fraction contents. Consequently, CH<sub>4</sub> yield decreased significantly from up to 320 mL/g VS in the summer period to 180 mL/g VS in December, despite the relatively stable theoretical methane potential of 405–430 mL/g VS. This indicates that the process was limited by the biological availability of the substrate, resulting from the increased proportion of lignocellulosic fractions, rather than by its total energy potential.

The highest process efficiency was achieved in the summer period, when the biomass was characterized by a favourable chemical composition and a lower degree of lignification. As the proportion of fibrous fractions and the C/N ratio increased, the course of anaerobic digestion deteriorated. At the same time, it was demonstrated that although biomass yield influenced total energy production, it was not the dominant factor. Despite a 13.6% higher biomass yield in September, the best net operating return, 1789 EUR/ha, was obtained for the July harvest, representing an 8.3% increase compared with September. This indicates the limiting role of biomass quality in determining energy yield and economic performance.

The obtained results confirm that the energy–economic effect results from a trade-off between biomass quantity and quality, with biodegradability playing a decisive role. Optimizing harvest timing may provide an effective means of increasing energy yield and the relative economic performance of biogas production, while reducing the need for additional process-intensification methods under the climatic conditions of Central Europe.

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