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Ultrasound-driven anaerobic digestion to improve energy production from crude glycerol

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Abstract: Anaerobic digestion of crude glycerol derived from biodiesel production is being studied as an alternative to valorization through methane production. The high organic load (1800 g COD L-1) of crude glycerol can cause kinetic stress, leading to inhibition of methanogenic microorganisms. To overcome this issue, an alternative approach is the use of ultrasound energy, which promotes cell wall and membrane disruption and releases intracellular material that enhances biodigestion. Considering this alternative, the main objective of this study was to test ultrasound pretreatment to facilitate the subsequent anaerobic digestion of crude glycerol.

Keywords: *Anaerobic digestion, Biogas production, Crude glycerol, Energy recovery, Ultrasound pre-treatment.*

1. Introduction

Biodiesel production represents a significant source of crude glycerol, a versatile by-product with a multitude of applications in diverse industrial processes. However, the purity of crude glycerol can vary considerably, with reported values ranging from 23% to 87% [1,2]. In order to meet the specific requirements for its intended use, it is necessary to effectively remove the impurities present, leading to increased selling costs and potential economic challenges [3,4]. Furthermore, the expansion of biodiesel production has resulted in an oversupply of glycerol, leading to a commercial devaluation that directly impacts the price of biodiesel. Therefore, it has become increasingly important to explore alternative methods for valorising glycerol [4].

One promising approach for valorising crude glycerol is biotransformation, whereby microorganisms are employed to convert it into compounds of higher economic value. Extensive research has been conducted into anaerobic digestion systems, which utilise crude glycerol as a valuable substrate for producing methane and hydrogen [5,6].

In order to enhance the efficiency of the digestion process, ultrasound treatment has emerged as a valuable physical pre-treatment method [7,8]. The application of ultrasound energy to microbial cells results in the disruption of cell walls and membranes, thereby facilitating the release of intracellular materials that promote biodigestion. Additionally, ultrasound treatment has been shown to enhance the disaggregation of biological flocs and the transformation of large organic particles into smaller, more easily digestible entities [9]. Therefore, the primary objective of this study was to investigate the energy valorization potential of crude glycerol through anaerobic digestion, with a focus on enhancing the overall efficiency by incorporating ultrasound pretreatment.

2. Materials and Methods

2.1. Complete Mixing Batch Digester

In a completely mixed batch digester (500 mL), 300 mL of inoculum and crude glycerol at concentrations of 0.2%, 1.7%, and 3.2% were added (Figure 1). Based on the characteristics of glycerol, potassium nitrate (C:N ratio of 25:1), disodium phosphate (C:P ratio of 120:1), and sodium bicarbonate (2

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g L^{-1}) were added. Anaerobic treatment was evaluated both with and without ultrasound pre-treatment. The physical pretreatment followed a methodology in which each sample was placed in ultrasound equipment (Selecta, 9 L) (Figure 2) for one hour per day, until reaching a total treatment time of 3, 9, and 15 h.

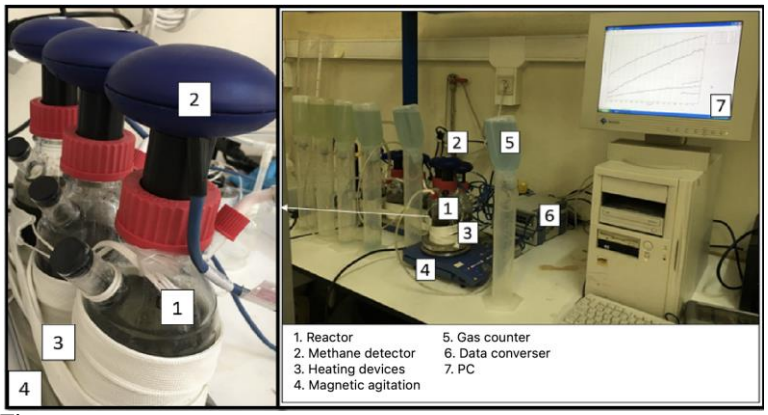

Figure 1.
Experimental layout (batch reactor and methane sensor).

Daily biogas production (% CH_4 and volume), COD (initial, after 15 days, and final-30 days), total solids (TS), volatile solids (VS), suspended solids (SS) (15 days and final-30 days) [10], and carboxylic acids in the digested material [11] were evaluated using a 2^2 factorial design. The experiment was conducted with temperature control in the mesophilic phase ranging from 35 °C to 40 °C.

Figure 2.
Ultrasound equipment.

2.2. Continuous Trials in an Upflow Anaerobic Sludge Blanket (UASB) Reactor

After conducting initial tests in a fully mixed batch digester, the most favorable experimental conditions were assessed in a UASB reactor with a capacity of 16.5 liters (Figure 3).

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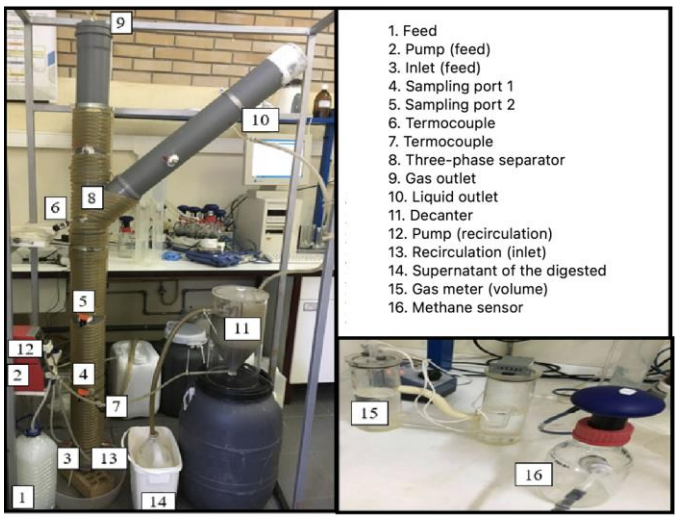

Figure 3.
Experimental setup of UASB reactor.

The UASB reactor was operated with an 11.5-day Hydraulic Retention Time (HRT). The reactor was continuously fed at an organic loading rate of 0.2% v/v glycerol; 0.34 kg COD m^{-3} d^{-1} (Condition A - weeks 1-3); 0.34 kg COD m^{-3} d^{-1} with an additional 15 h of ultrasound treatment (Condition B, weeks 4 and 5). The reactor feed (Figure 3 - 1 to 3) was pumped using a peristaltic pump controlled by a timer. The pump was operated for 15 min at a flow rate of 2 mL min^{-1}, followed by a 15-minute pause, and this cycle continued. This feeding pattern resulted in a daily flow rate of 1440 mL d^{-1} and an 11.5-day HRT. The internal temperature of the reactor was maintained between 40 °C and 45 °C. The effluent from a 7-liter settling tank was pumped (Figure 3 - 12) and recirculated back to the UASB reactor (Figure 3 - 13) at a rate of 16 mL min^{-1}. This recirculation process occurred four times daily, with each cycle lasting for 15 min. The pH and temperature of the reactor were closely monitored. Furthermore, various parameters were analysed and evaluated, such as gas volume, methane percentage, solids series, phosphorus, COD [10], and carboxylic acids in the residual material after anaerobic digestion [11].

3. Results and Discussion

3.1. Batch Experiments

The methane percentage (% CH4) was determined as an indicator of gas quality by utilising the glycerol exposure time to ultrasound and substrate concentration values. Statistical analysis employing a 2^2 factorial design resulted in Equation 1, which exhibited an R^2 value of 99.88% and a p-value of 0.003, thereby demonstrating the rejection of the null hypothesis.

$$CH_4(\%) = 27.943 + 0.01\,T_0 - 3.11\,T_3 + 3.03\,T_9 + 0.07\,T_{15} + 47.16\,S_{0.2\%} - 22.85\,S_{1.7\%} - 24.32\,S_{3.2\%} \quad (1)$$

where: $CH_4(\%)$, Methane percentage; T, ultrasound exposure time (h); $S_{0.2\%}$, 0.2% glycerol substrate; $S_{1.7\%}$, 1.7% glycerol substrate; $S_{3.2\%}$, 3.2% glycerol substrate.

Based on Figure 4, a lower glycerol concentration in the feed and a higher specific energy input (E_{sp}) they contributed to improved gas quality (C) and increased methane production, both in terms of glycerol mass (A) and SV (B). Bougrier et al. [12] have noted that an increase in E_{sp} leads to a decrease in the d50 value (representing particles with a diameter equal to or smaller than the original size).

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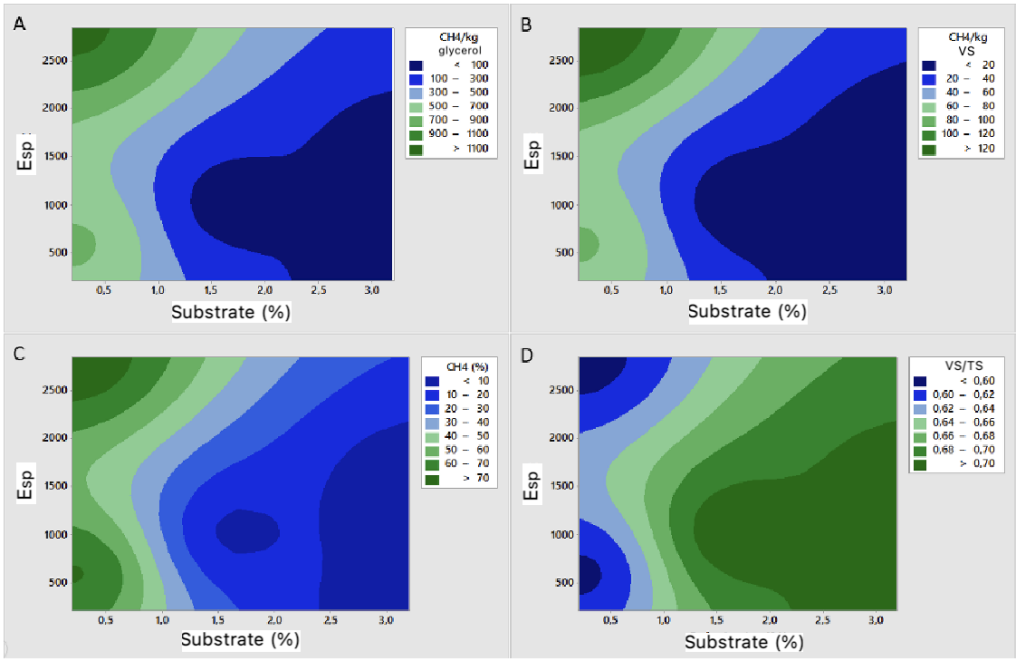

Figure 4.
Relation between E_{sp} and substrate concentration with the analysed parameters.

This decrease in particle size enhanced the contact area between the particles and microorganisms. Additionally, the VS/TS ratio (Figure 4 - D) decreases with higher E_{sp} and lower glycerol loading, indicating a less stabilised sludge during the microorganism growth phase [13,14].

According to Weemaes and Verstraete [9], the exposure of microbial cells to ultrasound energy releases intracellular materials. For the 0.2% glycerol concentration, the methane volume increased from 575 to 707 mL after 15 h of substrate exposure to ultrasound, representing a 23% increase. In contrast, for 1.7% glycerol concentration and nine h oursof ultrasound exposure, the methane volume obtained was 55 mL. For the 3.2% glycerol concentration, the methane volume was 23.5 mL for 3 hours.

The experimental trials in which glycerol was pretreated with ultrasound showed significant COD removal, ranging from 30% to 80%, as depicted in Figure 5. These removal values were similar to those obtained by Ma et al. [15], who achieved 75% COD removal in the anaerobic co-digestion of glycerol and potato processing wastewater (2 mL glycerol L^{-1} wastewater) in a continuous UASB reactor and by Silvestre et al. [16], who achieved 57% COD removal in the co-digestion of glycerol (1.25% v/v) and sewage sludge in a continuous complete mixing reactor. However, these values were lower than those reported by Athanasoulia et al. [17] and achieved COD removal rates ranging from 88% to 96.4% in the co-digestion of crude biodiesel glycerol and domestic wastewater sludge.

The production of the three identified acids was highest when using a glycerol concentration of 1.7% and a 9-hour ultrasound treatment. Only oxalic acid was detected in the case of 0.2% glycerol, whereas for 3.2% glycerol, both oxalic acid and glutaric acid were produced (Figure 6). During anaerobic digestion, glycerol is converted to propionic acid. If acetogenic or methanogenic microorganisms do not metabolize this acid, it accumulates in the final digestion product.

Additionally, propionic acid concentration increases with higher glycerol loading because excessive organic loading affects the subsequent stages of acidogenesis [17,18]. The formation of glutaric acid is directly linked to the activity of the lipase enzymes released by microorganisms [19]. Glutaric acid was not detected at low glycerol concentrations (0.2%); however, it was produced at concentrations of 1.7% and 3.2% glycerol.

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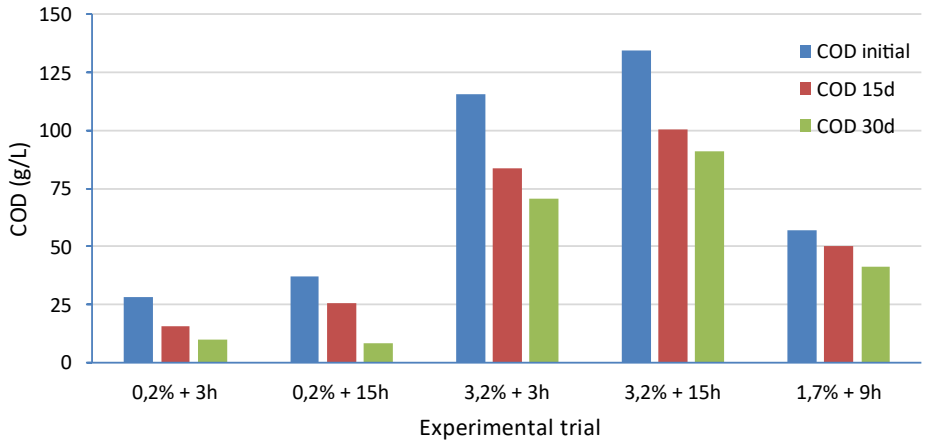

Figure 5.
COD removal: ultrasound pretreatment of glycerol.

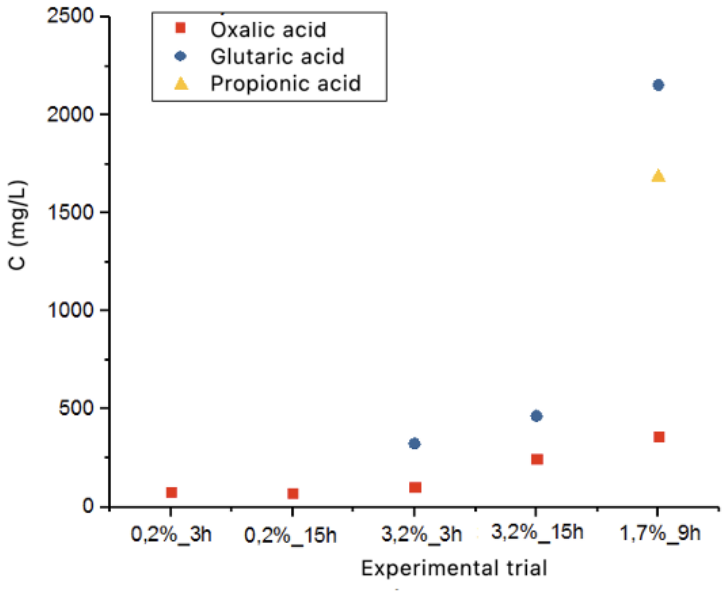

Figure 6.
Carboxylic acid in digestate, with substrate pre-treatment by ultrasound.

The results obtained for a glycerol concentration of 0.2% showed the most satisfactory outcomes in terms of both biogas volume and quality, with a methane percentage of 78%. Increasing the ultrasound exposure from 3 h to 15 h led to a significant increase in methane production: 70% based on volatile solids (VS) concentration, 60% based on feed chemical oxygen demand (COD) mass, and 60% based on glycerol mass.

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Table 1.
Biogas and methane production in experimental trials with glycerol pretreatment by ultrasound.

Time ultrasound (h)	Substrate (%)	E_{sp}	CH_4 (%)	COD (g L^{-1})	L biogás kg^{-1} COD	L CH_4 kg^{-1} COD	VS (g L^{-1})	L biogas.kg^{-1} VS	L CH_4 kg^{-1} VS	L biogás kg^{-1} glycerol	L CH_4 kg^{-1} glycerol
-	0.2	-	75	1.1	705	530	5.9	129	97	1248	938
3	0.2	586	70	1.1	581	409	5.4	116	82	1028	723
15	0.2	2846	77	1.1	848	652	5.1	181	139	1501	1153
-	3.2	-	3.6	17.4	35.4	1.3	13.5	46	1,7	63	2.3
3	3.2	215	2.2	17.4	62	1.4	12	89	2	110	2.4
15	3.2	1387	2.1	17.4	7.5	0.16	9.1	14	0.3	13	0.3
-	1.7	-	5.1	9.2	34.7	1.8	14.4	22	1.1	61	3.1
9	1.7	1029	8.1	9.2	73	5.9	6.4	105	8.5	129	11

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Comparing biogas production without ultrasound pretreatment to that with 15 h of pretreatment at a glycerol concentration of 0.2%, there was an increase of 23% based on VS concentration, 40% based on feed COD mass, and 23% based on glycerol mass (Table 1).

Wang et al. [20] demonstrated that anaerobic digestion coupled with ultrasound pretreatment, using a power of 200 W and a frequency of 9 kHz for various periods, resulted in methane production increases of 15%, 38%, 68%, and 75% for 10, 20, 30, and 40 min, respectively. Similarly, Martinez et al. [8] observed a 14% increase in methane production in a batch anaerobic reactor utilising ultrasound pre-treatment with sewage sludge. This can be attributed to the ultrasound-induced floccule disaggregation and oxidative breakdown of recalcitrant compounds (Table 1).

However, for feed with glycerol concentrations of 3.2% and 1.7%, methane production was considerably low, indicating that ultrasound pre-treatment's impact was insignificant. The limiting factor appeared to be the production of volatile fatty acids during the intermediate stage, likely because of the high COD concentration in the feed, which adversely affected the overall digestion process performance (Table I). Athanasoulia et al. [17] also observed this inhibition of anaerobic digestion, who used 4% glycerol as the substrate.

3.2. Continuous Trials - UASB (Upflow Anaerobic Sludge Blanket) Reactor

In continuous experiments conducted using an Upflow Anaerobic Sludge Blanket (UASB) reactor, COD removal reached a maximum of 20% for a feed rate of 0.34 kg COD m^{-3} d^{-1} (A). Pre-treatment of glycerol with ultrasound significantly enhanced COD removal, resulting in removal efficiencies ranging from 60 to 69% (B) (Figure 7 - [1]). The average phosphorus consumption was 2 and 0.8 mg L^{-1} for A and B, respectively (Figure 7 - [2]). Pretreatment facilitated the initial breakdown of molecules during hydrolysis and acidogenesis, leading to higher COD removal and reduced phosphorus removal requirements in the anaerobic digestion process.

The VS/TS ratio, which represents the biomass concentration in the reactor, is typically considered optimal, around 0.67 for UASB reactors [13,14]. This study's ratio obtained was slightly lower than the literature values (ranging from 0.44 to 0.61). However, no direct correlation was observed between this ratio and the methane production. The average TS removal rates were 30% and 45% for A and B, respectively. Lin et al. [21] reported that the co-digestion of landfill leachate and sewage resulted in a 45% reduction in volatile solids (VS). In the experimental feed, volatile solids accounted for 64% and 74% of the feed composition in treatments A and B, respectively. Ultrasound treatment contributed to particle size reduction, as previously discussed.

In the case of the continuously operated Upflow Anaerobic Sludge Blanket (UASB) reactor, three specific carboxylic acids were formed, corresponding to the findings from the batch digestion experiments. Oxalic acid was consistently present throughout the experimental period, with concentrations ranging between 9 and 18 mg L^{-1} (equivalent to a loading rate of 0.34 kg COD m^{-3} d^{-1} in condition A) and 11 to 13 mg L^{-1} (0.34 kg COD m^{-3} d^{-1} with an additional 15-hour ultrasound treatment in condition B). The formation of glutaric acid, associated with microbial lipase activity [19], was observed at the 0.34 kg COD m^{-3} d^{-1} loading rate. The production of propionic acid under conditions A and B resulted from the metabolic pathways described by Silva et al. [22]. Notably, acid formation was more pronounced in condition A than in condition B, along with higher phosphorus consumption.

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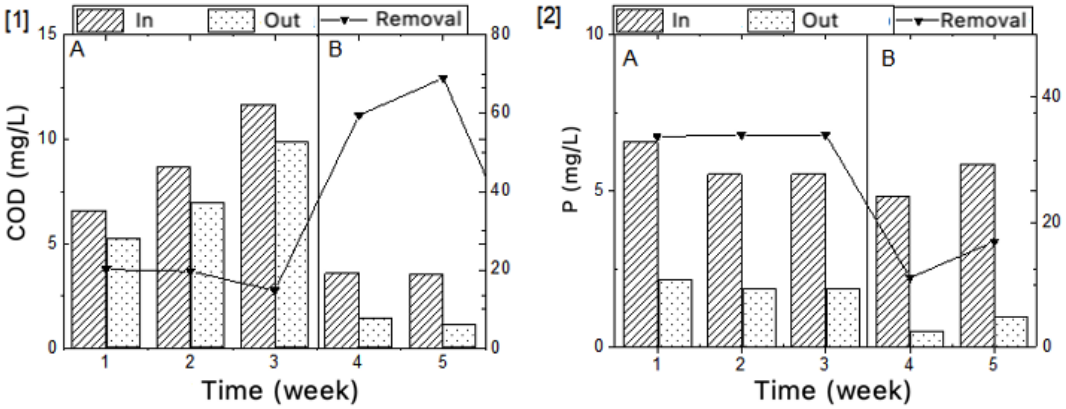

Figure 7.
Concentration at the inlet, outlet, and removal of [1] COD and [2] phosphorus in the UASB reactor.

In the continuously fed UASB reactor with a loading rate of 0.34 kg COD m^{-3} d^{-1}, the methane volume generated through anaerobic digestion varied between 1.3 and 3.6 m^3 CH_4 kg^{-1} glycerol d^{-1} (condition A). However, when an identical feed underwent a 15-hour ultrasound pretreatment, the daily methane production over 15 days ranged from 3.2 to 4.0 m^3 CH_4 kg^{-1} glycerol (condition B) with an average daily production of 3.6 m^3 CH_4 kg^{-1} glycerol. Ultrasound pretreatment resulted in a significant 43% increase in methane production compared to the control assay, surpassing the 23% increase observed in the batch system.

The higher methane production observed under condition B was directly correlated with increased removal rates of Chemical Oxygen Demand (COD) (60-69%) and Total Solids (TS) (74%), as well as a lower concentration of carboxylic acids in the digested material (88 g L^{-1}). The average methane production of 3.7 m^3 CH_4 L^{-1} glycerol and 560 L CH_4 kg^{-1} COD removed m^{-3} (condition B) exceeded the values reported in the literature. Nghiem et al. [23] achieved methane production of 1.3 m^3 CH_4 L^{-1} glycerol in an utterly mixed digester (50 L) during the co-digestion of 0.63% (v/v) glycerol with sewage sludge.

The quality of biogas, expressed as a percentage of methane, describes the variations observed in the digester throughout the process. For a feeding rate of 0.34 kg COD m^{-3} d^{-1}, the methane percentage ranged from 62% to 79% (Condition A) and 79% to 81% (Condition B), without and with ultrasound pretreatment, respectively. Athanasoulia et al. [17] studied the co-digestion of sewage sludge with different glycerol concentrations in a continuously stirred tank anaerobic digester and obtained 78% methane in the biogas. Using a continuous UASB reactor, Ma et al. [15] achieved a methane concentration of 59% during the co-digestion of potato processing wastewater and glycerol.

For the gas quality assessment, the energy gains were estimated according to Eriksson [24], considering the current price of electricity at 0.1652 € per kWh. The calculations related to biogas production per mass of glycerol and volatile solids (VS) concentration. A lower glycerol concentration yielded better results when comparing the energy gains in euros per amount of substrate added. Moreover, ultrasound pretreatment increased the energy gain by 42%, ranging from 4.70 to 6.40 € per kg of glycerol (average of 5.80 € per kg glycerol). The production concerning the VS concentration showed an 87% increase from Condition A to Condition B, corresponding to values between 157 € and 357 € per kg of VS (Condition A) (average of 306 € per kg of VS), represented in Figure 8.

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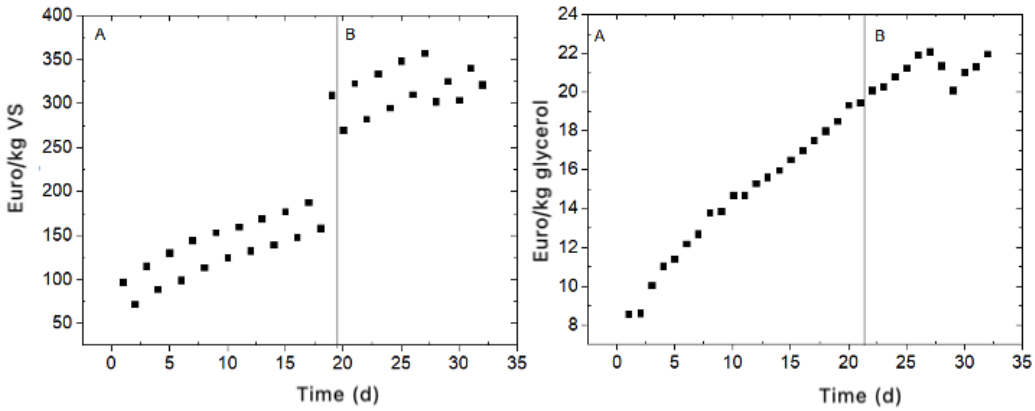

Figure 8.
Economic value of biogas generated in the UASB reactor.

4. Conclusions

The batch digestion experiments yielded the most satisfactory results when conducted with a glycerol concentration of 0.2% and subjected to a 15-hour ultrasound pretreatment.

The composition of the obtained biogas corresponded to 77% methane, and the volume reached 1153 L CH4 kg^{-1} glycerol. In the UASB reactor, with a feeding rate of 0.34 kg COD m^{-3} d^{-1} and ultrasound pretreatment, a methane volume of 4000 L CH_4 kg^{-1} glycerol was achieved.

Regarding the economic value of the biogas generated with ultrasound pretreatment, there was a 43% increase in the added value compared to the same loading rate without pretreatment (5.80 € per kg glycerol). This value exceeded the corresponding increase in energy valorisation observed in the batch experiment, which amounted to 23%. The batch experiment also recorded the highest volume of production and superior biogas quality (79–81% CH_4). Additionally, this experimental setup was associated with the highest removal rates of COD (60–69%) and TS (74%), along with the lowest concentration of carboxylic acids in the digested material (88 g L^{-1}).

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