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The Use of Solidified Carbon Dioxide in the Aerobic Granular Sludge Pre-Treatment before Thermophilic Anaerobic Digestion

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Abstract: The most common technology for the recovery of energy and valuable materials from sewage sludge is anaerobic digestion (AD). Ensuring thermophilic conditions during AD has been proven to cause process intensification and an improvement in its final outcomes. Nonetheless, the search is underway for other methods to bolster the effectiveness of the AD of aerobic granular sludge (AGS), which is characterized by a compact and complex structure. A prospective AGS pre-treatment technology entails the use of solidified carbon dioxide (SCO_2). The present study focused on an evaluation of the AGS pre-treatment with SCO_2 on the thermophilic AD technological effects. It evaluated the effect of the SCO_2 pre-treatment method on changes in the concentrations of organic and biogenic compounds in the dissolved phase and the yield and kinetics of biogas and methane production in periodical reactors, as well as enabled the development of an empirical organizational model of biogas production. SCO_2 introduced to AGS caused an increase in the content of COD, N-NH_4^+, and P-PO_4^{3-} in the AGS dissolved phase at SCO_2/AGS volumetric ratios ranging from 0 to 0.3. A further increase in the SCO_2 dose did not cause any statistically significant differences in this respect. The highest biogas and methane yields were obtained at SCO_2/AGS of 0.3 and reached 482 ± 21 cm^3/gVS and 337 ± 14 cm^3/gVS, respectively. The higher SCO_2 doses used led to a significant decrease in the pH value of the AGS, which, in turn, contributed to a decreasing CH_4 concentration in the biogas.

Keywords: aerobic granular sludge (AGS); pre-treatment; solidified carbon dioxide (SCO_2); anaerobic digestion; thermophilic conditions; biogas; methane

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1. Introduction

The management of sewage sludge generated during the biodegradation of contaminants has undergone substantial transformation [1]. Until recently, these contaminants were perceived as onerous to the environment and difficult to safely manage [2], having high concentrations of heavy metals, posing a sanitary risk, being highly loaded with organic compounds susceptible to putrefaction, and causing odor nuisance and aerosol emissions [3]. Continuously modified and upgraded economic processes, saving raw materials and energy, minimizing material losses, and reducing the emissions of hardly degradable and toxic substances into the natural environment significantly influenced the characteristics and improved the quality of raw sewage discharged into sewage systems [4]. All these changes had a direct positive impact on the quality of sewage sludge, which is currently treated as a practical source of fertilizing substances and energy [5]. These transformations have contributed to the successful implementation of the bio-economy assumptions in municipal and industrial wastewater treatment facilities [6].

The most common technology for the recovery of energy and valuable materials from sewage sludge is anaerobic digestion (AD) [7]. It is a well-known and optimized method, as indicated by a vast number of closed digesters exploited in the technical scale. It is noteworthy, however, that despite high technological advancement and the immense diversity of the available solutions for sewage sludge AD, the search is still underway for solutions ensuring AD intensification [8]. The main focus of interest among scientists, technologists, and operators of such bio-energetic systems is enabling the undisturbed exploitation of digesters and ensuring high technological effectiveness of AD at a high organic load rate (OLR) and short hydraulic retention time (HRT) [9]. Optimization works conducted in this respect would enable bio-methane production and the stabilization of significantly greater volumes of sewage sludge in small-cubature digesters, which, in turn, would directly contribute to an increased energy gain [10,11].

Increasing the temperature of methane fermentation and conducting it under thermophilic conditions are well-known technological means that ensure expected outcomes [12,13], like, e.g., an increased rate of biochemical reactions, improved effectiveness of the biodegradation of organic substances and the mineralization of sewage sludge, increased effectiveness of biogas and methane production, significantly shortened HRT and increased OLR, and also partial hygienization of fermented substrates [14]. The process weaknesses relate to the sensitivity of anaerobic microflora to slight fluctuations in technological parameters and changes in environmental conditions, which necessitates the use of substrates with a stable quantitative and qualitative composition [15]. The implementation of thermophilic fermentation is facilitated by the possibility of using the usually sparingly manageable and often lost waste heat generated in commonly applied co-generation systems [16].

The continuous search for, and the observed evolution of, AD technology is associated primarily with the increasingly common co-digestion of sewage sludge with other organic waste in biorefinery systems, as well as new wastewater treatment technologies, resulting in modified characteristics of the sewage sludge produced [17,18]. An example of a new and dynamically developing technology is the method based on aerobic granular sludge (AGS). In this case, the sludge formed is a compact structure, in which an important role is played by filamentous bacteria and extracellular polymeric substances (EPS) [19]. On the one hand, it has been proven that the taxonomic and chemical composition of AGS, and also the structure, allows for the improvement of wastewater treatment efficiency, and, on the other hand, it may pose difficulties in the typical AD process performed in the existing infrastructure [20]. So far, only a few studies have focused on the verification of AGS parameters that affect the final effects of methane fermentation [19,21]. The complex morphology, intricate structure, size, and chemical composition of AGS have been proven to influence the course of AD. In the case of AGS, the hydrophobicity, density, granule compactness, EPS composition, and a high proportion of filamentous bacteria in the structure significantly reduce degradation and AD conversion rates [22].

For this reason, pre-treatment methods are recommended that would allow for the disintegration of granules, an increase in the solubility of molecules and, consequently, the efficient course of anaerobic stabilization of sewage sludge [20,21]. A promising and prospective pre-treatment technology consists of low-temperature conditioning with the use of solidified carbon dioxide (SCO_2) [21]. This is an innovative technology in which SCO_2 is expected to be recovered during cryogenic enrichment and the upgrading of biogas to bio-methane. Cryogenic technologies are based on the fact that different raw biogas components have different liquefaction temperatures. Since SCO_2 is stable in atmospheric conditions and has many different applications, its production becomes the preferred method of capturing and recovering CO_2 from biogas [23]. Bio-methane can also be cooled to a liquid state, and in this form, it can be distributed and consumed via existing LNG systems [24,25]. Biogas purification is associated with the improvement of the technical functionality and energy efficiency of the process. This feature not only increases the technology's potential to improve the efficiency and cost-effectiveness of fermentation processes but can also help reduce CO_2 emissions into the atmosphere, which is a significant advantage in terms of environmental protection and is in line with the assumptions of the current climate policy [26].

This manuscript is the first ever to present research on the thermophilic methane fermentation of AGS pre-treated with SCO_2. The present study focused on an evaluation of the AGS pre-treatment with SCO_2 on the thermophilic AD technological effects. It analyzed the effect of this disintegration technology on the changes in the concentrations of dissolved organic and biogenic compounds and the yield and kinetics of biogas and methane production in periodical reactors, as well as enabled the development of an empirical organizational model of biogas production.

2. Materials and Methods

2.1. Experiment Organization

The study was conducted in two stages (S1, S2). In S1, AGS was pre-treated using SCO_2, whereas, in S2, it was introduced to anaerobic digestion (AD) under thermophilic conditions. S1 and S2 were divided into variants (V), depending on the volumetric ratio of SCO_2/AGS: (control without SCO_2) V1, (1:10) V2, (1:5) V3, (1:3) V4, (1:2.5) V5, and (1:2) V6. The SCO_2/AGS proportion used was estimated based on the results from the literature [27,28] and previous research [21,29]. Figure 1 presents an organizational scheme of the research.

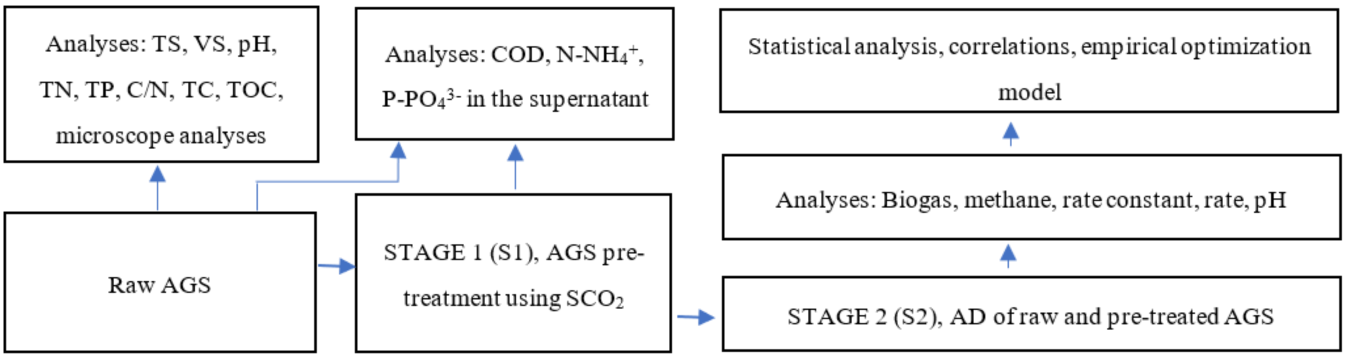


Figure 1. Scheme of research.

2.2. Materials

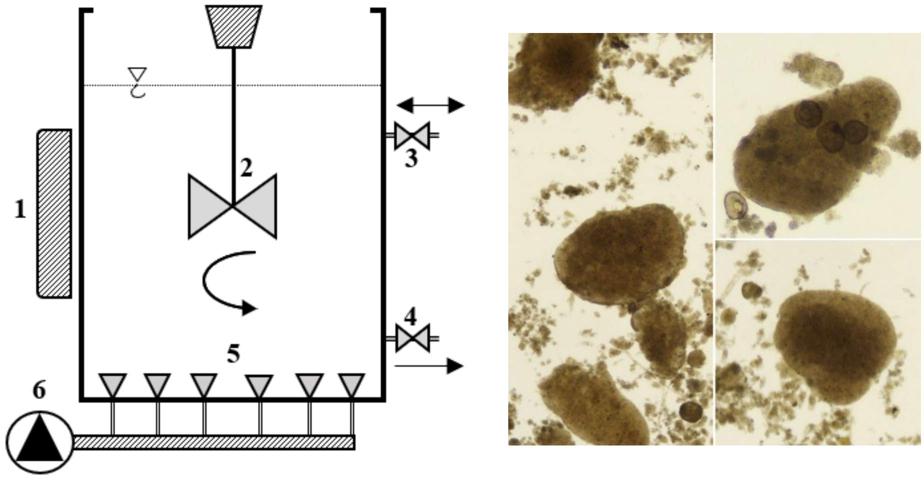
2.2.1. Aerobic Granular Sludge (AGS)

The AGS culture process was conducted for 120 days in a sequencing batch reactor (SBR) [21,30] under laboratory conditions. Suspended activated sludge (SAS) from the municipal WWTP for 2500 people with an average capacity of 400 m^3/d was used as the inoculum. The plant operates based on anaerobic technology with elevated phosphorus and nitrogen removal. Mature granules were used in the laboratory work. Figure 2 presents the SBR reactor used for granulation and a microscopic image of the AGS.

The inoculum used in the fermentation experiment was anaerobic sludge (AS) sourced from an anaerobic digestion reactor (ADR) with a capacity of 7300 m^3 located in the WWTP (Białystok, Poland), which operates at OLR = 2.0 gVS/dm^3·d, HRT = 21 days, and a temperature of 35 °C. Raw AS was adapted to the temperature of 55 °C for 40 days (HRT = 20 days) and OLR = 1.0 gVS/dm^3·d. Table 1 presents the characteristics of the AGS and AS inoculum.

2.2.2. SCO_2

In S1, the pre-treatment was performed with SCO_2 (Sopel Ltd., Bialystok, Poland) in the form of granules of 3.0 ± 1.0 mm in diameter. It is a natural product that is allowed to have contact with food and is tasteless, odorless, non-toxic, and non-flammable [31].



(a) (b)

Figure 2. (**a**) SBR used for AGS culture (1—programmable logic controller (PLC), 2—mechanical stirrer, 3—wastewater introduction and discharge valve, 4—excess AGS valve, 5—diffusers, 6—aeration pump), (**b**) microscopic image of AGS used in the study (×100 magnification).

Table 1. Parameters of AGS and AS inoculum used in the research.

Indicator	Unit	AGS	AS
Total solids (TS)	[%]	7.38 ± 0.1	3.35 ± 0.1
Volatile solids (VS)	[%TS]	93.88 ± 4.5	56.49 ± 6.2
Mineral solids (MS)	[%TS]	6.12 ± 1.3	43.51 ± 5.1
Total carbon (TC)	[mg/gTS]	652 ± 22	311 ± 11
Total organic carbon (TOC)	[mg/gTS]	601 ± 13	296 ± 11
Total nitrogen (TN)	[mg/gTS]	101 ± 6.2	35.5 ± 4.1
C/N ratio	-	6.46 ± 0.1	8,76 ± 0.2
Total phosphorus (TP)	[mg/gTS]	7.1 ± 1.5	1.9 ± 0.1
pH	-	7.82 ± 0.1	7.30 ± 0.1

2.3. Experimental Reactors

The pre-treatment was conducted using open reactors (JLT 6, VELP Scientifica, Milano, Italy) [21]. In total, 200 cm^3 of AGS (20 °C) with a SCO_2 dose assumed for a given experimental variant were mixed (50 rpm). When the SCO_2 was completely sublimated and the sample temperature reached 20 °C, the samples were subjected to AD under thermophilic conditions.

The volume of biogas was measured in a set of respirometers (Hornik Ltd., Poznań, Poland) [21]. To this end, 200 cm^3 of AS was introduced to the laboratory chambers with an appropriate dose of AGS. Anaerobic conditions inside the reactors were ensured for 3 min by N_2 purging. AD was conducted at a temperature specific for thermophilic fermentation, i.e., 55 °C at an initial OLR = 5.0 gVS/dm^3 [29]. The volume of generated biogas was controlled every day until its production stopped. The biogas composition was determined at the end of the fermentation.

2.4. Analytical Procedures

The concentrations of the TS, VS, and MS were measured using the gravimetric method [32]. The TC and TOC content were determined using a Multi NC 3100 analyzer (Analytik Jena, Jena, Germany). The supernatant was determined for the COD (chemical oxygen demand) and contents of TN, $N\text{-}NH_4$, and $P\text{-}PO_4$ using a Hach DR6000 spectrometer (Hach, Loveland, CO, USA). It was obtained after AGS centrifugation (10 min, 5000 rpm) in an MPW-251 laboratory centrifuge (MPW Med. Instruments, Warsaw, Poland). The pH value was determined using the potentiometric method. The biogas composition was determined using a DP-28BIO gas analyzer (Nanosens, Wysogotowo, Poland).

2.5. Statistical, Calculation and Optimization Methods

The fermentation rate constants (k) and biogas generation rate (r) were calculated by means of the non-linear regression iterative method [21]. The experiments were carried out in three repetitions. Statistical analysis was performed using Statistics 13.3 software (Statsoft, Inc., Tulsa, OK, USA). The normality of the distribution of the variables was determined using the Shapiro–Wilk test. The significance of the differences between the variances was determined with the ANOVA test, and the homogeneity of variance in the groups was assessed using Levene's test. Tukey's HSD test was applied to determine the significance of the differences between the analyzed variables. Empirical equations were developed using the stepwise progressive multiple regression method. The key predictors of the changes in the values of the estimated parameters were identified in model systems. The model fit to the empirical data was verified by means of determination coefficients. The results were considered significant at $\alpha = 0.05$.

3. Results and Discussion

3.1. Stage 1

In V1, the COD concentration in the supernatant was 150 ± 11 mgO_2/dm^3, whereas the concentrations of the nutrients were 82 ± 4 mg $N\text{-}NH_4^+/dm^3$ and 63 ± 3 mg $P\text{-}PO_4^{3-}/dm^3$ (Table 2). The concentrations of COD, $N\text{-}NH_4^+$, and $P\text{-}PO_4^{3-}$ in the supernatant were observed to significantly increase in V2–V4. The COD concentrations ranged from 330 ± 13 mgO_2/dm^3 in V2 to 435 ± 13 mgO_2/dm^3 in V4. In V2, the $N\text{-}NH_4^+$ concentration reached 157 ± 7 mg/dm^3 and that of $P\text{-}PO_4^{3-}$ reached 68 ± 4 mg/dm^3 (Table 2). In V4, the concentrations of both these indicators increased ultimately to 273 ± 10 mg/dm^3 and 77 ± 4 mg/dm^3, respectively (Table 2).

Table 2. Changes in content of organic compounds and nutrients in AGS dissolved phase caused by AGS pre-treatment with SCO_2, in particular, technological variants.

Indicator	Unit	V					
		1	2	3	4	5	6
COD	[mgO_2/dm^3]	150 ± 11	330 ± 16	412 ± 17	435 ± 15	440 ± 14	445 ± 15
$N\text{-}NH_4^+$	[mg/dm^3]	82 ± 4	157 ± 7	225 ± 8	273 ± 10	288 ± 9	312 ± 11
$P\text{-}PO_4^{3-}$	[mg/dm^3]	63 ± 3	68 ± 4	73 ± 3	77 ± 4	78 ± 3	79 ± 3

The increase in the COD content is due to the destruction of the SAS structures by the disintegration of the microorganisms' cells [29]. This process also leads to the distribution of the enzymes from their protoplasts, whose biodegradation activity results in transformations of organic and biogenic compounds. This, in turn, contributes to increased concentrations of the mineral forms of nitrogen and phosphorus in the dissolved phase [30].

Likewise, Zawieja (2018) [28] determined an increase in the COD concentration in the SAS supernatant. The SCO_2/SAS increase from 0.05/1.0 to 0.75/1.0 resulted in a COD concentration increase from 119 mgO_2/dm^3 to 296 mgO_2/dm^3 [28]. In another study by Zawieja (2019) [33], the $N\text{-}NH_4^+$ concentration in the raw SAS supernatant was approximately 43 mg/dm^3 and was observed to increase along with an increasing SCO_2 dose, reaching ca. 102 mg/dm^3 at a SCO_2/SAS volumetric ratio of 0.75/1.0 [33]. In another study [29] addressing the impact of pre-treatment with SCO_2 on dairy SAS, the COD concentration in the raw SAS supernatant reached 400.5 ± 23.8 mg/dm^3. The highest COD values, from 490.6 ± 12.9 to 510.5 ± 28.5 mg/dm^3, were noted at SCO_2/SAS ratios ranging from 0.3 to 0.5. Disintegration with SCO_2 caused an increase in the $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ concentrations in the supernatant from 155.2 ± 10.2 mg/dm^3 and 198.5 ± 23.1 mg/dm^3 in the raw sludge to 185.9 ± 11.1 mg/dm^3 and 300.6 ± 35.9 mg/dm^3, respectively, at a SCO_2/SAS volumetric ratio of 0.5 [29]. In experiments performed by Machnicka et al.

(2019) [34], the COD content in the dissolved phase increased to 205 mgO_2/dm^3 at a SCO_2/SAS volumetric ratio of 0.25 and peaked at 889 mgO_2/dm^3 at a SCO_2/SAS ratio of 1.0, whereas it reached 63 mgO_2/dm^3 in the non-pre-treated sludge.

A rapid decrease in temperature directly affects the external and internal cellular structures of microorganisms. Frozen microorganisms are mechanically destroyed, resulting in positive phenomena for sludge management, including the dissociation of cellular lipoproteins and other organic substances, the dissolution of intracellular substances, and the release of bound water into the dissolved phase [35]. In the next stage of the SCO_2 pre-treatment, the medium and the sediment are gradually heated, which increases the volume of water from the previously formed ice crystals. This phenomenon leads to the further disintegration of bacterial cells and the transfer of organic matter and improves AD efficiency [34]. Flash freezing followed by gradual thawing destroys flocs and granules of activated sludge, promotes biomass fragmentation, facilitates the effective dispersion of molecular compounds, modifies cell morphology, and denatures macromolecules. These mechanisms can be used for sludge decontamination and dewatering as well as the pre-treatment of sludge biomass prior to aerobic or anaerobic stabilization [33].

3.2. Stage 2

3.2.1. AD Efficiency

The biogas yield in V1 (raw AGS) reached 319 ± 22 cm^3/gVS (Figure 3, Table 3) at r = 203.5 cm^3/d. The CH_4 concentration in the biogas was 66 ± 3%, which yielded 211 ± 11 cm^3CH_4/gVS (Figure 3, Table 3). After disintegration with SCO_2, the biogas and CH_4 yields were observed to increase, reaching 482 ± 21 cm^3/gVS biogas at r = 457 cm^3/d and 337 ± 14 cm^3CH_4/gVS at r = 223.9 cm^3/d in V4 (Figure 3, Table 3). The successive increase in the SCO_2 dose diminished the AD yield and significantly reduced the CH_4 concentration. In V5, the biogas production reached 442 ± 21 cm^3/gVS (r = 390.7), and the biogas produced contained 60 ± 3% of CH_4, which yielded 265 ± 11 cm^3CH_4/gVS (r = 40.7) (Figure 3, Table 3). In V6, the respective values were 436 ± 22 cm^3/gVS biogas (r = 380.2) and 253 ± 11 cm^3/gVS methane (58 ± 1%) (r = 127.9) (Figure 3, Table 3).

Table 3. Yield of biogas and CH_4 generation efficiency, production rates (r), and reaction rate constants (k) in individual technological variants.

Indicator	**Unit**	**V**					
		1	**2**	**3**	**4**	**5**	**6**
Biogas	[cm^3/gVS]	319 ± 22	343 ± 21	445 ± 22	482 ± 21	442 ± 21	436 ± 22
r_{biogas}	[cm^3/d]	203.5	235.3	396.1	457	390.7	380.2
k_{biogas}	[1/d]	0.64	0.69	0.89	0.96	0.88	0.87
CH_4	[%]	66 ± 3	68 ± 3	69 ± 2	70 ± 2	60 ± 3	58 ± 1
CH_4	[cm^3/gVS]	211 ± 11	233 ± 14	307 ± 12	337 ± 14	265 ± 11	253 ± 11
r_{CH4}	[cm^3/d]	88.65	108.8	188.5	223.9	140.7	127.9
k_{CH4}	[1/d]	0.42	0.47	0.61	0.67	0.53	0.51

The pre-treatment is expected to boost the AD effectiveness [33]. The disintegration of complex biomass macromolecules and the effective transfer of particles to the dissolved phase increase substrate availability to the population of anaerobic microorganisms [34]. In our previous study, AGS disintegrated using SCO_2 was subjected to AD under mesophilic conditions at a temperature of 42 °C [21]. The highest yields of biogas and methane production, reaching 476 ± 20 cm^3/gVS and 341 ± 13 cm^3/gVS, respectively, were obtained at a SCO_2/AGS volumetric ratio of 0.3. These findings are, thus, very similar to the results obtained in the present study under thermophilic conditions. Also, Gavala et al. [36] did not notice any significant differences in the volumes of biogas and CH_4 produced during SAS fermentation under mesophilic and thermophilic conditions. During AD performed at a temperature of 37 °C, they achieved 406 ± 68 cm^3/d biogas with a CH_4 content of 61.6 ± 4.9%, whereas during AD performed at 55 °C, the respective values reached

426 ± 56 cm^3/d and 60.2 ± 5.7% [36]. In turn, the rate of fermentation conducted under thermophilic conditions is higher than that performed under mesophilic conditions. Also, other authors observed a beneficial effect of sewage sludge pre-treatment with SCO_2 on its AD. As reported by Nowicka et al. (2014) [27], the use of SCO_2 for SAS pre-treatment prior to AD enabled a 49% increase in biogas production in the most effective variant compared to the crude SAS [27]. At a SCO_2/SAS ratio of 0.55/1, Zawieja (2019) [33] obtained 0.62 dm^3/gVS biogas with a CH_4 concentration reaching ca. 78% [33], while the respective values achieved for raw SAS were 0.43 dm^3/gVS biogas and ca. 75% CH_4.

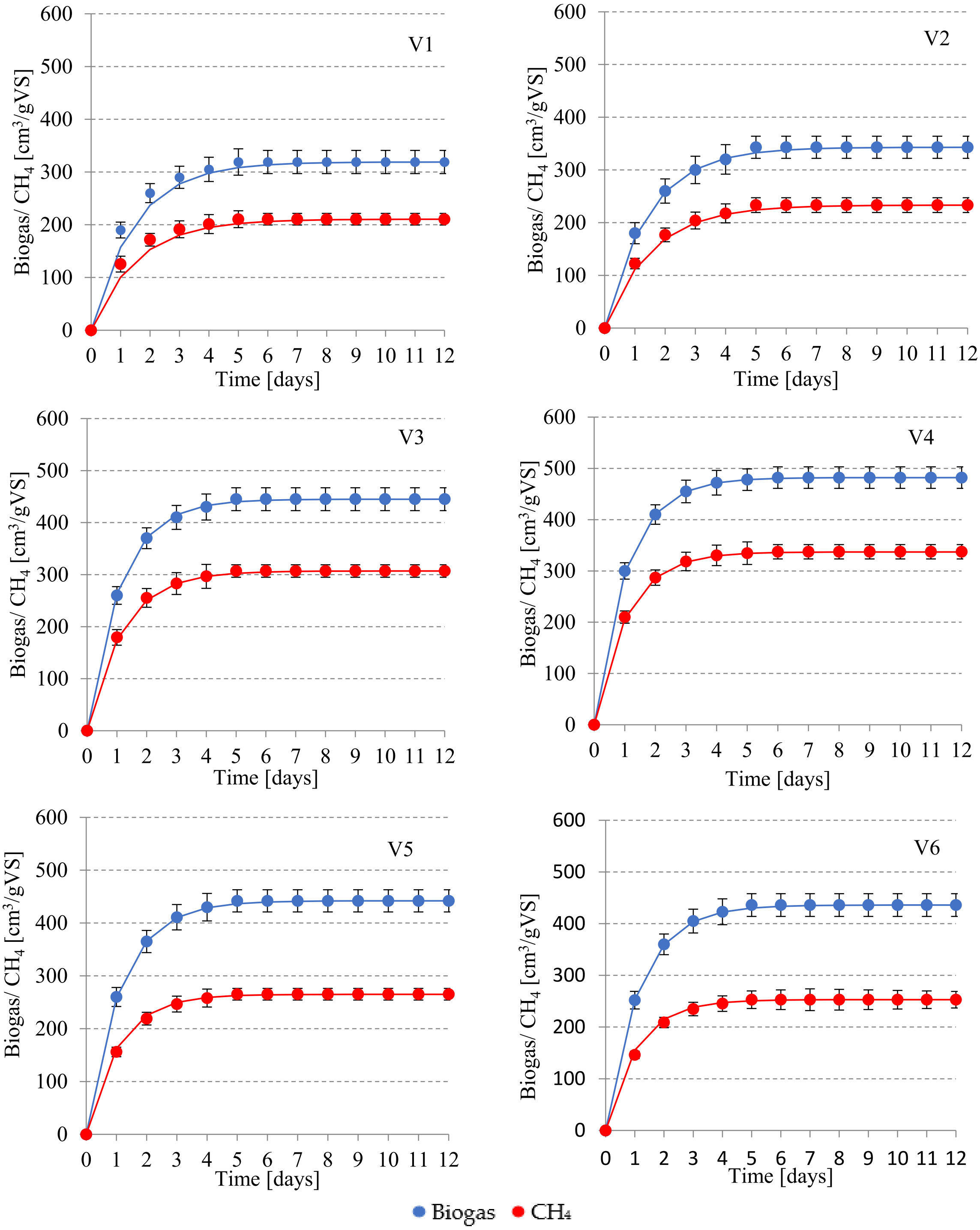


Figure 3. The biogas and CH_4 production kinetics.

3.2.2. pH

The pH of the AGS was 7.82 ± 0.1 in V1. The disintegration of the AGS using SCO_2 caused its pH to decrease to 6.95 ± 0.1 (V4) (Figure 4). An increase in the SCO_2 dose decreased the pH of the AGS from 6.43 ± 0.1 (V5) to 6.33 ± 0.1 (V6). The pH measured in the respirometers after AD decreased from 7.5 ± 0.1 to 7.08 ± 0.1 (V1) (Figure 4). The AGS pH ranged from 7.39 ± 0.1 (V2) to 7.16 ± 0.1 (V4) and decreased after AD to 6.89 ± 0.1 (V2) and 6.76 ± 0.1 (V4) (Figure 4). In turn, a sharp decrease in the pH values was observed after AD in the variants V5 and V6, i.e., from 6.96 ± 0.1 to 6.46 ± 0.1 in V5 and from 6.87 ± 0.1 to 6.31 ± 0.1 in V6 (Figure 4). As proved in other authors' investigations, pH decline leads to the reduction of the methanogen population and CH_4 production [21]. Zawieja (2019) [33], who also pre-treated SAS with the use of SCO_2, reported a pH decrease that could be associated with an AD yield reduction.

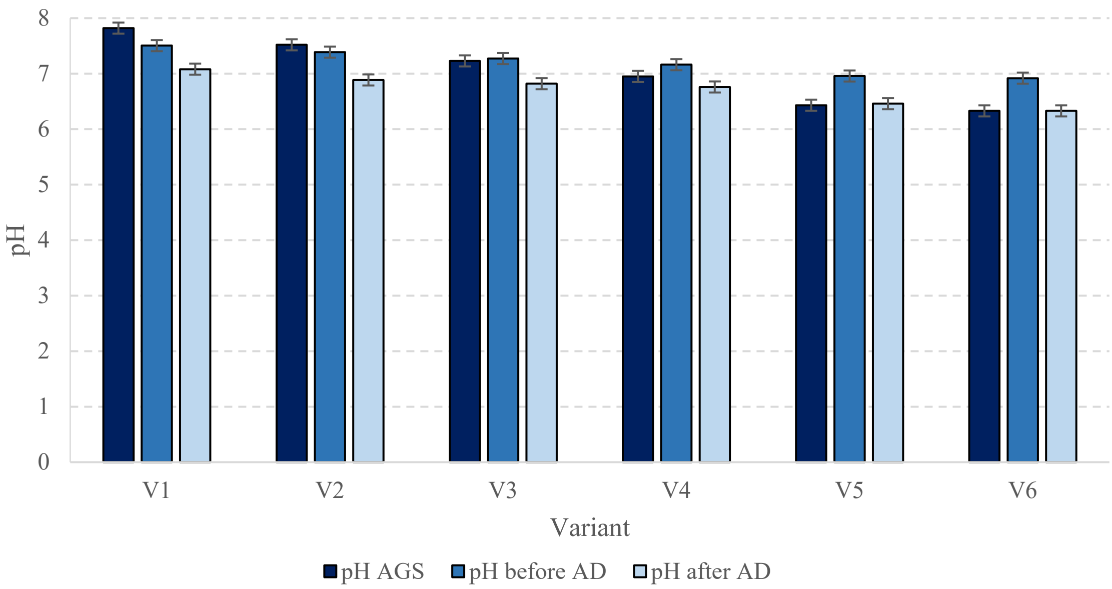


Figure 4. pH values after SCO_2 pre-treatment and AD.

CO_2 is well soluble in aqueous solutions, and its solubility reaches 2900 mg/dm^3 at a temperature of 25 °C [37]. During CO_2 sublimation, its part dissolves in the supernatant, resulting in the formation of hydrogen ions, H^+, carbonate ions, CO_3^{2-}, and bicarbonate ions, HCO_3^-, which contribute to a pH decrease [38]. This pH decrease negatively influences the AD effectiveness, including gaseous metabolite production [39]. Hence, the pH values measured in the anaerobic chambers were a result of the initial conditions after pre-treatment with SCO_2. The pH value is an important factor of the anaerobic process because it determines its stability and may contribute to its inhibition [40].

3.2.3. Correlations and Empirical Models

Strong correlations were noted between the SCO_2 dose and the production of biogas/CH_4. In the variants V1–V4, the determination coefficients reached R^2 = 0.8755 for biogas and R^2 = 0.8881 for CH_4 (Figure 5a). In the variants V4–V6, their values reached R^2 = 0.8459 for biogas and R^2 = 0.8567 for CH_4 (Figure 5a). In V1–V4 and V4–V6, strong correlations between the pH value of the AGS and the biogas volume were found, as indicated by the determination coefficients reaching R^2 = 0.9409 and R^2 = 0.9991, respectively (Figure 5b). Similar correlations were noticed in the case of CH_4, with determination coefficients of R^2 = 0.9558 and R^2 = 0.9998, respectively (Figure 5b). The increase in the biogas and CH_4 yields observed in V1–V4 was due to the improvement of the AGS biodegradability as a result of its conditioning with SCO_2. The substrate availability and biodegradability in V4–V6 were similar due to the concentrations of the compounds determined in the dissolved phase. However, the high SCO_2 doses applied resulted in a pH decrease, which, in turn, had an inhibiting effect on the populations of methanogenic microorganisms, which exhibit optimal metabolic activity at a pH of approximately 7.0 (neutral pH) and are very sensitive to environmental changes [41].

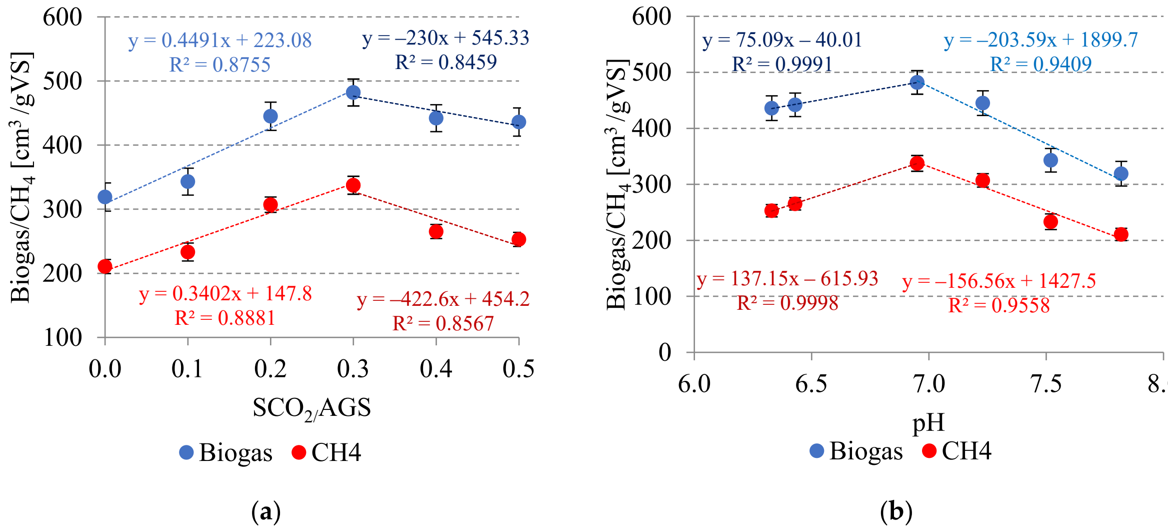


Figure 5. Correlations between (**a**) SCO_2 dose, (**b**) pH of AGS, and biogas and methane volumes.

The results obtained in V1–V4 demonstrated a correlated surface effect of the COD concentration and pH value of the supernatant on the yields of biogas (Figure 6a) and CH_4 (Figure 6b).

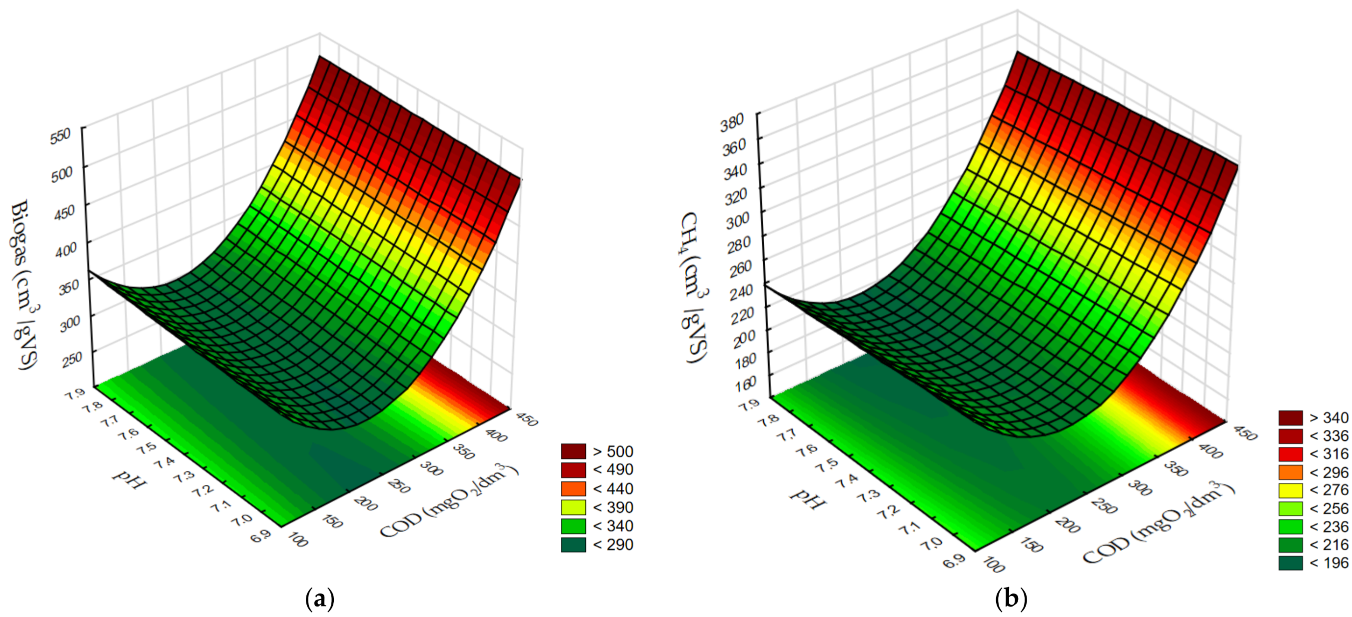


Figure 6. Correlation between pH, dissolved COD concentration, and the (**a**) biogas and (**b**) CH_4 yields.

A mathematical model was developed that enabled the estimation of biogas production. Because a linear correlation was found in the variants V1–V4, they were considered in the estimation. It was found that dependent variables such as the COD concentration and pH value of the supernatant had a statistically significant effect on the volume of biogas produced. The developed model of biogas production (1) reflects ca. 88.41% of the changes in the process of biogas acquisition (determination coefficient R^2 = 0.8841) and is characterized by an estimation error of ±38.987.

$$BIOGAS = 0.4921COD - 0.3065pH + 230.33 \quad (1)$$

BIOGAS—the amount of produced biogas (cm^3/gVS),
COD—COD concentration in the dissolved phase, mgO_2/dm^3,
pH—pH value (−)

The monitoring of the organic compound concentrations in the supernatant is one of the ways to assess the effectiveness of the sludge preparation processes of a given substrate subjected to disintegration [42,43]. The possibility to state reliable mathematical models to estimate the AD course based on the organic compound content in the supernatant was proven [44]. Hence, such an assessment is essential from the practical standpoint, as it allows for the omission of highly advanced measurements that enable an estimation of the effectiveness of the applied disintegration methods.

As in any technology, in the case of the use of the AGS pre-treatment with SCO_2, weaknesses can also be identified. Certainly, a limitation is the low reconnaissance and low technological readiness level (TRL) based only on laboratory-scale work. The lack of data from installations operated on a pilot or fractional technical scale limits the possibility of a reliable and credible estimation of both technological efficiency and economic profitability [21,29]. The production of SCO_2 in the biogas upgrading process is also not well known, which may favor, and significantly support, the profitability of the entire technological system. Obtaining SCO_2 from installations dedicated to its production may eliminate this method of sewage sludge conditioning due to the investment and operating costs incurred [26]. From a technological point of view, the introduction of doses of SCO_2 that are too high to the mass of processed AGS may cause a decrease in the pH of the biomass, which implies technological problems related to the activity of methanogenic microorganisms [10,28].

Although little information has appeared in the world literature so far on the feasibility of low-temperature sludge conditioning with SCO_2, it seems that there is no doubt that it is an environmentally friendly approach, especially since SCO_2 can be recovered by upgrading biogas. The method also avoids secondary pollution, often introduced by chemical breakdown, and the additional energy input required for mechanical processing. It can also be included in the closed CO_2 cycle of biogas production—biogas upgrading—SCO_2 production—sludge disintegration—fermentation—biogas production, which is a significant advantage in terms of environmental protection [26].

This new approach to the production and use of SCO_2 largely counteracts the existing limitations, which are mainly related to the low profitability of the production process. The use of SCO_2 to treat sludge may prove to be an increasingly attractive alternative to other methods, given the current emphasis on the circular economy and the reduction of CO_2 emissions.

4. Conclusions

It was proven that an increasing portion of SCO_2 introduced to AGS biomass with volumetric ratios ranging from 0 to 0.3 led to an increase in the concentrations of organic and biogenic compounds in the dissolved phase. Increasing the SCO_2 dose had no significant effect on increasing the content of the indicators in the supernatant.

The highest CH_4 yields were achieved at a SCO_2/AGS volumetric ratio of 0.3, i.e., 337 ± 14 cm^3/gVS. The study proved that SCO_2 doses exceeding 0.3 had no effect on the AD effectiveness expressed in the biogas and CH_4 volumes. This was most likely due to a significant decrease in the pH of the AGS, which, in turn, contributed to a decreasing CH_4 concentration in the biogas. Optimization procedures demonstrated the COD concentration and pH value to be reliable predictors of estimated biogas volume.

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