

Article

Study on the Effect of Dedicated Microelement Mixture (DMM) on the Kick-Off Phase of the Digester and Stabilization of the Methane Fermentation Process

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Abstract: The kick-off process is an important aspect of the proper operation of an agricultural biogas plant. At this stage, various operational problems may arise, mainly related to the stabilization of the fermentation process and reaching the full biogas production capacity. This paper presents the results of research on the kick-off of the fermentation process carried out on three selected biogas plants located in Poland. For the experiments, titration, potentiometric, and spectroscopic methods (ICP-MS) were used. The biogas plants during the kick-off period operated on the following substrates: a mixture of cattle and pig manure, corn silage, and whey liquor. Special attention was paid to the dosing process of the formulation developed by the authors (CMP-S1) containing Mo, Co, Ni, Se, and Mn for the fermentation chambers, to which the mixture of the selected microelements was not dosed. The study was carried out under real conditions on an engineering scale. The study showed that supplementing a dedicated mixture of microelements (DMM) in the first days of fermentation chamber kick-off has a positive effect on stabilizing the methane fermentation process and allows a faster and higher loading of fermenters with dry organic matter. The above translates into shortening the time (by more than half) of the kick-off process in the fermentation chamber, as well as brining about a significant reduction in costs.

Keywords: anaerobic digestion; microelements; biogas; methane yield



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1. Introduction

The agricultural biogas plants that are built globally require, first of all, a proper fermentation chamber kick-off process, which has a direct impact on the stable operation of the plant and biogas production. The proper operation of a biogas plant is also influenced by appropriately selected and prepared substrates used for fermentation, as well as the careful control of individual process parameters. Many authors point to the possibility of the proper supplementation of the fermentation process with properly selected microelements that use methanogenic archaeons. The issue of macronutrients such as Mg, K, Na, and Ca has already been explained in many publications, such as [1–4], though micronutrients are more complicated to explain. Publications such as [4–7] deal with micronutrients but in the context of a specific fermentation material or a specific fermentation process. Our paper contains information about the process carried out in natural conditions. Mo, Co, Ni, Se, and Mn were chosen because the data on these micronutrients are not up to date and there is little correlation between them [3,7–11].

The concentration of metals in bacterial cells ranges from 10^{-7} to 10^{-3} mol·dm⁻³ for macronutrients and from 10^{-15} to 10^{-6} mol·dm⁻³ for microelements [1–4]. Microelements essential to the function of a bacterial cell, known as trace elements, are included

as cofactors in enzyme systems and perform various catalytic functions in respiratory cycles. A corrinoid such as cyanocobalamin, which contains cobalt (Co), can bind to the coenzyme methylase that catalyzes methane production in acetoclastic methanogens and hydrogenotrophic bacteria [12]. In extracts from *Methanobacterium thermoautotrophicum*, a low-molecular weight coenzyme F430 was detected that contains significant amounts of nickel (Ni) [13]. This coenzyme (F430) is contained in the enzyme methylcoenzyme M reductase, which reduces it to methane in all methanogenic pathways [14]. Mangan (Mn) stabilizes methyltransferase in methane bacteria and acts as an electron acceptor in anaerobic respiration processes. It is often exchanged with magnesium (Mg) in kinase reactions. As with copper, the role of Mn in methanogenesis has only been studied through supplementation in trace metal mixtures [15]. The results showed that the stimulating effect was significant with Mn, and the degradation activity of acetate and iso-butoate ions increased [16]. Selen (Se) is a highly immunomodulating element [17]; it has a very important effect on the methanogenic hydrogenotrophic pathway. This pathway contains selenoproteins that include formylmethanefuran dehydrogenase, formate dehydrogenase, and formate hydrogenase [18]. Molybdenum (Mo) is involved in the enzymatic reaction of formylmethanefuran dehydrogenase (hydrogenotrophic methanogens) and formate dehydrogenase (syntrophic oxidizing bacteria and hydrogenotrophic methanogens). It is thought to be chemically analogous to tungsten in the formation of enzymes [19]. Molybdenum cannot be replaced by other trace elements in any methanogenic species. Previous researchers have suggested that clarifying the role of the rare trace elements (Mo, Se, and Mn) required for anaerobic fermentation is extremely important for the process to proceed properly [20]. Therefore, the purpose of this study was to demonstrate the effect of selected microelements, such as Mo, Co, Ni, Se, and Mn, on a selected phase of the methanogenic process occurring in fermentation chambers under mesophilic (35–37 °C) conditions, namely, the kick-off phase. Most of the research on the fermentation process that results in biogas production is concerned with the effect of microelements on the stabilized or collapsed fermentation process [21–26]. This paper presents the results of tests from the kick-off of the fermentation process carried out on the selected three biogas plants with a particular reference to the dosing of a formulation (CMP-S1) containing Mo, Co, Ni, Se, and Mn in relation to the lack of dosing of a mixture of selected microelements. The study was carried out under real conditions on an engineering scale.

2. Study Methodology

To determine the effect of microelements on the process of methane fermentation carried out under actual conditions on an engineering scale, a series of experiments were conducted at three Polish agricultural biogas plants: Falknowo (φ 53°41'0.87" N, λ 19°23'48.89" W), Wicie (φ 52°11'27.76" N, λ 19°59'42.38" W), and Kupin (φ 53°36'52.28" N, λ 19°14'59.35" W), each with an electrical output of 0.999 MW_{el}.

The plan of each biogas plant is shown in Figure 1.

Figure 1 shows the plan of the biogas plant where the experimental work was carried out. Marked: 1—car scale; 2—storage silo No. 1; 3—storage silo No. 2; 4—silo effluent tank; 5—dosing tank; 6—fermentation tank No. 1; 7—fermentation tank No. 2; 8—post-fermentation tank; 9—post-fermentation mass storage tank; 10—digestate collection point; 11—biogas treatment plant; 12—cogeneration system container; 13—pumping station, heat distribution center, and control room; 14—flare; 15—transformation station; 16—social container; and 17—rainwater reservoir.

The study was conducted under mesophilic fermentation conditions during the biotechnological kick-off of six digesters. The working volume of the chambers ranged from 3165 to 3255 m³, the fill height ranged from 7.00 to 7.20 m with a diameter of 24.0 m, the target organic dry matter (ODM) load ranged from 2.6 to 3.5 kg·m⁻³·d⁻¹, and the hydraulic retention time (HRT) was dependent on the parameters of the dosed substrates and ranged from 33 to 60 days. Target process parameters: the process temperature was

314.15–315.15 K, pH 7.7–8.1, FOS/TAC < 0.20. The expected biogas production in each digester per 100% was CH_4 –125 $\text{N}\cdot\text{m}^3\cdot\text{h}^{-1}$.

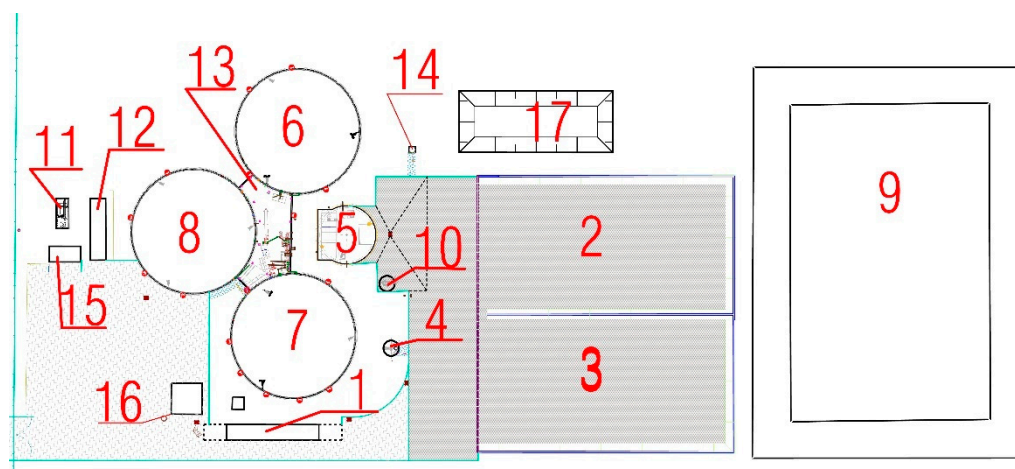


Figure 1. A map of each biogas plant where the research was conducted. Description is in the text.

Based on the experiences of various authors, in the first stage of biotechnological kick-off, a mixture of cattle and pig slurry was used as substrates, with a predominance of pig slurry (DM 3.6%, ODM 2.7%) [27–29], corn silage (DM 31–33%, ODM 29–31%) [30,31], and whey liqueur (DM 26–31%, ODM 25–30%) [32–34].

A baseline restriction has been adopted for corn silage, specifying that it cannot account for more than 25% m/m of substrates used for the digestion process. For all biogas plants, throughout the experiment, substrates were dosed into the F1 and F2 digesters (fermentation chambers) from an underground dosing tank with a volume of 350 m^3 , equipped with two medium-speed mixers and a submersible pump. The substrates were controlled for their quantity and dry matter content before being applied to the dosing tank. A certified TAMTRON truck scale was used for this purpose, and a moisture balance (RADWAG MA.110.X2.A.WH, Radwag, Poland) and a muffle furnace (7.0SM/1150 Conbest, Poland) were used to determine the dry matter. Once the batching tank was filled with the substrate mixture, it was mixed to a homogeneous feed that could be pumped. The volume of substrate mixtures dosed into the digesters was controlled using a flow meter (PEM-1000ALW/DN150, Aplisens, Warsaw, Poland). The dosing process was alternated every 2 h, 12 times a day, to each of the two digesters at a given location. Based on the above assumptions, it was presumed that a mixture of substrates with identical proportions and quality parameters was dosed into both digesters.

The dosed formulation, CMP-S1, contained Mo(VI) in the form of a complex of Mo- γ -CDx and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ in the amount of 50–58 $\text{g}\cdot\text{dm}^{-3}$, Co(II) in the form of a complex of Co- α -CDx and CoCl_2 in the amount of 250–290 $\text{g}\cdot\text{dm}^{-3}$, Ni(II) in the form of a complex of Ni- α -CDx and NiCl_2 in the amount of 140–180 $\text{g}\cdot\text{dm}^{-3}$, Se(VI) in the form of a complex of Se- β -CDx and Na_2SeO_4 in the amount of 30–40 $\text{g}\cdot\text{dm}^{-3}$, and Mn(II) in the form of a complex of Mn- α -CDx and MnCl_2 in the amount of 310–340 $\text{g}\cdot\text{dm}^{-3}$, where: α -CDx is (alpha)-cyclodextrin, 6 glucose subunits; β -CDx is (beta)-cyclodextrin: 7 glucose subunits; and γ -CDx (gamma)-cyclodextrin: 8 glucose subunits. The formulation additionally contained organic chelating substances assimilable to bacterial microflora. The dosing of microelements in chelated form was carried out through a dedicated installation directly into the digesters. The CMP-S1 formulation was diluted with tap water at a ratio of 1:100. The CMP-S1 formula contained the analyzed micronutrients in a chelated form. Such a formula was proposed by analyzing the shortages of fermentation processes [2,12,17,18,20,26,35,36].

Supplemented micronutrients are involved in a number of enzymatic reactions during the transformation of anaerobic digestion [37]. The dosage of CMP-S1 is significantly different from that of other researchers [18,38–40]. The preparation is dosed at daily

intervals so that the concentration of micronutrients does not exceed $1/3$ of the obligatory value given in the specification of the preparation expressed in mg dm^{-3} . The concentration of trace elements in the fermentation mixture was much lower than the concentration described by [16,18,38,40,41]. The dosing process of the preparation is more complicated, because the release from CDx complexes is an additional advantage that increases the bioavailability [42] of micronutrients and their lower excretion. The concentrations of the tested micronutrients in each case did not exceed IC_{50} [38,43]. The range of concentrations of microelements that stimulate, inhibit, or have no effect on the process of anaerobic digestion is presented in detail in [44]. All values presented in the paper are below or within the stimulus concentration.

In the course of the testing procedure, FOS/TAC was determined using the Nordman technique [45,46] and a titrator (TitraLab AT1000, HACH Lange, Wrocław, Polska). The FOS/TAC ratio is a commonly used indicator for assessing the fermentation process within biogas reactors, where FOS is a measure of the volatile organic acids (measured in $\text{mg CH}_3\text{COOH} \cdot \text{dm}^{-3}$) and TAC is a measure of the total inorganic carbon, i.e., the alkaline buffer capacity of the system (measured in $\text{mg CaCO}_3 \cdot \text{dm}^{-3}$). In simple terms, this ratio looks at acid vs. alkaline in the system, which helps to assess the risk of acidification in the biogas reactor.

Dry organic matter was determined using the thermal method [8] according to PN-EN 15935:2022-01.

CH_4 , CO_2 , H_2S , H_2 , and O_2 were measured using GFM-416 IR and an electrochemical sensor (GFM-416, GasData Ltd., Coventry, UK).

The content of the selected microelements was analyzed using an ICP-MS 7500 CX mass spectrometer with a collision chamber (ICP-MS 7500 CX Agilent Technologies Ltd., Tokyo, Japan). The methodology consisted of preparing the solution along with its microwave-assisted wet mineralization, and subjecting the samples to a further analysis using a spectrometer. All the results on the micronutrient concentration are the arithmetic means of five measures; the confidence interval for these values shall not exceed 3%. All the gas concentration results are the arithmetic means of five measures; the confidence interval for these values shall not exceed 2%. The results of potentiometric analyses are the arithmetic averages from five measures; the confidence interval for these values shall not exceed 4%.

3. Study Results and Their Interpretation

3.1. Description of the Kick-Off of the Falknowo Biogas Plant

For the first 6 days of the process, a mixture of cattle and pig slurry (proportions according to local availability) was accumulated in digesters FF1 and FF2, and heating began. On the 7th day of the process, inoculum was dosed in the amount of 60 Mg from a model agricultural biogas plant in the village of Buczek (φ 53°31'49.16'' N, λ 18°25'2.71'' W). On the 10th day of the process, the feeding of varied substrates began, those including maize silage and whey liqueur. On the 26th day of the fermentation process, the fermentation mixture reached such a volume that digestate began to be systematically removed from the FF1 and FF2 digesters in an amount that made it possible to dose the daily substrate feed and keep the filling of the digesters at a constant level of 3200 m^3 . The detailed dosing procedure and selected substrate parameters are shown in Figure 2. The dosing parameters for FF1 and FF2 were identical; the same substrates were dosed in the same quantity.

As shown in Figure 2, on day 19 of the process, the amount of maize silage dosed began to increase from 3 $\text{Mg} \cdot \text{d}^{-1}$ to 8 $\text{Mg} \cdot \text{d}^{-1}$. Similarly, with whey liqueur, its dosage was increased to 17 $\text{Mg} \cdot \text{d}^{-1}$ on day 36 of the fermentation process. A mixture of cattle and pig manure was dosed at a constant level throughout the experiment at a rate of 72 $\text{Mg} \cdot \text{d}^{-1}$. All the substrates used were characterized by stable dry matter and dry organic matter contents. In both FF1 and FF2 fermenters, the kick-off course and achieved parameters differed slightly. It was observed that after the start of dosing with CMP-S1, the basic process parameters stabilized very quickly to the expected values, i.e., FOS decreased, TAC increased, the FOS/TAC ratio decreased, and the pH increased to a stable value in the range of 7.9–8.1, as shown in Figure 3.

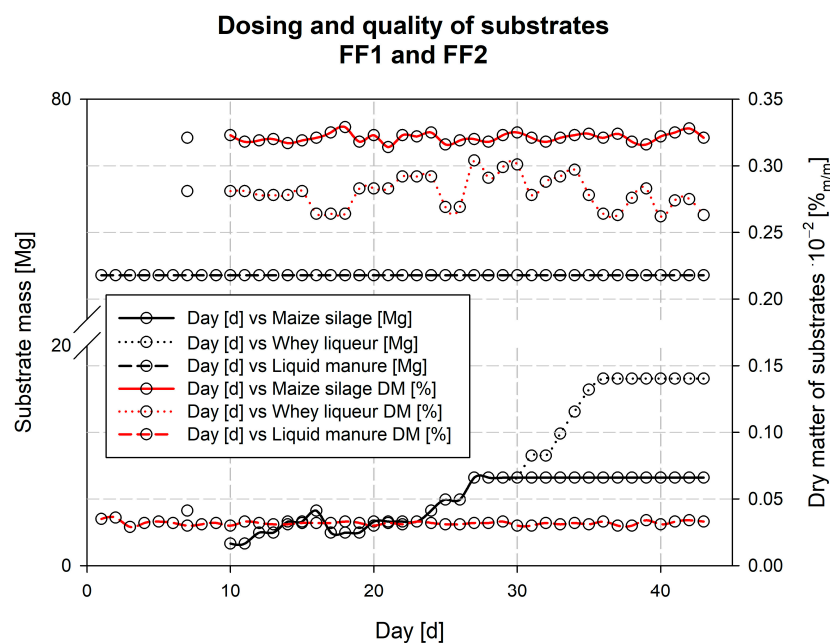


Figure 2. Dosage and quality of substrates fed to FF1 and FF2 digesters.

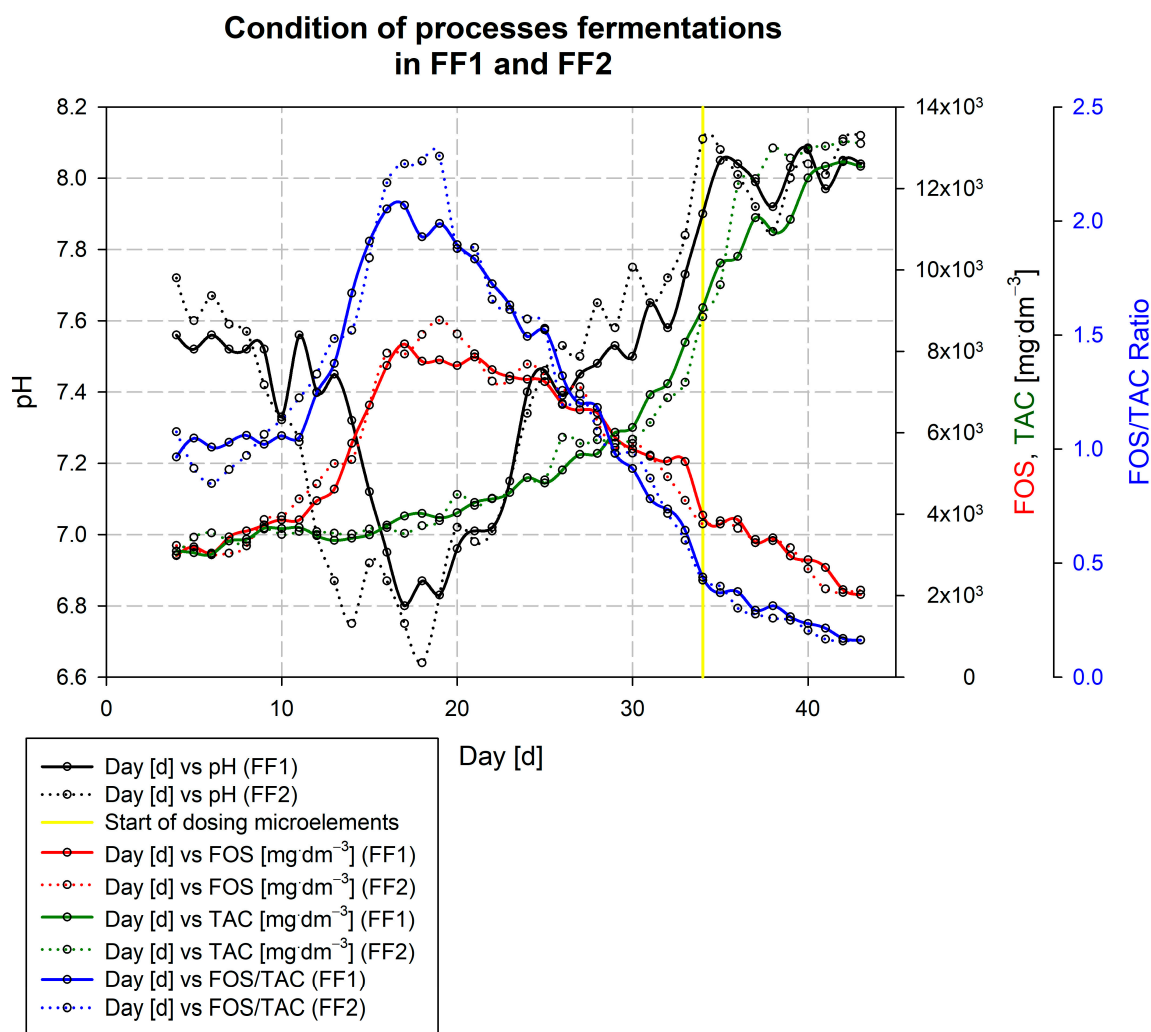


Figure 3. Condition of processes fermentation in FF1 and FF2 chambers.

On the 34th day of the trial and for 4 consecutive days, doses were administered at 1000 cm³, and from the 38th day of the process, for the next 6 days, doses of CMP-S1 were provided at 250 cm³ per day. Figure 4 shows the changes in the microelement concentrations at different stages of fermentation occurring in FF1 and FF2 digesters. All concentration levels were determined in µg·dm⁻³ (ppb).

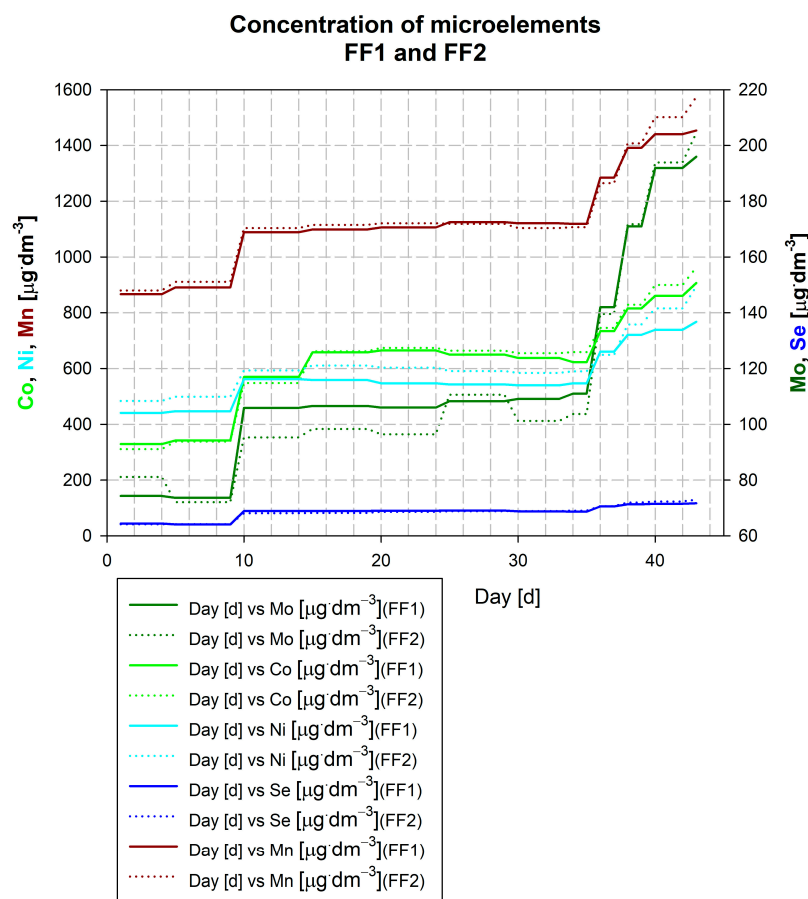


Figure 4. Microelements concentration in fermentation chambers FF1 and FF2.

In the course of conducting the digestion processes in FF1 and FF2, a typical acidification phase occurred. In FF1, the acidification phase occurred about 5 days earlier. As reported by [47], it is also worth noting the kinetics of the increase in TAC values (Figure 3). In FF2, the TAC values increased slightly faster than in FF1, i.e., the equilibrium of the carbonate buffer was reached faster, which is safer for the fermentation process. The results presented in Figure 3 became the reason for conducting a study of the effect of CMP-S1 on the kick-off process of methane fermentation, carried out under mesophilic conditions during the kick-offs of the next two biogas plants WF and KF. An important aspect of the research conducted was to reduce the digester kick-off time and achieve the stabilization of the fermentation process. To determine the productivity and stability, as well as the quality of the fermentation process' performance, a continuous monitoring of the parameters, such as HRT and OLR, was carried out. The obtained values of these parameters for the FF1 and FF2 digesters are shown in Figure 5. In both analyzed cases, the OLR and HRT parameters stabilized around the 35th day of the fermentation process. There were no significant differences between the OLR and HRT values for the processes carried out in FF1 and FF2 digesters. Many authors point out that attention should be paid to the concentration profile of methane, hydrogen, and hydrogen sulfide in biogas during the conduct of fermentation processes [48–50]. As shown in Figure 6, the concentration values of CH₄, O₂, H₂, and H₂S were not significantly different. Starting from day 5 of the fermentation process, the methane concentration in the biogas began to increase steadily to a value of 59.55% v/v

around day 33 of the fermentation process. By the end of the process, it remained above 56% *v/v*. The removal of oxygen from the system occurred during the first 8 days of the process, which confirmed the tightness of the fermentation system. By the end of the process, it did not exceed 0.2% *v/v*. The hydrogen concentration in both chambers did not exceed the 100 ppm value, indicating the good condition of the hydrogenotrophic process. The hydrogen sulfide concentration varied in both digestion processes (FF1 and FF2) but stabilized at around 1.800 ppm in the last 17 days of the digestion process. The maximum H_2S concentration value for FF1 was 2300 $\text{mg}\cdot\text{N}\cdot\text{m}^{-3}$ on day 24 of the process and that for FF2 was 2800 $\text{mg}\cdot\text{N}\cdot\text{m}^{-3}$ on day 20 of the fermentation process.

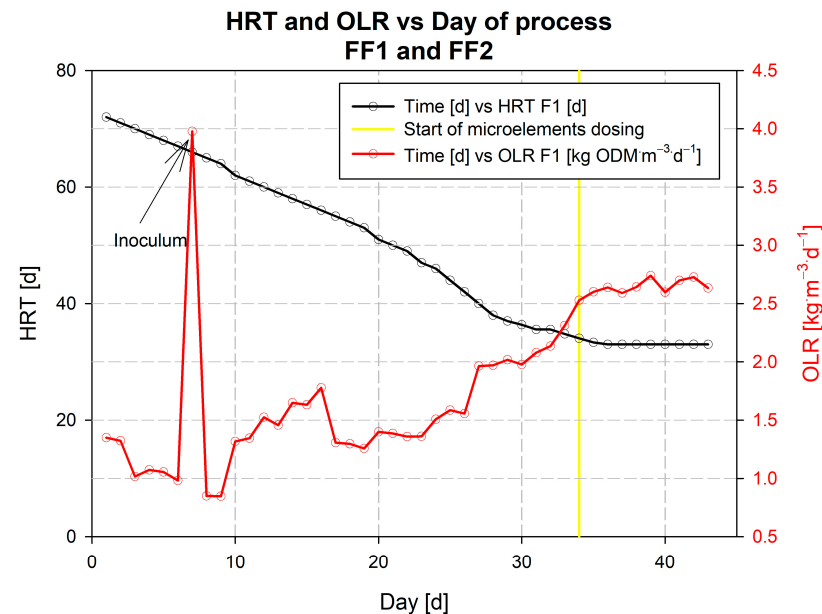


Figure 5. Process parameters FF1 and FF2.

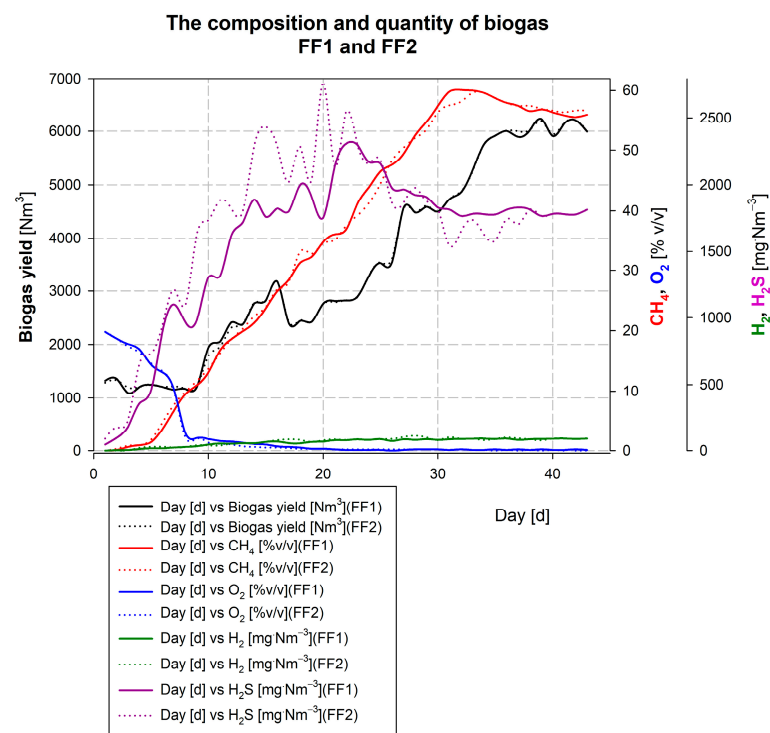


Figure 6. The composition and quantity of biogas in fermentation chambers FF1 and FF2.

3.2. Description of the Kick-Off of the Kupin Biogas Plant

Analyzing the results obtained from the engineering-scale fermentation processes in the FF1 and FF2 digesters, it was planned to dose both digesters at the Kupin biogas plant (KF1 and KF2) with identical substrate feedstocks, whereas the CMP-S1 formulation was additionally dosed to the KF2 digester. In the period before the digesters started warming, a mixture of cattle and pig slurry was accumulated in both digesters in amounts of 1.300 m³ each.

On day 4 of the process, the systematic dosing of substrates to both KF1 and KF2 began, and inoculum was applied in the amount of 60 Mg each from the model agricultural biogas plant in Falknowo. The process of dosing substrates in KF1 and KF2 is shown in Figure 7. The quality of the substrates dosed into digesters KF1 and KF2 at the KUPIN biogas plant was almost identical. The process parameters (Figure 8) of both digesters (KF1 and KF2) on day 4 of the process, i.e., before the start of dosing CMP-S1 to KF2, were very similar.

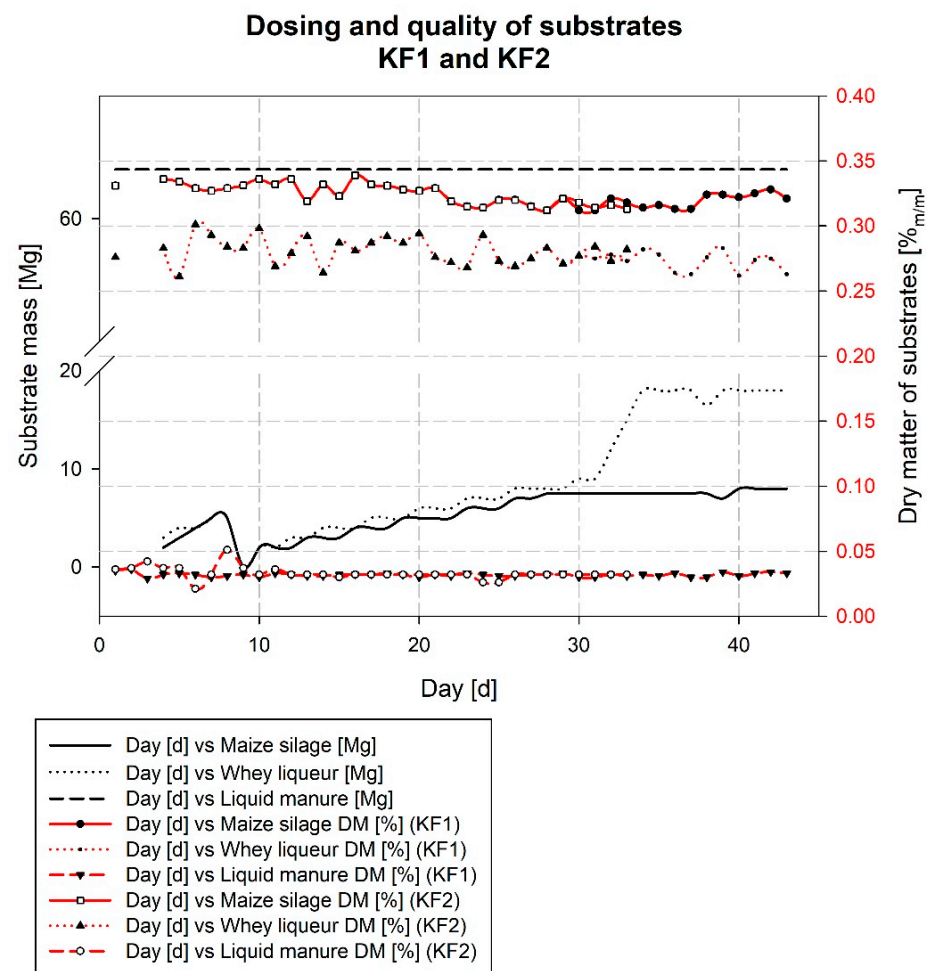


Figure 7. Dosing and quality of substrates fed to KF1 and KF2.

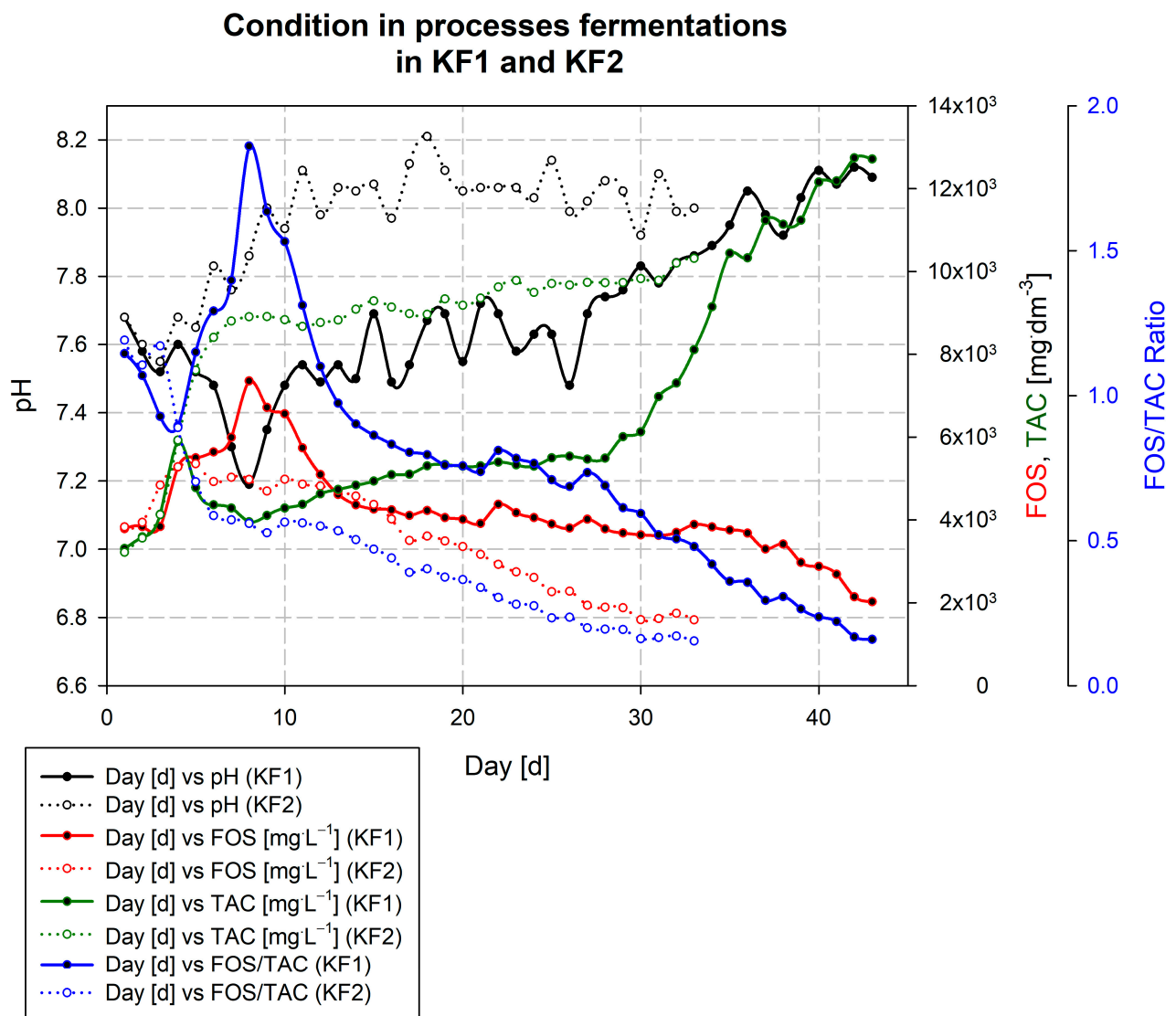


Figure 8. Condition of processes fermentation in KF1 and KF2 chambers.

From day 5 of the process, a significant deterioration in the parameters of the KF1 compared to the KF2 digester was observed (higher FOS values, lower TAC values, and lower pH). On day 9 of the process, out of fear of the deteriorating process parameters in KF1 stopping or critically disrupting the fermentation process, a decision was made to significantly reduce the amount of substrate dosage until the process parameters improved. On day 14 of the process, a gradual improvement in the process parameters was observed, which allowed for an increase in the OLR loadings in the digester. On day 34 of the process, the expected biogas production volume was achieved, and on day 42, the process parameters were fully stabilized (pH in the range of 7.9–8.1 and FOS/TAC ratio ≤ 0.2), as shown in Figure 8.

Starting on day 4 of the process, 1.000 cm³ per day was dosed into the KF2 fermenter for 4 days, and starting on day 8 of the process, 250 cm³ each of the CMP-S1 formulation was dosed for the following 20 days. The formulation contained microelements in a chelated form, bioavailable to methanogenic microorganisms, as described in the introduction. The dosage of CMP-S1 was adjusted according to the process parameters. The concentration of microelements was controlled throughout the fermentation process and is shown in Figure 9.

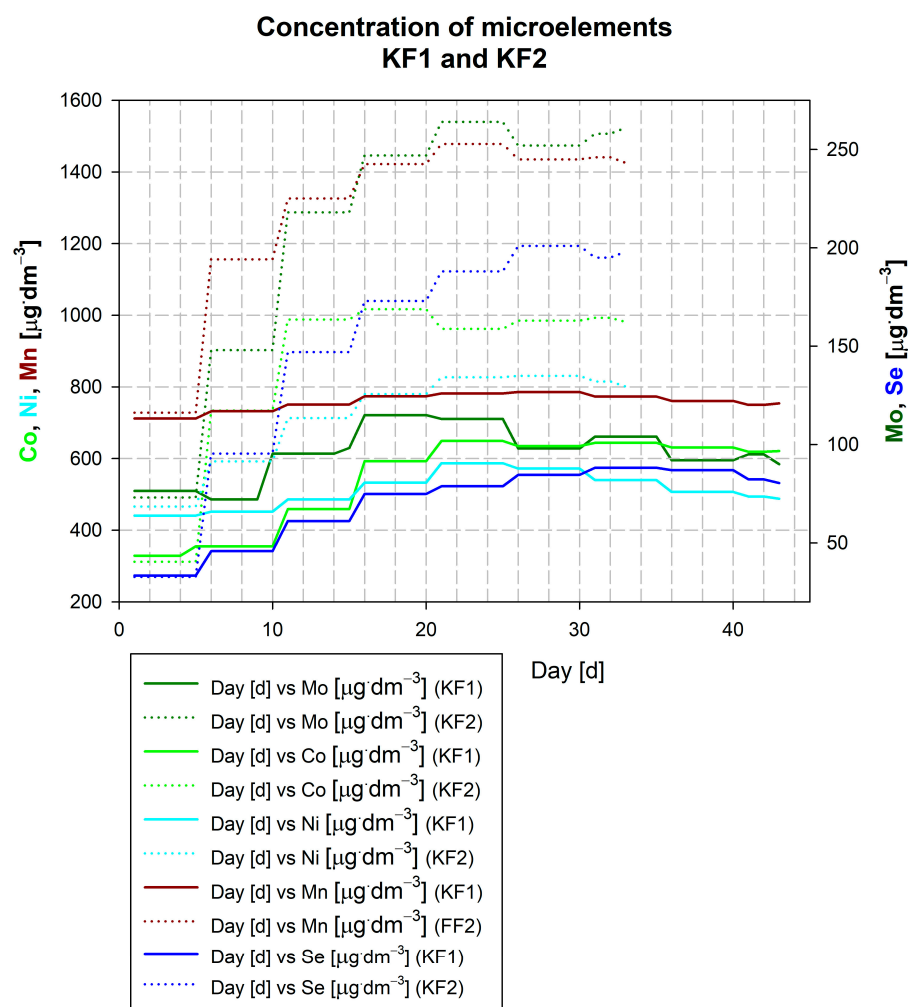


Figure 9. Microelements concentration in fermentation chambers KF1 and KF2.

It was found that the concentration of microelements in KF2 was higher than in KF1 at each stage of fermentation (Figure 9). The minimum Mo concentration of $72.1 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 6 of the fermentation process in KF1, and the maximum Mo concentration of $115 \mu\text{g}\cdot\text{dm}^{-3}$ was also recorded on day 17 of the fermentation process in KF1. The average Mo concentration during the fermentation process in KF1 was $96.2 \mu\text{g}\cdot\text{dm}^{-3}$, which was within the range defined by [35,41]. A minimum Mo concentration of $73.1 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 1 of the fermentation process in KF2, whereas the maximum concentration of this element, at $264 \mu\text{g}\cdot\text{dm}^{-3}$, was recorded on day 21 of the fermentation process. The average Mo concentration during the fermentation process in KF2 was $206 \mu\text{g}\cdot\text{dm}^{-3}$, which slightly exceeded the values provided by [35,41], but was within the range given by [38,51]. The average molybdenum concentration in the process carried out in KF2 was 2.14 times higher than in KF1.

Similarly, the minimum Co concentration of $329 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 1 of the fermentation process in KF1, and the maximum concentration of $649 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 21 of the fermentation process. The average concentration of Co during the fermentation process in KF1 was $543 \mu\text{g}\cdot\text{dm}^{-3}$, which is within the range defined by [9,52]. The minimum concentration of Co in KF2 of $312 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 1, and the maximum concentration of $1017 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 16 of the fermentation process. The average concentration of Co during the fermentation process in KF2 was $847 \mu\text{g}\cdot\text{dm}^{-3}$, which exceeded the values reported by [52,53]. The average concentration of cobalt in the process carried out in KF2 was 1.56 times higher than in KF1.

The lowest Ni concentration, $441 \mu\text{g}\cdot\text{dm}^{-3}$, was recorded on day 1 of the fermentation process in KF1, and the highest concentration, $587 \mu\text{g}\cdot\text{dm}^{-3}$, was recorded on day 21 of the fermentation process in this chamber. The average Ni concentration during the fermentation process in KF1 was $513 \mu\text{g}\cdot\text{dm}^{-3}$, which was within the range defined by [54,55]. In contrast, the KF2 chamber had the lowest Ni concentration of $466 \mu\text{g}\cdot\text{dm}^{-3}$, which was recorded on day 1 of the fermentation process, and the maximum value of $831 \mu\text{g}\cdot\text{dm}^{-3}$, which was recorded on day 26 of the fermentation process. The average Ni concentration during the fermentation process in KF2 was $711 \mu\text{g}\cdot\text{dm}^{-3}$, which did not exceed the values given by [56,57]. The average nickel concentration of the process carried out in KF2 was 1.21 times higher than in KF1.

The minimum Se concentration of $33.4 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 1 of the fermentation process in KF1. The highest Se concentration of $88.2 \mu\text{g}\cdot\text{dm}^{-3}$ was found on day 31 of the fermentation process in KF1. The average Se concentration during the process in KF1 was $70.1 \mu\text{g}\cdot\text{dm}^{-3}$, which was within the range defined by [10,44]. In the second KF2 tested, the lowest Se concentration of $32.7 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded, as with the other elements tested, on day 1 of the fermentation process. In contrast, the highest concentration of Se, $201 \mu\text{g}\cdot\text{dm}^{-3}$, was recorded on day 26 of the fermentation process. The average Se concentration during the process in KF2 was $145 \mu\text{g}\cdot\text{dm}^{-3}$, which did not exceed the values given by [10] and was within the range given by [58]. The average molybdenum concentration in the process carried out in KF2 was 2.07 times higher than in KF1.

The last element studied was Mn, whose concentration in KF1 from day 1 of the process ($712 \mu\text{g}\cdot\text{dm}^{-3}$) increased up to the value of $786 \mu\text{g}\cdot\text{dm}^{-3}$, which was recorded on day 26 of the fermentation process. The average Mn concentration during the fermentation process in KF1 was $758 \mu\text{g}\cdot\text{dm}^{-3}$, which was below the scope presented by [59,60]. The second KF2 chamber recorded a minimum Mn concentration of $728 \mu\text{g}\cdot\text{dm}^{-3}$ on day 1 of the fermentation process, and a maximum of $1478 \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 21 of the fermentation process. The average Mn concentration during the fermentation process in KF2 was $1274 \mu\text{g}\cdot\text{dm}^{-3}$, which did not exceed the values provided by [36,38] and was within the range given by [61]. The average molybdenum concentration of the process carried out in KF2 was 1.68 times higher than in KF1.

The process in KF2 proceeded without any disturbance, allowing it to be systematically loaded with a higher OLR. These values for KF1 and KF2 are shown in Figure 10. In the cases analyzed, OLR stabilized at $2.67 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for KF2 on the 25th day of the process and oscillated near this value until the end of the process. For KF1, the OLR value settled at a similar level 9 days later, i.e., on day 34 of the fermentation process. HRT stabilized at 36 d for KF2 on day 25 of the process and 37 d for KF1 on day 34 of the process. Significant differences were noted between the OLR values for the processes carried out in the KF1 and KF2 chambers. As early as on day 14 of the process, the expected volume of biogas production was achieved (Figure 11), and on day 27, the process parameters were fully stabilized (pH in the range of 7.9–8.1 and FOS/TAC ratio ≤ 0.2), as shown in Figure 8. Analyzing the parameters shown in Figure 11, it was found that the methane concentration in biogas for the process conducted in KF2 settled at an average level of 53.5 % v/v on day 14 of the process. For KF1, the average methane concentration in the biogas settled at a level of 52.2 % v/v much later, at around day 29 of the process. The oxygen content in both chambers (KF1 and KF2) decreased in a similar manner to the value of 0.1 % v/v. The hydrogen content of the biogas during the conduct of the experiment reached a value of about 90 ppm in the biogas from KF1 and KF2, with the difference being that in KF2, there were greater fluctuations in the concentration of H₂ in the analyzed biogas.

The content of hydrogen sulfide in the analyzed biogas from KF1 and KF2 was different. The maximum content in the biogas from KF1 was 1197 ppm on the 15th day of the process, and in KF2, it reached 954 ppm on the 19th day of the process. It was found that fluctuations in the concentration of H₂S in the biogas from KF1 and KF2 were significantly different in nature (Figure 11), with a favorable result being obtained in KF2 due to the supplementation with the CMP-S1 preparation.

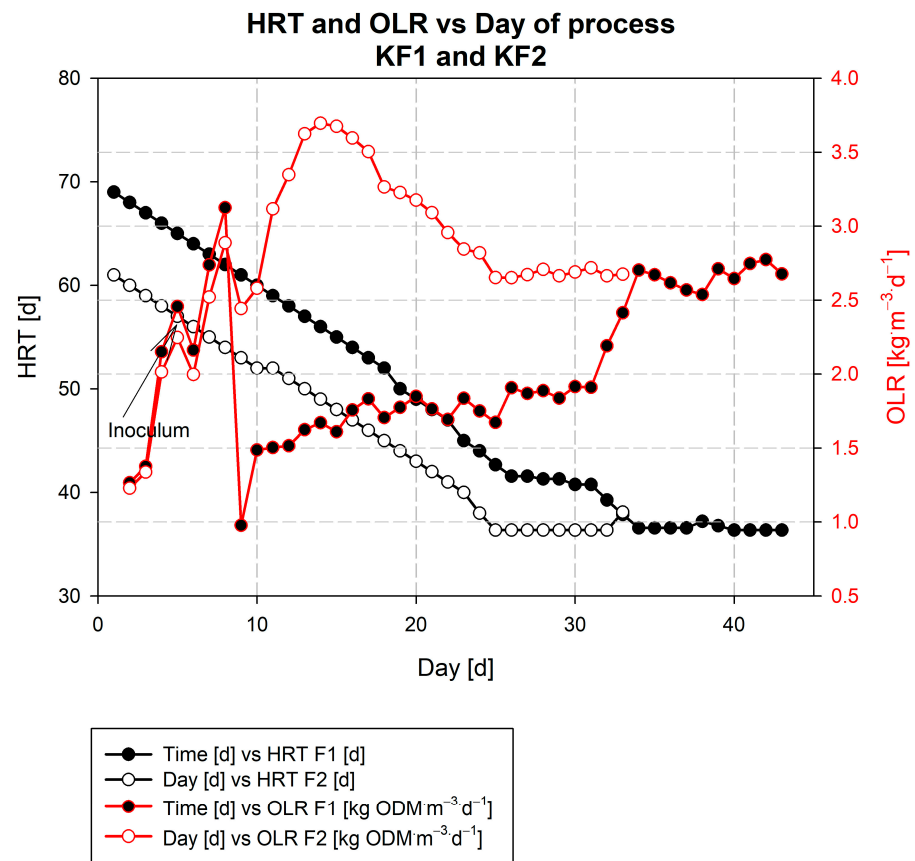


Figure 10. Process parameters KF1 and KF2.

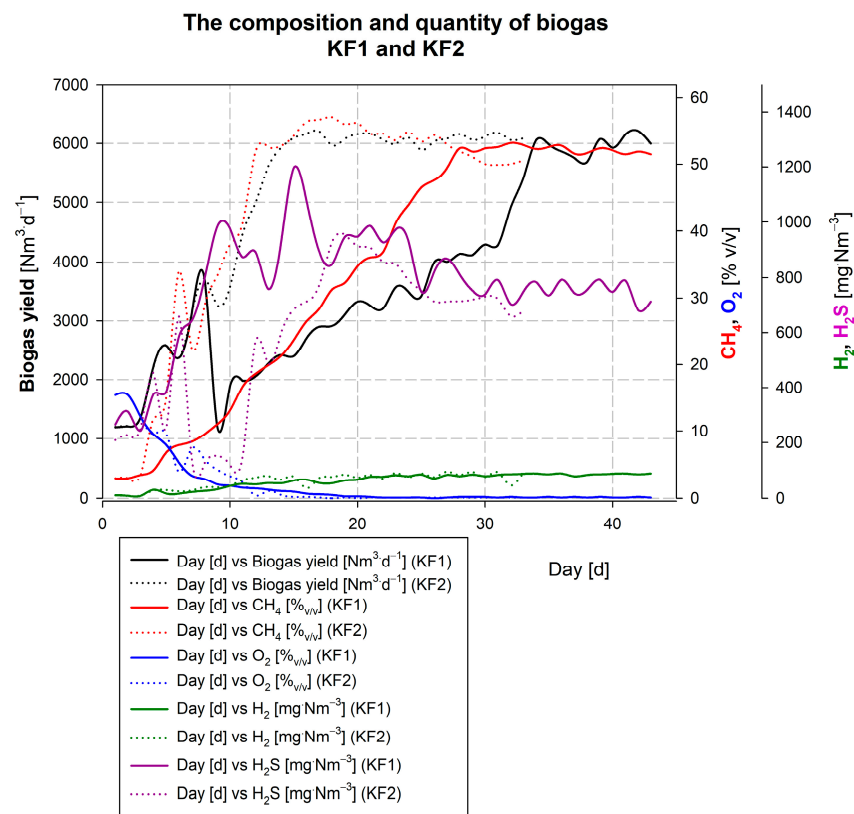


Figure 11. The composition and quantity of biogas in fermentation chambers KF1 and KF2.

3.3. Description of the Wicie Biogas Plant Kick-Off

The promising results of the experiments carried out at the Kupin biogas plant induced us to repeat them, unmodified, during the kick-off of the fermentation processes at the Wicie biogas plant in WF1 and WF2. It was planned that identical substrate feedstocks would be dosed to both digesters, and CMP-S1 preparation would be additionally dosed to the WF2 digester (Figure 12). In the period before the digesters began to warm, a mixture of cattle and pig slurry was accumulated in both digesters at a rate of 1300 m³ each. On day 5 of the process, the systematic dosing of substrates into both digesters began. On day 6 of the process, inoculum was dosed into WF1 and WF2 in the amount of 60 Mg each, from a model agricultural biogas plant in Tończa (φ 52°27'15.39'' N, λ 21°58'59.22'' W). In addition, from the 6th day of the process, 1.000 cm³ daily for WF2 for 4 days, and from the 10th day of the process, 250 cm³ of CMP-S1 preparation was dosed for the next 20 days, containing microelements in a chelated form with cyclodextrins and with the organic compounds bioavailable to methanogenic microorganisms.

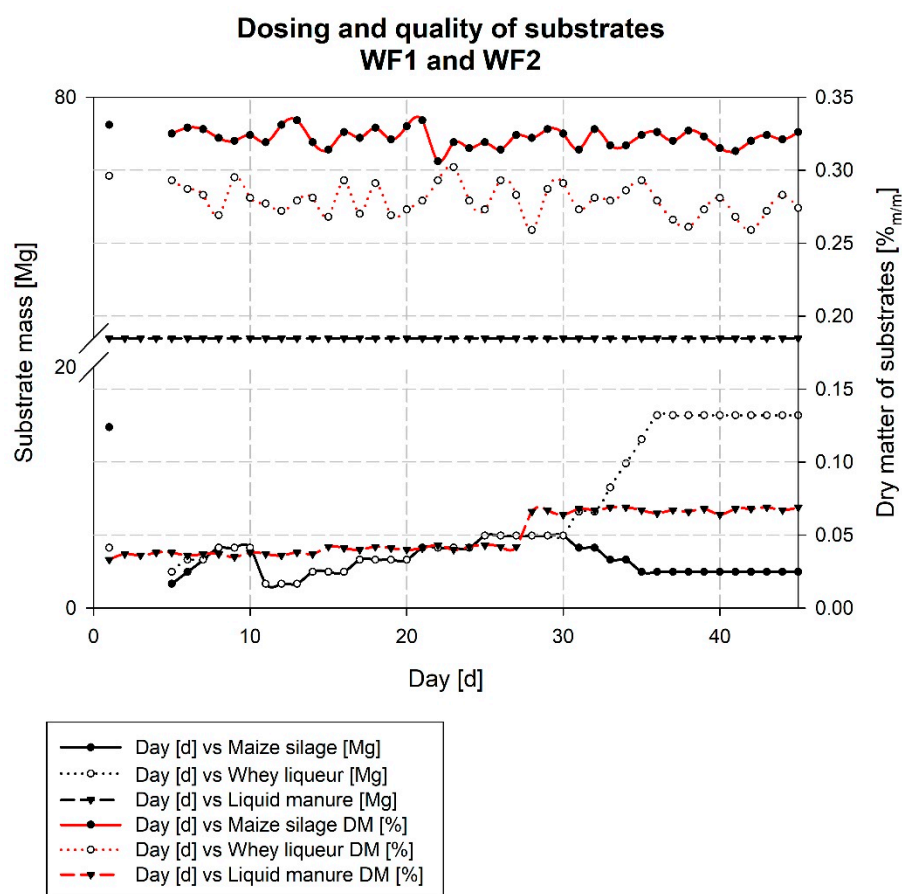


Figure 12. Dosing and quality of substrates fed to WF1 and WF2.

The process parameters of both fermenters on day 5 of the process, i.e., before the start of dosing CMP-S1 into WF2, were similar. From day 7 of the process, a significant deterioration in the parameters of WF1 compared to WF2 was observed (higher FOS, lower TAC, and decreasing pH) (Figure 13). On the 11th day of the process, for fear of the steadily deteriorating process parameters in WF1 stopping or critically disrupting the fermentation process, the decision was made to significantly reduce the amount of substrate dosage until the process parameters improved. On day 16 of the process, an improvement in the process parameters was observed, which enabled a gradual increase in the OLR loading of the digester. On the 34th day of the process, the expected biogas production volume was

achieved, and on the 41st day, the process parameters in WF1 were fully stabilized (pH in the range of 7.9–8.1 and FOS/TAC ≤ 0.2).

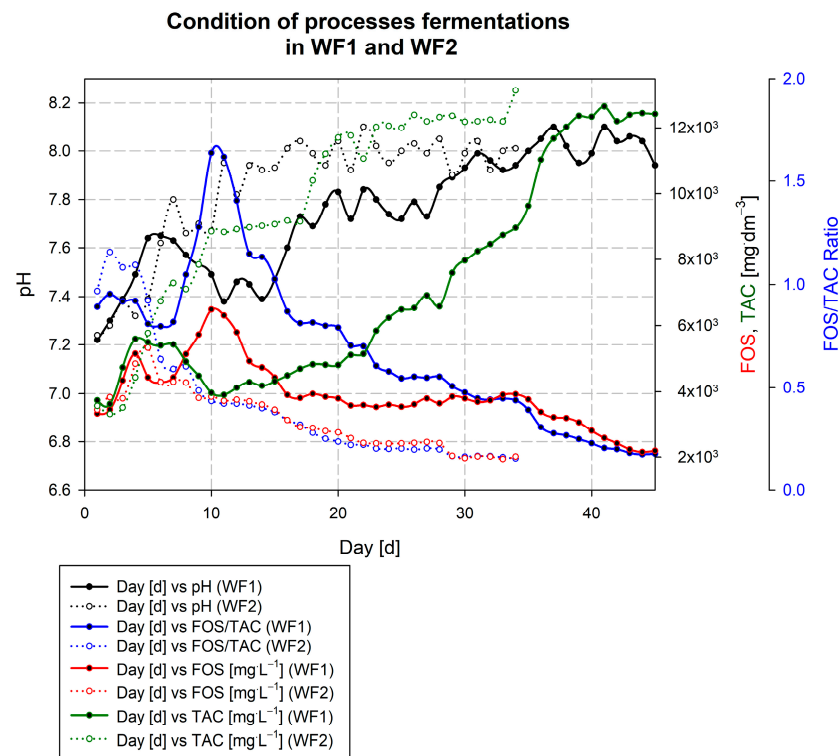


Figure 13. Condition of processes fermentations in WF1 and WF2 chambers.

At the same time, the process in WF2 ran smoothly, allowing it to be systematically loaded with a higher OLR. On the 14th day of the process, the expected volume of biogas production was already obtained, and on the 23rd day, the full stabilization of the process parameters was noted (pH in the range of 7.9–8.1 and FOS/TAC ratio ≤ 0.2) (Figure 13). At the same time, microelements in the form of CMP-S1 were dosed. The concentration of microelements in WF1 and WF2 during the fermentation processes is shown in Figure 14.

It was found that the concentration of microelements in WF2 was higher than that in WF1 (Figure 14) for each stage of the fermentation process. In the WF1 chamber, on the first day of the fermentation process, the lowest concentrations of the tested elements were recorded: Mo— $63.1 \mu\text{g}\cdot\text{dm}^{-3}$; Co— $277 \mu\text{g}\cdot\text{dm}^{-3}$; Ni— $398 \mu\text{g}\cdot\text{dm}^{-3}$; Se— $41.8 \mu\text{g}\cdot\text{dm}^{-3}$; and Mn— $911 \mu\text{g}\cdot\text{dm}^{-3}$. The maximum Mo concentration in WF1 of $99.6 \mu\text{g}\cdot\text{dm}^{-3}$ was found on the 45th day of the fermentation process, and the average value of the concentration of this element was $83.0 \mu\text{g}\cdot\text{dm}^{-3}$.

At the same time, the process in WF2 proceeded without any disturbance, allowing it to be systematically loaded with a higher OLR load. As early as day 14 of the process, the expected volume of biogas production was achieved, and by day 23, the process parameters were fully stabilized (pH in the range of 7.9–8.1 and FOS/TAC ratio ≤ 0.2) (Figure 13). At the same time, the dosing of microelements in the form of CMP-S1 was carried out. The concentration of micronutrients in WF1 and WF2 during fermentation processes is shown in Figure 14.

It was found that the concentration of micronutrients in WF2 was higher than in WF1 (Figure 14) for each stage of the fermentation process. The lowest concentrations of the tested elements were recorded in the WF1 chamber on the first day of the fermentation process: Mo— $61.8 \mu\text{g}\cdot\text{dm}^{-3}$; Co— $271 \mu\text{g}\cdot\text{dm}^{-3}$; Ni— $387 \mu\text{g}\cdot\text{dm}^{-3}$; Se— $40.5 \mu\text{g}\cdot\text{dm}^{-3}$; and Mn— $924 \mu\text{g}\cdot\text{dm}^{-3}$.

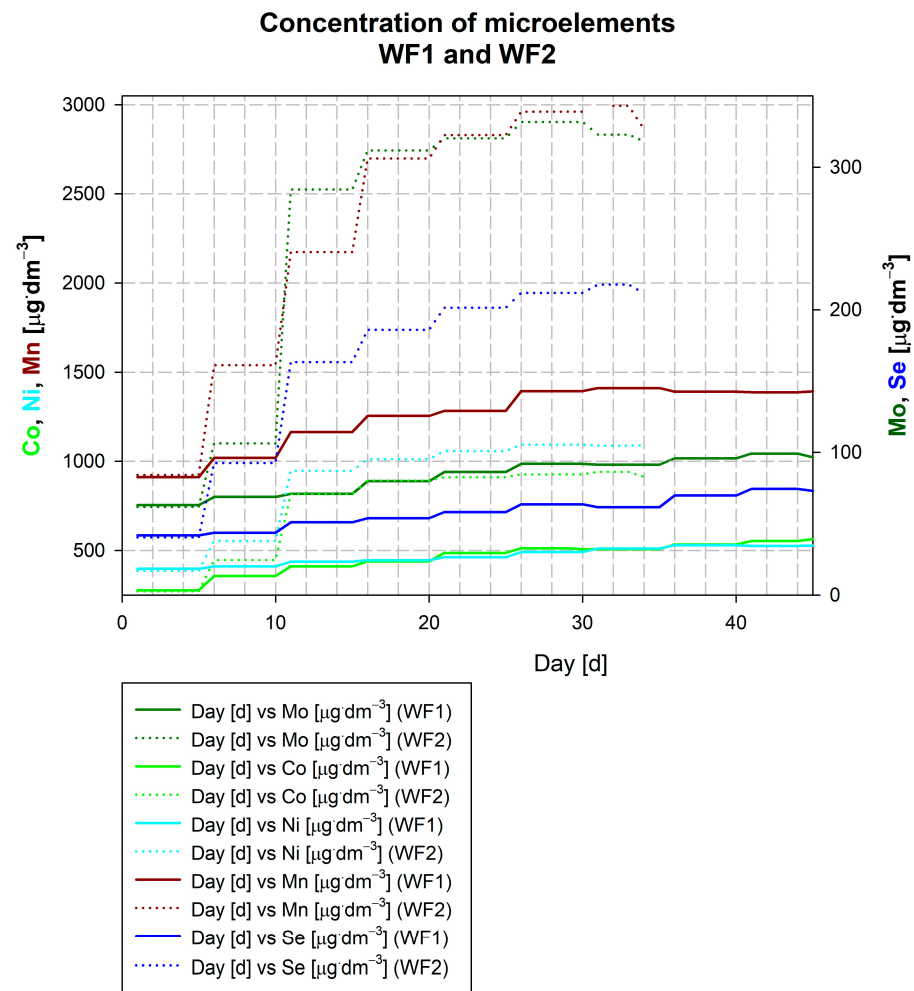


Figure 14. Microelements concentration in fermentation chambers WF1 and WF2.

The maximum Mo concentration, which was $331\ \mu\text{g}\cdot\text{dm}^{-3}$, was recorded on day 26 of the fermentation process in WF2, and the average concentration of this element during the fermentation process was $246\ \mu\text{g}\cdot\text{dm}^{-3}$ and was 2.96 times higher than in WF1.

The highest concentration of Co of $564\ \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 45 of the process in WF1, and in chamber WF2 on day 31 of the process, the value of $941\ \mu\text{g}\cdot\text{dm}^{-3}$ was recorded. The average concentration of Co during the fermentation process in WF1 was $453\ \mu\text{g}\cdot\text{dm}^{-3}$ and it was $737\ \mu\text{g}\cdot\text{dm}^{-3}$ in WF2. The average cobalt concentration in the process carried out in KF2 was 1.63 times higher than in WF1.

The maximum Ni concentration of $529\ \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 36 of the fermentation process in WF1, and the average concentration during the fermentation process was $468\ \mu\text{g}\cdot\text{dm}^{-3}$.

In WF2, the maximum Ni concentration of $1094\ \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 26 of the fermentation process, and the average concentration was $871\ \mu\text{g}\cdot\text{dm}^{-3}$. The average nickel concentration in the process carried out in WF2 was 1.86 times higher than in WF1.

Another element tested was selenium, whose highest concentration of $74.4\ \mu\text{g}\cdot\text{dm}^{-3}$ was recorded on day 41 of the fermentation process in WF1, and the highest concentration in KF2 of $218\ \mu\text{g}\cdot\text{dm}^{-3}$ was found on day 31 of the digestion process. The average Se concentration during the process in WF1 was $57.5\ \mu\text{g}\cdot\text{dm}^{-3}$, and in WF2 it was $157\ \mu\text{g}\cdot\text{dm}^{-3}$; therefore, the average selenium concentration in the process carried out in WF2 was 2.73 times higher than in WF1.

The last element studied was manganese. The maximum concentration of Mn, i.e., $1411\ \mu\text{g}\cdot\text{dm}^{-3}$, was registered on the 31st day of the digestion process in chamber

WF1, and in chamber WF2, the maximum concentration of this element reached a value of $2994 \mu\text{g}\cdot\text{dm}^{-3}$, which was recorded on day 31 of the fermentation process.

The average Mn concentration during the fermentation process in WF1 was $1246 \mu\text{g}\cdot\text{dm}^{-3}$, and it was $2279 \mu\text{g}\cdot\text{dm}^{-3}$ in WF2. The average molybdenum concentration of the process carried out in WF2 was 1.83 times higher than in WF1.

The process in the WF2 proceeded without any disturbance, allowing it to be systematically loaded with a higher OLR. These values for WF1 and WF2 are shown in Figure 15.

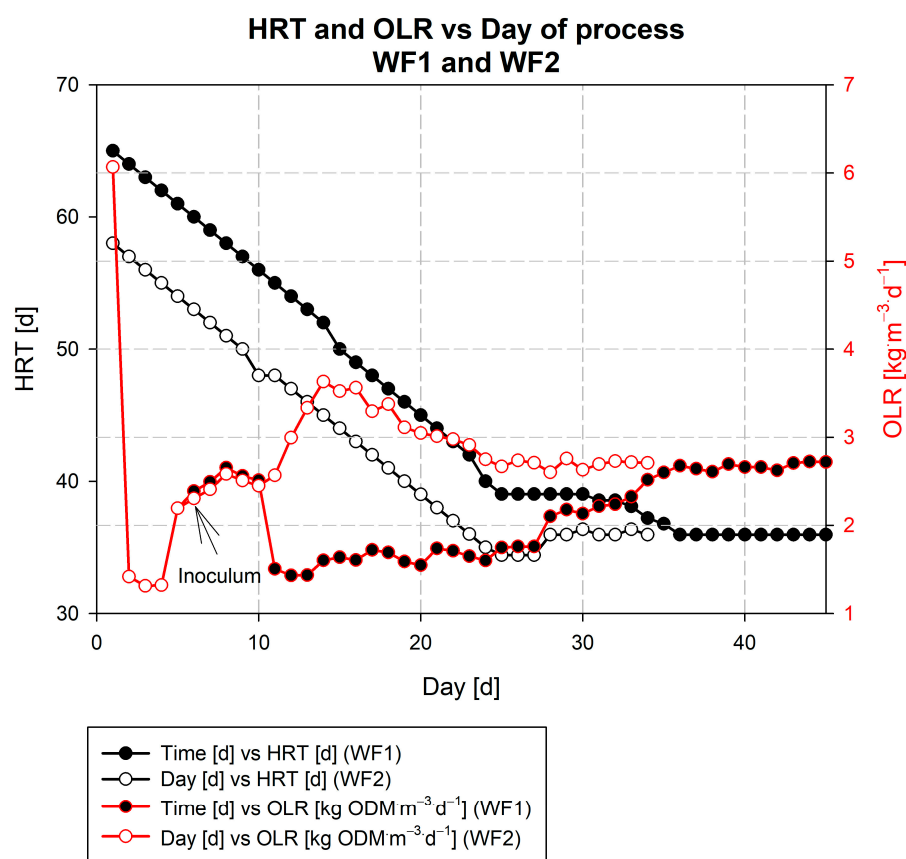


Figure 15. Process parameters in WF1 and WF2.

The OLR value settled at $2.70 \text{ kg}\cdot\text{m}^{-3}\cdot\text{d}^{-1}$ for WF2 on the 25th day of the process and remained at a similar level until the end of the process run. At WF1, the OLR value settled at a similar level on day 36 of the fermentation process. HRTs stabilized at 36 d for WF2 on day 28 of the process and 36 d for WF1 on day 36 of the process. There were significant differences between the OLR values for the processes carried out in the WF1 and WF2 chambers. On the 14th day of the process in the WF2 chamber, the expected volume of biogas production was achieved (Figure 16), and on the 28th day, the process parameters were fully stabilized (pH in the range of 7.9–8.1 and for FOS/TAC ratio ≤ 0.2), as shown in Figure 13. It was found that the methane concentration in biogas for the process conducted in WF2 settled at an average level of 56.5% v/v from day 16 of the fermentation process. In WF1, the average methane concentration in the biogas settled at 56.2% v/v from day 15 of the process. The oxygen content in both digesters (WF1 and WF2) decreased in a similar manner to a value of about 0.1% v/v . The hydrogen content of the biogas during the conduct of the experiment remained close to the value of 55 ppm in the biogas from WF1 and WF2.

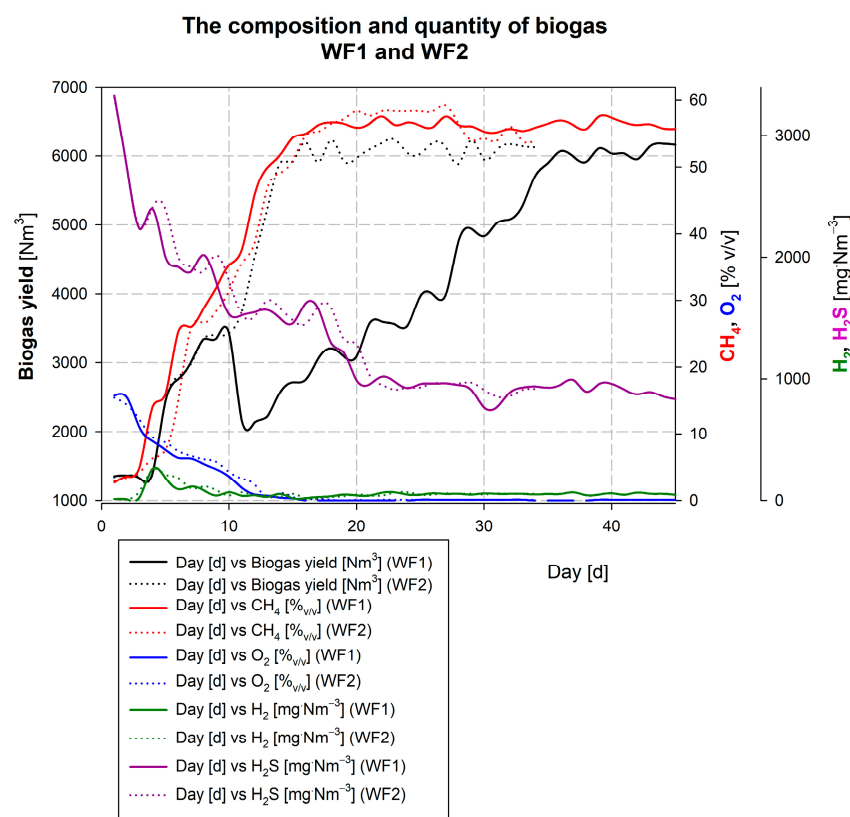


Figure 16. The composition and quantity of biogas in fermentation chambers WF1 and WF2.

4. Conclusions

The assessment of agricultural biogas plants is one of the essential processes in any newly built facility. This process is very vulnerable, and the factors determining its effectiveness when using different mixtures of substrates are poorly recognized and unpredictable. Hence, there is a need for experimental research in a laboratory on a semi-engineering scale but especially on an engineering scale. Very often, in newly built installations, the destabilization of the methane digestion process already occurs in the initial kick-off phase as a consequence of the overloading of the digester with dry organic matter or an improper balancing of macro and microelements, which stretches the process over time, generates additional costs and, in the worst case, can result in the need to replace the entire reactor contents and reinitiate the digestion process.

Reducing the kick-off stage of a biogas plant and stabilizing the parameters that determine a properly conducted fermentation process is the primary task facing any investor.

This paper presents the results of a study contributing to the knowledge of the impact of a dedicated mixture of microelements (DMM) on shortening the digester kick-off phase and stabilizing the methane fermentation process for its process and cost optimization.

The aim of the analysis was to determine the impact of an appropriately selected composition of microelements that utilize methanogenic archaeans on improving the stability and efficiency of the process and shortening the kick-off phase of the biogas plant, which was carried out based on three newly built facilities in Falknowo, Wicie, and Kupin. We conducted real-scale experiments on six fermenters (each with a working volume of 3200 m^3), which permitted the evaluation of the effectiveness of the CMP-S1 formulation that included the microelements Mo, Co, Ni, Se, and Mn.

From the data obtained, it can be seen that in all experiments/studies, the dosing of the CMP-S1 formulation resulted in:

- The rapid elimination of microelement deficiencies;
- The activation of methanogenic microorganisms in the fermenter; and
- The stabilization of the microbial metabolic process.

In addition, the present study showed that in fermenters KF2 and WF2, in which the preparation was dosed during the first days of kick-off compared to KF1 and WF1:

- Reaching full power took place over a span of 20 days;
- An average of 52% less substrate was used until full power in biogas production was achieved; and
- 27% less fuel oil required to power mobile boiler plants warming the digesters was used.

In conclusion, it can be said that the use of CMP-S1 in the initial stage of kick-off accelerates the process of biotechnological commissioning and the stabilization of the process parameters (indicators such as pH, alkalinity, the concentration of volatile fatty acids, and the methane content in biogas were used as a measure of the methane fermentation stability), which translates into a significant cost reduction.

The aim of the research in three locations was to determine the repeatability of the obtained results on an engineering scale and to average the fermentation material. At the Falkowo site, research was carried out to compare the starting state of the fermentation process. Two fermentation chambers (FF1 and FF2) were used to obtain the same process conditions. At the Kupin site, fermentation was carried out with and without the addition of CMP-S1 to capture changes in the fermentation process, especially its starting part. At the Wicie location, the dosage of micronutrients was repeated to achieve the repeatability of results. Further engineering-scale research should focus on the essence of releasing micronutrients from CDx complexes under process conditions. It may be interesting to determine the kinetics of use and excretion of individual micronutrients by a complex of fermentation microorganisms. The authors aim to determine the dynamics of change in the concentration of individual micronutrients during the fermentation process in subsequent studies. Of particular interest is the kick-off process.

To summarize, the experiments carried out in FF1 and FF2 allowed us to conclude that there are no significant differences when conducting processes with the same CMP-S1 dosage under the same conditions of fermentation processes. In WF1 and KF1, processes were carried out without CMP-S1 dosing, whereas WF2 and KF2 were conducted with CMP-S1 dosing. This form of experiment ensured the correctness of the experiment conducted, with a view to ensuring minimal differences in the composition of the substrates.

Author Contributions: Conceptualization, M.C. and S.L.; methodology, M.C. and A.B.; software, S.L. and M.C.; validation, M.C. and A.B.; formal analysis, M.C. and U.K.; data curation, S.L.; writing—original draft preparation, M.C.; writing—review and editing, U.K.; visualization, S.L. and A.B.; supervision, M.C.; project administration, S.L. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CMP-S1	Name of the formulation developed by authors.
DM	Dry matter (%).
DMM	Dedicated mixture of microelements.
ODM	Organic dry matter (% DM).
HRT	Hydraulic retention time (d).
IC50	The half-maximal inhibitory concentrations
FOS	Value corresponds to the volatile fatty acids content (De: Flüchtigen Organischen Säuren) ($\text{mg} \cdot \text{dm}^{-1}$).
TAC	Estimation of the buffer capacity of the sample (De: gesamt Anorganischen Carbonat) ($\text{mg} \cdot \text{dm}^{-1}$).
OLR	Organic loading rate ($\text{kg ODM} \cdot \text{m}^{-3} \cdot \text{d}^{-1}$).
FF1	Falknowo fermenter 01.
FF2	Falknowo fermenter 02.
WF1	Wicie fermenter 01.
WF2	Wicie fermenter 02.
KF1	Kornin fermenter 01.
KF2	Kornin fermenter 02.

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