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Anaerobic Digestion Remediation in Three Full-Scale Biogas Plants through Supplement Additions

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Abstract: Additives can improve the efficiency of anaerobic digestion by increasing biogas production, reducing air pollution, and preventing ammonia inhibition. Biological or chemical supplementation can also improve the economic efficiency of anaerobic digestion. However, the effects of specific additives on biogas production can vary, depending on the type of supplement used. This research utilizes the additives on an industrial scale and monitors the optimization of the anaerobic digestion operating parameters after their addition. The various AD additives were examined in a sufficient cycle of operation for three biogas plants located in northern Greece. In this manner, the effectiveness was investigated in multiple initial feeds and unstable operating situations caused by the seasonality of specific feedstocks. The existing operation state in the three biogas plants was recorded before and after adding the supplements. The addition of zeolite contributed to the reduction in the total ammoniacal nitrogen values in BG01 and BG03 plants. 8.4 tn of zeolite were added to the BG01 and BG03 plants over a period of two months. Low levels of trace element concentrations were observed in the BG02 plant; this issue was addressed by adding 5 kg of a trace element mixture every week over a period of 60 days. Introducing additives proved to be a stabilization factor in AD performance and an inhibition mediator.

Keywords: additives; anaerobic digestion; digestate; trace elements; zeolites

1. Introduction

Additives can have a significant impact on the efficiency of anaerobic digestion (AD) [1–3]. The dosage and types of additives used can influence the efficiency of AD, which mainly depends on the substrate material [1]. Inhibitors such as volatile fatty acids accumulation, high levels of total ammoniacal nitrogen (TAN), sulfur, and heavy metals can slow down the AD process and reduce its efficiency. However, the use of additives can also have advantages, such as reducing greenhouse gas emissions and converting carbon dioxide energy to methane [2]. Enzyme additives have been shown to increase the efficiency of AD. Overall, using additives in AD can be an effective way to improve biogas production and increase the energetic efficiency of biogas plants [1,3].

The performance of a biogas production plant depends on the conditions prevailing inside the bioreactor during AD. Anaerobic digestion is the decomposition and stabilization of organic materials by microbial organisms under anaerobic conditions, which leads to the production of biogas (a mixture of carbon dioxide and methane, a renewable energy source) and microbial biomass [4]. Anaerobic digestion is a synergistic process that uses mainly anaerobic microbes, and consists of four phases: (1) hydrolysis, (2) acidogenesis, (3) acetogenesis, and (4) methanogenesis. Figure 1 exhibits the progression and metabolic chemical compounds for each phase [5].
Anaerobic digestion of organic wastes including animal manure, food waste, and sewage sludge generates biogas. Biogas consists mainly of methane (CH$_4$, 45–70%) and carbon dioxide (CO$_2$, 30–45%), with traces of their gases and vapors, including water vapor, hydrogen sulfide (H$_2$S), ammonia (NH$_3$), hydrogen (H$_2$), nitrogen (N$_2$), and carbon monoxide (CO) [6]. Many variables can limit anaerobic digestion, including unstable feed, temperature and pH variations, a shortage of trace elements that are required for the “community” of bacteria to function, and the presence of interfering factors such as ammonia and other substances. One of the most critical parameters of anaerobic digestion is ammonia. Its non-polar chemical structure has the ability to easily pass through the cell membrane, causing fluctuations in the chemical balance of protons and loss of potassium (K). Methanogenic microorganisms are considered to be the most sensitive to the increase in ammonia concentration. The concentration of free ammonia and ammonium are inextricably linked through chemical equilibrium [7]. Through rising temperatures, the ratio of free ammonia to ammonium increases. The main destabilizing factors of ammonia are pH and temperature [8]. pH is a fundamental parameter that directly affects anaerobic digestion. The consistency of the pH value reveals the stability of the system and the lack of any interfering elements. Potential pH variations might be a sign of existing toxicity and a lack of buffering power. Free ammonia and ammonium are in equilibrium in aqueous solution, and high pH values favor the formation of free ammonia, which is the most toxic form of ammonia for the methanogens, instead of ammonium, limiting methane production [9], while low pH values inhibit the action of methanogenic bacteria and, by extension, methane production [10].

Determining the buffering capacity of digester contents is the most direct and reliable method for evaluating the smooth operation of the process. The total of the various volatile fatty acids produced during the acidogenesis phase is expressed through the FOS index (mg CH$_3$COOH/L). The total carbonate in the reactor is the TAC, and consists of the fraction of carbonate derived from microbial metabolism plus carbonate derived from the reactor feed. The pH inside the reactor is controlled by the concentration of carbon dioxide in the gas phase and the concentration of carbonate ions in the liquid phase. In case of low buffering capacity (low TAC) of the digester material, it is recommended to reduce the supply, add salts to carbonate production, or directly add carbonate to the supply. Volatile fatty acids produced during the acidogenesis phase is expressed through the FOS index (mg CH$_3$COOH/L). The total carbonate in the reactor is the TAC, and consists of the fraction of carbonate derived from microbial metabolism plus carbonate derived from the reactor feed.
acid (VFA) concentrations and the acetic acid equivalent are quite critical parameters for
the anaerobic digestion process, as they give an accurate picture of the prevailing situation
inside the digesters. VFAs are a product of the metabolic acidogenesis stage of the anaerobic
digestion process. It is necessary to check the profile of volatile fatty acids, as there is a
possibility of some acid accumulation. Accumulation of some acid is, more often than not,
capable of causing a decrease in efficiency in the unit as supply accumulates, which cannot
be degraded by the micro-organisms [11].

Products based on zeolite, trace elements, tri-valent iron, activated carbon, enzymes,
buffer solutions (for pH adjustment) and bacteria cultures are additives or supplements
that can improve the performance of anaerobic digestion. Zeolite has been shown to
have a good adsorption effect on ammonia nitrogen in anaerobic digestion [12] and, there-
fore, has been proposed as an additive to alleviate ammonia toxicity. Natural zeolites are
three-dimensional frameworks of SiO$_4$–4 and AlO$_4$–5 tetrahedra joined by shared oxygen
atoms [13]. They are crystalline, hydrated aluminosilicates of alkali and alkaline earth
cations, and porous substances with the capacity to reversibly lose and gain water, adsorb
molecules with the proper cross-sectional diameter (functioning as molecule sieves), and ex-
change their constituent cations without significantly altering their structural integrity [14].
Granular zeolite can be used as an auxiliary soil substance for the improvement of the
physical–chemical properties of soils, as a sorbent of aggressive substances from, or as a
universal sorbent of, odors and moisture intended for use in the household, as a carrier of
nutrients and trace elements in manufacturing the fertilizers, and as a carrier of effective
substances [15]. Zeolites are utilized in a variety of industrial, agricultural, and contamina-
tion prevention applications, including adsorption and separation, ion exchange, ammonia
removal in the treatment of municipal and other industrial wastewaters, the removal of
cesium and strontium from radioactive wastes, the removal of heavy metals from industrial
wastewaters as well as in animal feed additives, deodorizing animal installations, chemical
filtration, and more [13]. Trace element supplements, such as iron, cobalt, nickel, and
selenium can improve the economic viability and conductivity of anaerobic digesters [2],
especially under deficit conditions of these cations, while buffer solutions have been used to
regulate the pH [12]. It is well known that in anaerobic processes, trace elements typically
act as micronutrients for different enzymatic reactions as co-factors, promoters of microbial
aggregation which increases the activity of the anaerobic microbes in the case of syntrophy,
agents binding carrier proteins and/or nutrients such as phosphates, help to overcome
sulfide toxicity through metal sulfide precipitation, and may become toxic to the microbial
biomass at increasing amounts [16]. These different trace element impacts are influenced by
the surrounding environment, background concentrations, bioavailability, and microbial
uptake. Their speciation, or the distribution of an element among various chemical species
in a system, is typically associated with their bioavailability [17]. These additives can pro-
mote gas production and improve process stability, system recovery time from volatile fatty
acids inhibition, and microbial community dynamics in anaerobic digestion [18]. Although
there is extensive literature concerning the use of these supplements in laboratory scale
reactors, reports about their use in full-scale biogas plants are limited. In this study, the
application of specialized supplements for the stabilization of the anaerobic digestion process
in two cases of full-scale biogas plants operating under high ammonia concentrations and
high pH, as well as a case where a deficiency of trace elements, was observed.

2. Results
2.1. Operating Conditions of Biogas Plants and Additives Application

Data were collected from three different biogas plants (BG01, BG02, and BG03), in
order to monitor the pre-additive state of phases (Tables S1–S4). Each plant exhibited
different operational issues, for which a plant-individual approach was used to provide so-
lutions. Analyses were performed on digester material samples (digestate), and operational
parameters such as pH, total ammonia nitrogen concentration, FOS/TAC, VFA profile, and
trace elements concentration were verified. Table 1 exhibits the operating conditions of the three examined biogas production plants.

**Table 1.** Technical specifications and operational parameters amongst the three biogas plants (BG01, BG02, and BG03).

<table>
<thead>
<tr>
<th>Biogas Plant</th>
<th>BG01</th>
<th>BG02</th>
<th>BG03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical Power</td>
<td>1 MW</td>
<td>2 MW</td>
<td>1 MW</td>
</tr>
<tr>
<td>Capacity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-tank</td>
<td>350 m³</td>
<td>550 m³</td>
<td>290 m³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>160 m³</td>
</tr>
<tr>
<td>Daily Supply</td>
<td>100 tn/d: cow slurry</td>
<td>79 tn/d: corn silage</td>
<td>1 tn/d: cow manure</td>
</tr>
<tr>
<td></td>
<td>20 tn/d: olive mill waste</td>
<td>4 tn/d: glycerol</td>
<td>90 tn/d: cow slurry</td>
</tr>
<tr>
<td></td>
<td>20 tn/d: cheese whey</td>
<td></td>
<td>17 tn/d: chicken manure</td>
</tr>
<tr>
<td></td>
<td>15 tn/d: corn silage</td>
<td></td>
<td>3 tn/d: vegetable residue</td>
</tr>
<tr>
<td></td>
<td>First, 20 tn/d: chicken manure Then, 5 tn/d: chicken manure and 15 tn/d: sheep manure</td>
<td>45 tn/d: waste residues</td>
<td>30 tn/d: cheese whey</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.5 tn/d: corn silage</td>
</tr>
<tr>
<td>Feeding Rate</td>
<td>8.1 tn/h</td>
<td>2.3 tn/30 min</td>
<td>5.9 tn/h</td>
</tr>
<tr>
<td>1st Digester (D1)</td>
<td>4000 m³</td>
<td>4000 m³</td>
<td>5130 m³</td>
</tr>
<tr>
<td>Temperature</td>
<td>37 °C</td>
<td>45 °C</td>
<td>40–42 °C</td>
</tr>
<tr>
<td>HRT</td>
<td>21 days</td>
<td>31 days</td>
<td>36 days</td>
</tr>
<tr>
<td>Stirring</td>
<td>45 min</td>
<td>Constantly</td>
<td>30 min</td>
</tr>
<tr>
<td>2nd Digester (D2)</td>
<td>4000 m³</td>
<td>2.800 m³</td>
<td>2300 m³</td>
</tr>
<tr>
<td>Temperature</td>
<td>40 °C</td>
<td>39.5 °C</td>
<td>54–56 °C</td>
</tr>
<tr>
<td>HRT</td>
<td>21 days</td>
<td>22 days</td>
<td>17 days</td>
</tr>
<tr>
<td>Recirculation of Digested Residue</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

A customized monitoring plan was implemented to enhance each plant’s performance and address any issues. The usage of the proper additive was advised for each plant, either on a daily or weekly basis. Zeolite was added in plants BG01 and BG03 to reduce total ammoniacal nitrogen levels, and a mixture of citric and phosphoric acid mixture (CPmix) was added to adjust the pH. A trace element mixture was applied to plant BG02. The quantity and frequency of use for each plant are detailed in Table 2.

**Table 2.** Frequency and amount of additives supplement in plants BG01, BG02, and BG03.

<table>
<thead>
<tr>
<th>Biogas Plant</th>
<th>Additive</th>
<th>Quantity</th>
<th>Frequency</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG01</td>
<td>Zeolite</td>
<td>8.4 tn</td>
<td>500 kg/day for 6 days, and 100 kg/day for 54 days</td>
<td>60 days</td>
</tr>
<tr>
<td></td>
<td>Buffer solution</td>
<td>25 kg citric acid and 100 lt phosphoric acid 5% v/v</td>
<td>Every 5 days</td>
<td></td>
</tr>
<tr>
<td>BG02</td>
<td>Trace Elements mixture</td>
<td>5 kg</td>
<td>Weekly</td>
<td>60 days</td>
</tr>
</tbody>
</table>
2.2. The Effect of Zeolite and Acidic Buffer Solution (Citric and Phosphoric) in Biogas Plant BG01

The pH and total ammoniacal nitrogen (TAN) values were decreased by utilizing zeolite and the buffer solution in plant BG01.

The BG01 biogas plant operated under unstable conditions, as shown in Figure 2. Along with the increase in pH to 8.5, there was also a significant rise in ammonia exceeding 4500 ppm which, in combination, affected the significant reduction in methane production. At high pH values, ammonia transforms into free ammonia, which is the toxic form of ammonia for methanogenic populations, which contributed to the significant reduction in methane. To address this situation, the biogas plant reduced feeding quantities with poultry manure which, due to its protein composition, is rich in ammonia and, at the same time, zeolite and CPmix were introduced into the reactor, as shown in Table 2. The average values (for the period of 120 days) of all monitored parameters during additives application at BG01 exhibited an improvement, presenting a decrease in pH from 8.2 to 8.0 and in TAN from 3492 to 3166 ppm (Figure 2c). FOS ranged from 3031 to 2506 mg/L, TAC from 13,446 to 13,937 mg/L, while FOC/TAC ratio ranged from 0.227 to 0.180 (Figure 2a). Meanwhile, methane production increased from 57.3% to 58.8%, while biogas production ranged from 9620 m$^3$ to 10,092 m$^3$ (Figure 2d). The introduction of additives combined with the discontinuation of poultry manure supplement helped to reduce the ammonia concentration in the biogas plant and to stabilize the pH resulting in the treatment of the ammonia toxicity that had occurred. The reduction in ammonia is due in part to the addition of the zeolite, which adsorbed some of the ammonium ions. It is noteworthy that the reactor maintained steady conditions long after the additives’ inclusion.

Figure 2b also shows the VFAs of the plant, which were elevated during the toxicity period, and which decreased with the introduction of the additives. Prior to the application of additives, a high accumulation of VFAs was observed, specifically acetic acid (>1500 ppm). It should be noted that increased VFAs in a reactor, especially propionic acid, are an indicator of anaerobic degradation instability. During the implementation of zeolite and CPmix, VFAs’ values were decreased with the average acetic acid values ranging from 976 ppm to 1915 ppm. Anaerobic digestion involves several crucial intermediates, including acetic (AA), propionic (PA), and isobutyric (IBA) acids. These acids are produced by a variety of microbes as organic matter breakdowns down in anaerobic conditions, such as in a biogas digester. The stability and functionality of the digester can be observed using the concentration and ratio of VFAs. High quantities of VFAs may signal a microbial population imbalance or further issues with the digester [19]. The VFAs that are most frequently evolved during anaerobic digestion are acetic, propionic, and butyric acids [20]. Acetic acid is the most abundant VFA and is produced by a wide range of microorganisms. A particular type of microorganism produces propionic acid, which is a crucial step in the production of methane. Compared to acetic and propionic acids, isobutyric acid is generated by a narrower variety of microorganisms and is less prevalent [21]. Issues with anaerobic digestion are indicated by acetic acid levels greater than 800 mg/L, or a propionic to acetic ratio (PA/AA) greater than 1.4 [22].
2.2. The Effect of Zeolite and Acidic Buffer Solution (Citric and Phosphoric) in Biogas Plant BG01

The pH and total ammoniacal nitrogen (TAN) values were decreased by utilizing zeolite and the buffer solution in plant BG01. The BG01 biogas plant operated under unstable conditions, as shown in Figure 2. Along with the increase in pH to 8.5, there was also a significant rise in ammonia exceeding 4500 ppm which, in combination, affected the significant reduction in methane production. At high pH values, ammonia transforms into free ammonia, which is the toxic form of ammonia for methanogenic populations, which contributed to the significant reduction in methane. To address this situation, the biogas plant reduced feeding quantities with poultry manure which, due to its protein composition, is rich in ammonia and, at the same time, zeolite and CPmix were introduced into the reactor, as shown in Table 2. The average values (for the period of 120 days) of all monitored parameters during additives application at BG01 exhibited an improvement, presenting a decrease in pH from 8.2 to 8.0 and in TAN from 3492 to 3166 ppm (Figure 2c). FOS ranged from 3031 to 2506 mg/L, TAC from 13,446 to 13,937 mg/L, while FOC/TAC ratio ranged from 0.227 to 0.180 (Figure 2a). Meanwhile, methane production increased from 57.3% to 58.8%, while biogas production ranged from 9620 m³ to 10,092 m³ (Figure 2d). The introduction of additives combined with the discontinuation of poultry manure supplement helped to reduce the ammonia concentration in the biogas plant and to stabilize the pH resulting in the treatment of the ammonia toxicity that had occurred. The reduction in ammonia is due in part to the addition of the zeolite, which adsorbed some of the ammonium ions. It is noteworthy that the reactor maintained steady conditions long after the additives inclusion.

2.3. The Effect of Trace Elements in Biogas Plant BG02

Due to nutritional deficits in D1, the plant had operational issues with reduced yields and unstable operation. Low quantities of copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), calcium (Ca), and sodium (Na), six elements that are crucial to the process, were present in the biogas plant, as is shown in Figure 3a,b. The variation diagrams of trace elements, nutrients, biogas production and methane content, before and during their addition to the plant’s digester, are shown in Figure 3. The use of the additive contributed to the increase in trace elements critical for the plant (Cu, Zn, Fe, Mn, Ca, and Na), which are responsible for combating the toxicity that may occur in the plant and expanding to ensure its steady operating state [23–25]. Meanwhile, both biogas and methane production displayed no visible fluctuations (Figure 3c). The average values of the trace elements for the period before and after additives’ application, were calculated in order to compare with the optimal values of trace elements. It appeared that most trace elements and nutrients showed an increase in their concentration.

2.4. The Effect of Zeolite and Acidic Buffer Solution (Citric and Phosphoric) in Biogas Plant BG03

Since BG03 presented the same deficiencies as BG01, it was considered reasonable to test the same recipe as the prior plant. As shown in Figure 4a, the FOS/TAC ratio, which was initially at high levels, demonstrated a downfall with the addition of the supplements, averaging from 0.249 to 0.200, while FOS values ranged from 3476 mg/L to 2905 mg/L and TAC from 14,033 mg/L to 14,516 mg/L. During this period, average methane production ranged from 56.5 to 57.8% and biogas production from 8686 to 9565 m³ (Figure 4d). Furthermore, the use of zeolite and CPmix affected the concentration of ammonia, which initially appeared in high levels (>3000 mg/L) (Figure 4c).
Figure 3. (a1,a2) Trace elements, (b) nutrients, and (c) biogas production and methane content of plant BG02 over a period of 120 days.
Figure 4. Variation diagrams of (a) alkalinity buffer capacity, (b) volatile fatty acid (VFA) distributions, (c) total ammonia nitrogen (TAN) and pH, and (d) biogas production and methane content in plant BG03 over a period of 120 days.
3. Discussion

The performance of a biogas production plant depends on the conditions prevailing inside the bioreactor during anaerobic digestion (AD). The use of additives contributes to the better performance and operation of bioreactors. In this research, issues encountered by three biogas plants (BG01, BG02, and BG03) have been identified and AD supplements were offered to address them. The malfunctions displayed by the plants are mainly due to their seasonal feedstock variation, the temperature prevailing inside the digesters, and various operational parameters, such as the frequency of mixing the substrate material. In particular, in plant BG01, high pH and total ammoniacal nitrogen values were observed at the beginning of the recording of the plant’s digester conditions. The use of the CPmix to reduce the pH value, and the addition of zeolite to maintain the values at a constant level, resulted in the smooth operation of the digester. Accordingly, the combination of the two additives resulted in a reduction in the total ammoniacal nitrogen recorded in the digester.

The phenomenon of high pH values also appeared in plant BG03, where the pH value was above 8.2. Additionally, high ammoniacal nitrogen values and the accumulation of volatile fatty acids were observed. To improve the operation system of the plant’s digesters, CPmix and zeolite were used in a quantity and frequency that corresponded to the size and needs of the digesters. According to the methane variation, a slight increase in methane was observed during the period of both zeolite and CPmix (citric acid-phosphoric acid buffer) addition in the BG01 and BG03 plant.

BG02 biogas plant, however, showed deficiencies in trace elements that contribute to its improper functioning. Anaerobic digesters’ microbial diversity may be affected by the recirculation of digestate since it introduces additional nutrients and microbes that may change the composition of the microbial community [26]. This had a positive impact on AD process instability, which was observed during the period of their shortage, reducing the efficiency rate of the plant.

Accumulation of some acids can cause a decrease in efficiency in the plant most of the time, as feed accumulates which cannot be degraded by microorganisms. In a mesophilic reactor, the range of total VFA should be <1000 mgL\(^{-1}\) for a stable process [27–32]. However, individually, the optimum limit for acetic acid was reported as <1000 mgL\(^{-1}\), for propionic acid <250 mgL\(^{-1}\), and for longer chained VFA, such as butyric and valeric <50 mgL\(^{-1}\). During the monitoring of the BG01 plant, an accumulation of VFAs was observed. In particular, the levels of acetic acid were above 3000 mgL\(^{-1}\), as is shown in Figure 3. At the same time, an increase in ammonia was observed in the same plant (Figure 3). Ammonium nitrogen produced by protein degradation, in an aqueous environment such as in a digester, is present as both ammonium ions and as free ammonia. The percentage of NH\(_4\)-N present in the form of free ammonia (NH\(_3\)(aq)) increases at higher pH or temperature. Free ammonia is the main cause of inhibition since it freely passes through the cell membrane of the microbes [1]. In order to eliminate the aforementioned barriers and thereby improve the operating conditions of the BG01 plant, the use of zeolite was chosen as an additive, due to its properties as mentioned.

A comparative evaluation of different additives is in the scope of our upcoming research, in which the effectiveness of various additives, such as biochar or activated carbon in biogas production, will be examined. Nevertheless, different studies have investigated the performance of various supplements, such as carbon-based accelerants, biological additives, and alkali addition. The application of carbon-based materials, such as graphene, activated carbon, and biochar to anaerobic digestion systems, accelerates the direct interspecies electron transfer behaviors and helps in microbial immobilization and growth due to their favorable physicochemical properties [33]. Biological additives, such as microorganisms and enzymes, have been shown to be highly efficient [34]. However, the cost of commercial enzymes as an additive is high. The alkali (e.g., NaOH, lime, KOH) that are used to pretreat substrates with high cellulose can improve CH\(_4\) conversion [35].
4. Materials and Methods

The experimental setup was based on monitoring three biogas plants for a period of 120 days. During the monitoring period, the condition of the plants before (day 1 to day 60) and during the use of additives (day 61 to day 120) was systematically monitored. The individualized intervention was carried out in each biogas plant, adding the necessary supplement based on the deficiencies it presented. Throughout the trial period, samples were collected, at an average frequency of 5 days, from the D1 digesters of the three plants. Afterward, physicochemical characterization and determination of trace element concentrations followed. The results of the measurements contributed to recording the existing situation of all three biogas plants and implementing corrective actions by using supplements to improve their operating conditions.

Among the additives used for the treatment of anaerobic digestion destabilization in a biogas plant was zeolite. Zeolite is produced from natural zeolite of the clinoptilolite type (hydrated aluminosilicate of alkaline metals and alkaline earth metals). The used zeolite, which was quarried in the northern Greece, is a clinoptilolite of sedimentary origin with clinoptilolite content up to 85% (an average content of 80%), with the heavy metal content of lead, cadmium, and arsenic below the international specifications for a powder form with a grain diameter of 45 µm [16]. Zeolite is characterized as a mineral with a ratio of Si/Al from 4.8 to 5.4, pH from 6.8 to 7.2, and an effective pore diameter of 4 Å [36].

Citric acid monohydrate with ≥99.5% (C₆H₈O₇·H₂O) (food grade) and phosphoric acid (75–86% phosphoric acid and 14–25% water) (food grade) from Interchim (Interchim S.A., Thessaloniki, Greece) were also used.

The trace elements supplement, which was used for the BG02 plant, was a customized powder form consisting of manganese sulphate <25%, nickel sulphate <20%, cobalt acetate tetrahydrate <12.5%, zinc oxide <10%, sodium selenite <5%, copper sulphate <5%, and boric acid <3% (OLIgas by Methodo Chemicals, Italy). Its special formula accelerates the use of accumulated volatile organic acids by methanogenic bacteria (in the event of acidosis or alkalosis) [37].

Based on the technical specifications (e.g., capacity, feedstock quantity, HRT) and operation parameters (e.g., pH, ammoniacal concentration, VFAs) of each biogas plant, the proper additive was determined. Therefore, the plants BG01 and BG03 used a mixture of 25 kg citric acid and 100 lt phosphoric acid 5% v/v. The trace element deficiencies of the BG02 determined the selection of the added trace element mixture.

4.1. Determination of Trace Elements

An Agilent 7850 ICP-MS (Agilent Technologies, Santa Clara, CA, USA) equipped with the ORS collision cell was used for the analysis of macro-elements and trace metals. Sampling was performed using an Agilent SPS 4 autosampler. The 7850 ICP-MS was configured with the standard ISIS 3 injection system. The IntelliQuant function in the ICP-MS MassHunter 5.1 software provides the capability of a full mass-spectrum scan with only two seconds additional measurement time, though the samples were quantitated by internal standard seven-point calibration. The samples were prepared for analysis according to the digestion procedure outlined in ISO 17294 Part I and II and APHA 3125 [38].

The sample is decomposed in acid at a high digestion vessel pressure with the help of a Milestone Ethos Up microwave oven and the resulting solution is analyzed. First, an amount of sample (0.5–1.0 g) was weighed and HNO₃ and H₂O₂ were added to the sample followed by digestion gradually up to 210 °C. The sample was then diluted and analyzed by ICP-MS. Its concentration calculation occurs using templates.

4.2. Determination of Moisture

For moisture determination, an amount of sample (1 g) was weighed in a pre-weighed crucible and placed in an oven at 105 °C for 18 h. The sample was then removed from the oven and placed in a desiccator until it returned to ambient temperature, and reweighed. The moisture content as a percentage of the sample is calculated as follows:
Moisture = 100 − \left(\frac{(W_F - W_I)}{W_S}\right) \times 100

where \(W_I\): initial container weight (dry), in grams, \(W_S\): weight of the sample amount, in grams, and \(W_F\): final weight of the container with the dry sample, in grams.

4.3. Determination of Ash

For the determination of ash, an amount of sample (2 g) was weighed in a pre-weighed crucible and placed in a furnace at 550 °C for 3 h. The sample was then removed from the furnace and placed in a desiccator until it returned to ambient temperature, and reweighed. The crude ash content as a percentage of the sample is calculated as follows:

\text{CrudeAsh} = \frac{(W_F - W_A)}{W_I} \times 100

where \(W_I\): initial container weight (dry), in grams, \(W_S\): weight of the sample amount, in grams, and \(W_F\): final weight of the container with ash, in grams.

4.4. Determination of Total Ammoniacal Nitrogen (TAN) Concentration by Nessler Method

The determination of total ammoniacal nitrogen (TAN) concentration was held using a Vapodest (Vapodest 40s Gerhardt, S.N. 7340130001) for steam distillation of and receiving of ammonia in aqueous solution, based on a modified method according to A.P.H.A. The digester sample bottle was sufficiently stirred on a magnetic stirrer. A sample weighing 5 g (with an accuracy of ± 0.05 g) was taken and weighed in a 50 mL falcon tube and 45 mL of deionized water was added. Another identical falcon tube was prepared to balance the centrifuge. Centrifugation was applied at 6000 rpm for 20 min.

The samples were removed from the centrifuge, and a volume of 5 mL of the supernatant was extracted from them (without shaking) and brought up to the required volume in a 250 mL volumetric flask with deionized water. The flask was then vigorously shaken and a volume of 5 mL was transferred to a 25 mL volumetric flask with deionized water. Then, 3 drops of mineral stabilizer were added, 3 drops of polyvinyl alcohol dispersing agent, followed by agitation and, finally, 1 mL of Nessler reagent. The sample was left to rest for 15 min.

Using 10 mm optical path cells, the concentration of NH\textsubscript{4}\textsuperscript{+} was determined photometrically in the JASCO V-630 Spectrophotometer after 15 min. The range of the measurement is 0.1 mg/L to 2 mg/L. The Nitrogen (Ammoniacal) APHA 4500-NH3 B and C method was used to base the measurement (APHA, 2017) [38].

\[ C_{N-NH_4^+} = \frac{C_{NH_4^+}}{1.2871} = \frac{D_f \cdot C_{420nm}}{1.2871} \]

where \(C_{N-NH_4^+}\): Concentration of ammonium nitrogen (ppm), \(C_{NH_4^+}\): concentration of ammonia (ppm), \(D_f\): dilution factor (sample weight corrected), and \(C_{420nm}\): absorbance concentration at 420 nm (nm).

4.5. Determination of FOS/TAC Ratio

The FOS/TAC ratio is an indicator for assessing fermentation processes. The TAC value is an estimation of the total inorganic carbon, while the ratio corresponds to the alkalinity buffer capacity of the sample, and the FOS value corresponds to the volatile fatty acids content. It is calculated empirically according to the Nordmann method. A sample of 5 mL of fermentation substrate is titrated by 0.1 N of sulfuric acid solution (H\textsubscript{2}SO\textsubscript{4}) up to pH 5.0 to calculate the TAC value, expressed in mg/L of calcium carbonate (CaCO\textsubscript{3}). Then, the FOS value is obtained after a second titration step between pH 5.0 and pH 4.4. It is expressed in mg/L of acetic acid (CH\textsubscript{3}COOH) [39].
4.6. Determination of Volatile Fatty Acids (VFAs)

The Eppendorf minispin table centrifuge is used to centrifuge a 1.5 mL sample in a 2 mL Eppendorf tube for 10 min at 12,000 rpm. In order to have the VFAs (acetate, propionate, butyrate, iso-butyrate, valerate, and iso-valerate) in their acidic form and saturate the basic sites on the analytical column, the sample is acidified with 100 µL ortho-phosphoric acid to pH 2 prior to centrifugation. A total of 100 µL of the injection standard and 1 mL are added to the GC vial. A flame ionization detector (FID)-equipped GC Shimadzu GC–2010 Plus High-End gas chromatography system is used to inject the liquid phase. The column used was an Altmann Anaytik AS-FFAP EXT, 30 m × 0.25 mm × 0.25 µm. Helium (grade 99.999%) was used as a carrier gas at a flow rate of 1.9 mL/min. The injection volume was 1 µL with a split ratio of 1:10 and the injector temperature was 250 °C. The detector temperature was set at 250 °C. The temperature program employed was: initial oven temperature at 100 °C (hold time: 2 min), increasing at 10 °C/min to 220 °C (hold time: 0 min), then in the last step at 30 °C/min to 240 °C (hold time: 12 min). The total run time was 27 min. The VFA concentration is determined by a linear calibration curve obtained by calibration standards and adjusted by the injection standard.

5. Conclusions

The findings from the monitoring of the three examined biogas plants (BG01, BG02, and BG03) showed that the use of additives such as zeolite, which adsorbs ammonia and citric-phosphoric buffer solution to stabilize the pH, contributed to the immediate treatment of ammonia toxicity. Trace element applications can also contribute to combating the toxicity that may occur in the plant and, by extension, ensure its stable operating conditions. Before the use of additives, the problem of the instability of anaerobic degradation should be correctly diagnosed. It is important that the problem to be addressed should first be correctly diagnosed and then the appropriate additives should be used, as the supplements are customized for specific problems and on the individual technical specifications of each biogas plant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/methane2030018/s1, Table S1: Supplementary data for biogas plant BG01; Table S2: Supplementary data for biogas plant BG01; Table S3: Supplementary data for biogas plant BG02; Table S4: Supplementary data for biogas plant BG03; Table S5: Supplementary data for biogas plant BG03.

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