Maize Silage as Substrate for Biogas Production

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Abstract

The presented work studies the possibilities of using maize silage for biogas production in laboratory as well as in full-scale conditions. From the results of long-term operation of a mixed laboratory anaerobic reactor, it follows that processing of maize silage as a single substrate is an unstable process due to the low alkalinity of silage that has to be compensated by pH adjustment. Specific production of biogas was 0.655 m$^3$/kg of volatile solids. Start-up of a full-scale anaerobic reactor of a biogas plant with the volume of 2450 m$^3$ takes approximately 100 days. At the end of the start-up, the biogas plant reached the designed parameters—maize silage dose 6–7 t/d of total solids, the reactor load about 2.5 kg/(m$^3$/d) of volatile solids, the biogas production of 4200 m$^3$/d, electricity production of ca. 6600 kWh/d, and heat production of ca. 11,500 kWh/d. Processing of co-substrates in a biogas plant revealed both positive and negative effect on the biogas plant operation, for example, the meat and bone meal addition had a negative effect on the operation due to its high nitrogen content. Loading of crude glycerol (12.1% of the total volatile solids added) showed a positive and stabilizing effect.

Keywords: anaerobic digestion, biogas, biogas plant, maize silage, substrates for biogas production

1. Introduction

In order to replace fossil fuels by renewable energy sources, utilization of biofuels and renewable energy sources has been incorporated in the national and international legal standards and the government programs of developed countries. The EU in the Directive 2009/28/EC has defined a program of replacing 20% of total energy consumption with renewable energy sources and 10% of the consumption of liquid fuels with biofuels by 2020. One possibility of increasing the share of energy from renewable sources is biogas production
from energy crops. This option has gained wide application in particular in connection with government support of the electricity price produced from biogas in many countries of the EU. In addition, growing and utilization of energetic crops for biogas production is one of the alternatives of agriculture production diversification which can significantly improve farm economics. Energy from biogas produced by anaerobic digestion of energetic crops can be utilized to improve the energetic balance of a farm as excess energy can be sold (e.g. to the electric grid). Maize in the form of silage provides high yields (10–30 t of total solids—TS per hectare [1–3]) and is thus a suitable energetic crop for biogas production. More than 17,000 biogas plants, mostly using maize silage as the main substrate, are in operation in Europe; for example, in Germany, more than 8000 biogas plants have been in operation by the end of 2015 with the plant biomass utilization of more than 52 mass% and of livestock excrements of 43 mass% [4]. The rest are industrial and agro- and food processing waste as well as municipal biowaste. Advantages of plant biomass utilization are even pronounced by the fact that 52 mass% of the total substrates processed in biogas plants result in a 79% energy production. Maize silage represents 73 mass% of the plant biomass processed in the biogas plants, while the energy represents 72% of the total energy production. Thus, in 2014, 56.88% of energy produced by biogas plants in Germany originated from maize silage [4]. Even though no precise information on the species composition of the biogas plant substrates in other countries of the European Union is available, it is clear that the main substrate is maize silage. However, only little information on its anaerobic digestion is provided in literature. Generally, it can be stated that studies on the anaerobic digestion of fresh and ensiled materials did not show any significant differences between the biogas production from these materials [5, 6]. Concerning the anaerobic digestion, the main advantage of ensiling is the conservation of plant substrates to enable biogas production for the whole year. Zauner and Kuntzel [7] present anaerobic processing of maize silage in their work achieving methane production of 0.270–0.289 m$^3$/kg of TS in a laboratory batch reactor. The production was somehow lower, 0.181–0.184 m$^3$/kg of TS, in continuous laboratory reactors. Amon et al. [2] studied the biogas production from maize and clover grass in more detail. They focused on the biogas production of various species in different stages (milk, wax, and full ripeness). Also the influence of ensiling and drying on the methane production was studied. Various species had different optimal harvesting time in different ripeness stages. Specific methane production was in the range of 0.206–0.283 Nm$^3$/kg of the volatile solids—VS and the methane yield was in the range of 5300 to 8530 Nm$^3$/ha of the VS. These results were obtained by the batch tests of the anaerobic digestion in mesophilic conditions (40°C) for 60 days. Some maize varieties showed minimum difference in the methane production considering the ripeness stage and some showed a difference of more than 25% (variety Saxxo, wax ripeness) [2]. Specific methane production of 0.282–0.419 Nm$^3$/kg of the VS was achieved in the work of Schittenhelm [8], who studied the effect of maize composition and ripeness stage on the methane yield. Specific methane production from hybrids with late ripeness increased with the higher date of sampling more significantly than from climatically adapted “medium-early” hybrids, which reached the maximum methane production more quickly. These results are comparable with those provided by other authors [3, 9–14]. In [13], maximum yield of 9440.6 Nm$^3$/ha (hybrid maize,
ripe season FAO 400—FAO 500) and in [14], 10,401 m³/ha (20°C and normal pressure) were obtained.

To increase the specific methane production from silage, various methods of physical, chemical, or biological pre-treatment or their combination can be used [15, 16]. However, each pre-treatment method complicates the biogas production technology and increases the operation cost. Therefore, it is necessary to always consider if a higher amount of biogas produced has a relevant effect. Considering its properties and composition, maize silage is often used in co-fermentation with other substrates, for example, with manure, sludge from wastewater plants, various plant substrates, or industrial wastes [17–21].

Here, results obtained by anaerobic digestion of maize silage as the single substrate for biogas production in laboratory as well as in full-scale conditions are presented. Start-up of a biogas plant anaerobic reactor for maize silage processing as a single substrate and operation experiences using other co-substrates is presented.

2. Anaerobic digestion of maize silage in laboratory conditions

Within the laboratory experiments on anaerobic processing of maize silage, tests of the maize silage biogas potential were carried out, and a long-term operation of a biogas production model was monitored. The aim of these tests was to determine the specific biogas production.

2.1. Test of maize silage biomethane potential

It is necessary to emphasize that the tests of biomethane potential have only an informational character and the specific methane production reached by long-term operation of anaerobic digestion of biologically degradable substrates can differ considerably. Single biomethane potential test results are significantly affected by the anaerobic sludge used as inoculum. Used anaerobic sludge is not usually adapted to the substrate degradation and during batch test the adaptation is not carried out. Therefore, the biomethane potential test provides a value lower than that obtained by long-term anaerobic digestion, when the adaptation of the anaerobic sludge to the used substrate and thus deeper anaerobic digestion of the substrate can take place. If the substrate contains a toxic or inhibitory substance, its influence might not be demonstrated during the biomethane potential test, due to the sufficient dilution of the substrate by the anaerobic sludge used for inoculation, for example, in substrates with high nitrogen or sulfur content. In long-term anaerobic digestion process, when the substrate is repeatedly supplied to the reactor, nitrogen or sulfur can accumulate in the reactor, and ammonia or sulfide inhibition of anaerobic processes can occur gradually. However, the biomethane potential test is a suitable tool for the primary evaluation of anaerobic digestion of a substrate and the possible biogas production.

Maize silage produced at the STIFI farm in Hurbanovo was used in the biomethane potential test without particle size adjustment. The particle size was given by the harvesting machine as up to 5 cm in length. Silage was made in the traditional way. Harvesting took place at the
TS of the green maize about 30%. After the cropping, green maize was compacted by bulldozer in the silage pit with dimensions of 22 m × 75 m × 5 m. For tests, the silage after two month of ensiling was used. Content of TS of the used silage represented 35% with the VS content of 95.8%. Value of pH of maize silage water leachate (100 g of silage in 400 ml of tap water) was 3.7. Anaerobically stabilized sludge from the municipal wastewater treatment plant in Devínska Nová Ves (total suspended solids— TS of 37.23 g/L and volatile suspended solids— VS of 20.74 g/L) in the volume of 0.5 l and 7 g of fresh silage was used for the tests. The sludge mixture was completed to the total volume of 1 l with tap water. To determine the biogas production from the anaerobically stabilized sludge, a blank test was done. The tests were carried out in the mesophilic temperature regime (35°C) in three repetitions. The biomethane potential results are presented in Figure 1.

![Figure 1](image_url)

**Figure 1.** Test of biomethane potential of maize silage.

From the test results follows that 233 ml of methane per gram of TS (243 ml per gram of VS) respectively 0.206 Nm³/kg of TS (0.215 Nm³/kg of VS) were produced. It is in agreement with results provided by Amon et al. [2].

2.2. Long-term anaerobic digestion of maize silage in the laboratory conditions

Long-term maize silage processing was carried out in a mixed laboratory anaerobic reactor with the volume of 4 l. The reactor was filled to the half of its volume with the anaerobically stabilized sludge used for biomethane potential tests (TS of 37.2 g/L with VS of 55.7%) and was filled to the total volume of 4 l with tap water. Silage was processed in its raw form without any pre-treatment, that is, as taken from the silage pits in STIFI Hurbanovo, and stored at 4°C. The silage was loaded once a day into the laboratory model operated at 35°C. In the filtered samples of sludge water, parameters as chemical oxygen demand (COD), volatile fatty acids (VFA), ammonia nitrogen (NH₄-N), and pH were determined. Also the concentration of suspended solids and biogas production were monitored in the reactor. All analyses were...
carried out applying standard methods [22]. The analysis of VFA was done employing the method introduced by Kapp [23]. To determine the biogas composition (methane, CO$_2$, H$_2$, and H$_2$S), the apparatus GA 2000 Plus (Geotechnical Instruments, UK) was used.

Operation of the laboratory reactor for anaerobic digestion of maize silage started at the organic loading rate (OLR) of 1.68 kg/(m$^3$/d). The course of loading doses and the achieved parameters of the anaerobic reactor are provided in Table 1. Figure 2 shows the course of the specific biogas production per kg of added VS and the cumulative biogas production in the anaerobic reactor with gradual increase of OLR in the reactor. OLR increased from 1.68 to 6.71 kg/(m$^3$/d)—Table 1. Average specific biogas production at individual OLR values was in the range of 0.195–0.655 m$^3$ per kg of VS. Maximum specific biogas production was achieved at the OLR of 5.03 kg/(m$^3$/d). The course of COD and VFA is shown in Figure 3 and that of pH and NH$_4$-N in Figure 4. Instability of the processes was demonstrated by the decrease of pH and the increase of COD and VFA concentration, especially after the increase of OLR in the reactor (Figures 2 and 3).

<table>
<thead>
<tr>
<th>Organic loading rate (VS) [kg/(m$^3$/d)]</th>
<th>Day of operation</th>
<th>Dose of silage (raw material) [g/d]</th>
<th>Dose of silage (VS) [g/d]</th>
<th>Specific biogas production (VS) [m$^3$/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.68</td>
<td>0–20</td>
<td>20</td>
<td>6.71</td>
<td>0.195</td>
</tr>
<tr>
<td>2.52</td>
<td>21–40</td>
<td>30</td>
<td>10.06</td>
<td>0.230</td>
</tr>
<tr>
<td>3.36</td>
<td>41–80</td>
<td>40</td>
<td>13.42</td>
<td>0.430</td>
</tr>
<tr>
<td>4.19</td>
<td>81–120</td>
<td>50</td>
<td>16.77</td>
<td>0.530</td>
</tr>
<tr>
<td>5.03</td>
<td>121–220</td>
<td>60</td>
<td>20.12</td>
<td>0.655</td>
</tr>
<tr>
<td>6.71</td>
<td>220–300</td>
<td>80</td>
<td>26.83</td>
<td>0.420</td>
</tr>
</tbody>
</table>

Table 1. Anaerobic processing of maize silage—operation parameters of the anaerobic reactor.

![Figure 2](http://dx.doi.org/10.5772/64378)
Stabilization of COD and VFA took several days or even weeks depending on the destabilization degree after the silage load increase. At higher loads, the response to increased OLR was stronger and the stabilization period was longer. With the OLR increase, pH decreased below 6.5 (Figure 3) and sodium bicarbonate was used to adjust the pH. The pH value had to be adjusted not only after an OLR increase but throughout the anaerobic reactor operation because pH in neutral range is needed for methanogenic microorganisms. In total, ca. 6000 g of VS silage and 100 g of sodium bicarbonate were loaded into the reactor during its 300 day
operation. The average sodium bicarbonate consumption was 0.05 g/g of VS silage. Instability of the anaerobic processing of maize silage is related to it insufficient acid neutralizing capacity (alkalinity) due to the high C/N ratio in this substrate (30–46) [3]. Together with the carbonate buffer system (CO_3^2-/HCO_3^-), ammonia buffer system (NH_3/NH_4^+) also has an important role in the anaerobic processes. Results of long-term anaerobic reactor operation indicate that anaerobic digestion of maize silage as the only substrate requires the presence of alkaline reagents. From a practical point of view and that of nutrients demand, loading of a co-substrate with higher content of nitrogen, for example, sewage sludge, or manure, is required. At OLR of 6.71 kg/(m^3/d), the COD and VFA values exceeded 18,000 mg/L and 11,000 mg/L, respectively. It is thus clear that at this OLR value, the system was permanently overloaded and the COD and VFA values were stabilized at 6000 mg/L and 2800 mg/L, respectively. Also the specific biogas production was considerably lower (0.420 kg/kg of VS) as that at the load of 5.03 kg/(m^3/d) (0.655 kg/kg of VS)(0.655 m^3/kg of VS). Therefore, the optimal value of OLR has been established as 5.03 kg/(m^3/d), with the highest specific production of biogas.

During the stable operation of the reactor (days 121–220), the average concentration of suspended solids in the anaerobic reactor was 79 g/L. Daily amount of the suspended solids of excess sludge was 3.57 g. At the load of 21 g of TS silage (60 g of fresh silage with the TS content of 35%), the production of excess sludge was 0.17 g per 1 g of TS, which corresponds to the anaerobic silage digestion degree of 83%. The content of individual biogas components is provided in Table 2 and the parameters of anaerobic digestion of maize silage obtained from the laboratory model are summarized in Table 3.
Considering that from 1 ha of arable land, 30 t of TS silage (VS of 95%) per annum are obtained, methane production is 9006 Nm$^3$/ha. For a biogas plant with produced biogas incineration in a cogeneration unit with the electric power of 1 MW (electric energy production efficiency of 35%), the daily maize silage demand represents the area of 0.77 ha. This means that the annual operation of biogas plants needs 8431.5 t of TS silage, grown on 281 ha of arable land.

Conclusions of the anaerobic digestion of maize silage in laboratory conditions:

Biomethane potential tests provided the measured specific methane production of 0.215 Nm$^3$/kg of VS. For long-term maize silage processing in a mixed laboratory anaerobic reactor, the measured specific methane production was 0.316 Nm$^3$/kg of VSS. The higher value obtained for long-term reactor operation is due to the adaptation of the anaerobic microorganisms to the maize silage substrate.

Long-term operation of the anaerobic reactor for maize silage processing as the only substrate showed significant instability caused by the low alkalinity of maize silage (high C:N ratio). To stabilize the anaerobic processes, other alkaline reagents or a co-substrate with higher content of nitrogen (sewage sludge or manure) can be used.

Daily operation of a biogas plant with biogas incinerated in a cogeneration unit with the electric power of 1 MW requires the amount of silage from an area of 0.77 ha of arable land.

3. Start-up and trial operation of biogas plant for maize silage processing

Despite the 17,000 biogas plants in EU [24], many of which use maize silage as the main substrate, only a little information on their start-up and trial operation can be found in literature. Start-up and trial operation of a biogas plant for processing of maize silage as the main substrate are described.

Technology of a biogas plant is depicted in Figure 5. Effective volume of the used anaerobic reactor was 2450 m$^3$. Two high-speed blade mixers with horizontal rotational axis and with the immersion depth and mixing direction regulation were used. Fresh silage was loaded into the reactor by means of a conveyor belt. Silage pits were located next to the anaerobic reactor; an average TS of the silage was used during the start-up, and pilot plant operation was 35%; and the expected biogas production was 4200 m$^3$/d. Biogas was incinerated in a cogeneration unit (ELTECO, Slovakia) with the electric power of 276 kW (electric efficiency of 32%) and the heat power of 479 kW. The reactor was operated at the temperature of 37°C and the volume of the gasholder was 80 m$^3$, which was assumed as sufficient at stabilized production and consumption of biogas. Also a gas boiler enabling biogas as well as a natural gas incineration with the heat power of 470 kW was included in the technology. This boiler plays an important role during the start-up of the anaerobic reactor when biogas is not available and the reactor has to be heated to the operation temperature by natural gas.

The anaerobic reactor was inoculated with aerobically stabilized sewage sludge from a brewery, which is not often used as an inoculation medium for anaerobic reactors. Normally, for the
inoculation of the anaerobic reactor the anaerobically stabilized sludge is used, but the distance to the nearest wastewater plant with anaerobically stabilized sludge was 15 km, and the required amount of sludge could not be provided. Aerobically stabilized sludge from brewery wastewater plant was available for only as far as 2 km from the biogas plant. The amount of aerobically stabilized sludge added to the anaerobic reactor for inoculation before its start-up was 1700 m$^3$ with an average concentration of suspended solids (SS) of 30 g/L. After the inoculation, the reactor was heated to 37°C and gradually loaded with maize silage. During the start-up of the anaerobic reactor, biogas production, pH, VFA, NH$_4$-N, PO$_4$-P, and suspended solids concentration were monitored. The course of these parameters was also monitored during the first 200 days of the pilot plant operation (Figures 6–10).

Figure 5. Diagram of biogas plant for anaerobic digestion of the maize silage.

Figure 6. Course of silage dose and biogas production during the start-up of the anaerobic reactor.
The silage load was gradually increased (Figure 6), with the starting load of 2 t/d. As it follows from Figures 6 and 7 (biogas production and VFA concentration), anaerobic reactor operation was stable, and the biogas production was proportional to the increasing load approximately until the end of day 100. Maximum load of silage in this period was 20 t/d. This
amount was divided into six parts and every 6 hours 3.33 t was dosed. Average specific biogas production between days 50 and 100 of the reactor operation was 0.726 m³/kg of silage TS. Figure 8 presents the course of the NH₄-N and PO₄-P concentrations.

Increase of these concentrations within the first 20 days of operation is related to the degradation of sludge used as the inoculum (similarly as for the VFA concentration—Figure 7). NH₄-N concentration gradually decreased to approximately 200 mg/L and that of PO₄-P to below 20 mg/L. Low concentrations of ammonia nitrogen were followed by a pH decrease (Figure 9) due to the low alkalinity of the silage. Values of pH below 6.5 led to methanogenesis inhibition which increased the VFA concentration above 7500 mg/L (Figure 7) and decreased the biogas production significantly. From day 120, it was started with dosing of aerobically stabilized sludge (the same one that was used for inoculation) to increase the NH₄-N concentration and stabilize pH. The sludge dose was 7–10 m³/d (SS concentration of 30 g/L). As it is evident from Figures 7–9, the NH₄-N concentration increased to above 600 mg/L, VFA concentration decreased and pH was stabilized at around 7.2. The silage dose after stabilization of the reactor operation was increased to 24 t/d, specific biogas production reached 0.7 m³/kg of silage TS, and the cogeneration unit worked with its 100% capacity. OLR of the anaerobic reactor was in the range of 2.3–2.7 kg/(m³/d) and the SS concentration in sludge water of the anaerobic reactor after 200 days of operation was 60 g/L (Figure 10).

During the anaerobic reactor start-up, some interesting phenomena have been observed: after each silage dosing, a temporary increase in biogas production and the resulting increase in the cogeneration unit electrical power output, Figure 11 shows the response of the electrical power output for a silage dose every 3 h (16 t per day, each dose of 2 t), with the total biogas production of 2800 m³ and the cogeneration unit efficiency of 67% (day 140). The period of increased biogas...
production was ca. 1 h; the increase in biogas production showed in Figure 11 represents 5.13% of the total biogas production per silage dose. Such an increase is related to the content of readily biodegradable organic matter in maize silage (VFA, alcohols, lower saccharides, etc.), which can vary in the range of 2.1–11.1% (Table 4).

Figure 10. Concentration of suspended solids in the anaerobic reactor during the start-up.

Figure 11. Increase of power output of the cogeneration unit (biogas production) after silage dosing.
Table 4. Concentration of volatile and readily biodegradable matter in maize silage (% of TS).

<table>
<thead>
<tr>
<th>TS [%]</th>
<th>Acid</th>
<th>Ethanol</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactic</td>
<td>Acetic</td>
<td>Propionic</td>
<td>Butyric</td>
<td></td>
</tr>
<tr>
<td>39.2</td>
<td>6.2</td>
<td>2.6</td>
<td>0.2</td>
<td>&lt;1</td>
<td>1.1</td>
</tr>
<tr>
<td>36.0</td>
<td>4.2</td>
<td>1.5</td>
<td>0.32</td>
<td>1.04</td>
<td>1.5</td>
</tr>
<tr>
<td>31.0</td>
<td>5.21</td>
<td>1.28</td>
<td>–</td>
<td>–</td>
<td>0.29</td>
</tr>
<tr>
<td>41.0</td>
<td>2.12</td>
<td>0.86</td>
<td>0.05</td>
<td>0.24</td>
<td>0.17</td>
</tr>
<tr>
<td>28.5</td>
<td>0.56</td>
<td>0.47</td>
<td>0.01</td>
<td>0.01</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Between two doses, also the quality of the biogas produced changed; average methane concentration in biogas was 54.5% and that of hydrogen sulfide was 160 ppm. In the first two hours after the loading, the methane concentration decreased by ca. 2% (from 55 to 53%), which can be explained by higher CO₂ production due to the degradation of readily biodegradable matter. More significant changes in the biogas composition were observed for a five day silage loading interruption, when the methane concentration in biogas increased from 52.8 to 65%.

In the steady state, when the full capacity of the cogeneration unit was achieved, the biogas production of 4200 m³/d, electric energy production of ca. 6600 kWh/d and the heat production of ca. 11,500 kWh/d were provided at the daily maize silage dose of 6–7 t of TS. Electric energy was sold to the electric grid, produced heat was employed for the anaerobic reactor heating (12–13% of the produced heat), for greenhouses heating and also for drying maize grains between September and December produced on the premises as well as that produced by neighboring farmers. The digestate was stored and used if necessary as a fertilizer on the arable lands of the farm.

Conclusions from the start-up and trial operation of a biogas plant for anaerobic digestion of maize silage:

Results of the start-up of the anaerobic reactor have proved the suitability of aerobically stabilized sludge for the anaerobic reactor inoculation despite the substrate not being used for this purpose usually.

Start-up of the anaerobic reactor took approximately 100 days.

Results of the laboratory experiments were confirmed – the low alkalinity of maize silage and the need for additional substrates with higher nitrogen content to stabilize the reactor operation. In the present case, aerobically stabilized sludge from a brewery wastewater plant was used.

After ca. 150 days of the biogas plant operation, the designed parameters were stabilized. At the full capacity of the cogeneration unit, the biogas production of 4200 m³/d, electric energy production of ca. 6600 kWh/d and heat production of ca. 11,500 kWh/d were achieved. Daily dose of silage was 24 t/d, divided into six portions every 4 hours.
4. Influence of different substrates on operation of biogas plant for anaerobic digestion of maize silage as main substrate

Raw materials used for the biogas plant differ not only in their physical properties but also in their composition. From the anaerobic digestion point of view, organic carbon and its proportion to nitrogen are more appreciated. If the organic carbon is in the form of hardly degradable matter and hydrolysis or acidification is required, the effect of substrate dosing will differ from that observed for readily biodegradable matter. If the dosing effect of readily biodegradable substrate on the anaerobic processes is strong and the biogas production increases sharply immediately after the loading, together with other changes in the reaction mixture (pH change, VFA increase, etc.), it is recommended to divide the loadings to as many as possible during the day. Dosing optimization has a positive influence on the processes not only considering the degradation but also concerning the presence of toxic or inhibitory substances.

For efficient anaerobic processes, the balanced substrates composition, especially when considering the macronutrients (nitrogen and phosphorus) content, is also an important factor. Inhibition of ongoing processes can be caused by low nitrogen or phosphorus content, or by high nitrogen content. Optimum COD/N/P ratio for the anaerobic microorganisms growth is in the range of 1000:5:1 for acidified substrate (with low biomass production), and up to 350:5:1 for unacidified substrates (with high biomass production) [29]. For materials processed in biogas plants, COD determination is quite a complex problem; therefore, the ratio of organic carbon to nitrogen (C:N ratio) is usually applied. Generally, it can be stated that for materials with high nitrogen content (blood, meat and bone meal, rapeseed meal, chicken droppings), this ratio is up to 10–15, for materials with medium nitrogen content (maize silage, cereal straw) it is up to approx. 50, and for materials with low nitrogen content (e.g. wood biomass), the C:N ratio is above 50 [30].

At very high C:N ratios, methanogenic microorganisms are not sufficiently supplied with nitrogen to assimilate (growth and propagation) and conditions for organic carbon degradation are not achieved. Or, as in case of maize silage, at low alkalinity of the substrate, pH in the reactor decreases and the process becomes instable. At low values of pH (below 6.5) growth of methanogenic microorganisms is strongly inhibited, because optimal pH for their growth is in the neutral range. However, at very low C/N ratios, nitrogen accumulates in the sludge water in its ammonia form, which can result in a pH increase and anaerobic processes inhibition by undissociated ammonia.

Considering the biomass composition, the presence of sulfur is also important; sulfur in its organic as well as inorganic form is transformed to its reduced forms, mainly to sulfides and hydrogen sulfide, by anaerobic processes. Sulfides present in the anaerobic sludge water are toxic to the methanogenic microorganisms, and hydrogen sulfide causes problems with the biogas incineration in heaters of cogeneration units.

As an example of influent of different substrates dosing on a biogas plant operation, long-term monitoring of the biogas plant in Hurbanovo using maize silage as the main substrate can be provided. Its start-up and trial operation were described above.
From the beginning of the biogas plant operation, maize silage was used as the main and often the only substrate. Maize silage composition has changed depending on different factors, for example, the maize variety used. One of the most important factors is the ripeness season when maize is harvested for ensiling [3, 8]. In Table 5, selected parameters of substrates significantly influencing the biogas plant operation are provided. Except for the maize silage, also meat and bone meal, molasses stillage from bioethanol production—vinasse and a by-product of biodiesel production—crude glycerol characteristics are presented.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maize silage</th>
<th>Meat and bone meal</th>
<th>Vinasse</th>
<th>Crude glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>–</td>
<td>–</td>
<td>6.15</td>
<td>9.03</td>
</tr>
<tr>
<td>Chemical oxygen demand [g/g of TS]</td>
<td>1.22</td>
<td>1.32</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Chemical oxygen demand [mg/L]</td>
<td>–</td>
<td>–</td>
<td>332,930</td>
<td>1,870,000</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen [% of TS]</td>
<td>0.88</td>
<td>7.90</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total Kjeldahl nitrogen [mg/L]</td>
<td>–</td>
<td>–</td>
<td>19,254</td>
<td>–</td>
</tr>
<tr>
<td>NH₄-N [mg/L]</td>
<td>–</td>
<td>–</td>
<td>3390</td>
<td>–</td>
</tr>
<tr>
<td>Total nitrogen [% of TS]</td>
<td>2.69</td>
<td>8.85</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Total nitrogen [mg/L]</td>
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<td>–</td>
<td>–</td>
<td>1690</td>
</tr>
<tr>
<td>Total phosphorus [mg/L]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>192</td>
</tr>
<tr>
<td>PO₄-P [mg/L]</td>
<td>–</td>
<td>–</td>
<td>835</td>
<td>–</td>
</tr>
<tr>
<td>C:N</td>
<td>17.6</td>
<td>4.42</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dissolved anorganic salts [mg/L]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5150</td>
</tr>
<tr>
<td>Density [g/L]</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1080</td>
</tr>
<tr>
<td>Lactic acid [%]</td>
<td>1.85</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acetic acid [%]</td>
<td>1.77</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TS [%]</td>
<td>30.8</td>
<td>72.5</td>
<td>45.9</td>
<td>–</td>
</tr>
<tr>
<td>VS of TS [%]</td>
<td>94.1</td>
<td>79.9</td>
<td>71.8</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 5.** Characteristics of the used substrates.

During the biogas plant operation, other substrates were also processed, for example, rye silage and a mixture of oat and peas, which however did not significantly affect the reactor operation.

Co-substrate loading in the biogas plant had two main reasons: insufficient amount of the main substrate—maize silage, especially in spring; and stabilization of the anaerobic reactor operation, for example, in case of the brewery wastewater plant sludge. The course of various substrates loading is presented in Figure 12.

Loading of various substrates is discussed in relationship with the biogas production and pH changes. The course of biogas production in a biogas plant from the start of its operation is presented in Figure 13 and the pH values are shown in Figure 14.
Start-up and trial operation were discussed earlier. At this period of the anaerobic reactor operation, the disadvantage of the low alkalinity of maize silage processed as the only substrate was demonstrated. This disadvantage was suppressed by brewery sludge loading to the
reactor. The main effect of sludge dosing was the increase of N in the system. It is possible, that the other effects of sludge dosing was introducing micronutrients and minerals, but from Figures 8 and 9 it is obvious that main effect to pH stabilization is the increase of NH$_4$-N concentration.

From day 203 of the operation, meat and bone meal was dosed to the reactor, first to increase nitrogen concentration, then also to compensate the lack of maize silage. When the maize silage was not available and only low quality rye silage could be used, the average dose of this silage was 2 t and up to 3.2 t of meat and bone meal. Between days 180 and 250 of the operation, the biogas production achieved its maximum (designed) values. Long-term high loads of meat and bone meal (between days 203–255) had, however, a negative impact on the anaerobic reactor operation; therefore, dosing of this substrate was in day 255 stopped. Meat and bone meal is a substrate with low C:N ratio, of 4.42 (Table 5), and thus with high nitrogen content, which resulted in the increase in ammonia nitrogen concentration in the sludge water to above 2800 mg/L and that of pH to above 7.5; the process was probably inhibited by undissociated ammonia in the sludge water. Volatile fatty acids concentration was higher than 7500 mg/L (Figure 15) and the biogas production decreased (Figure 13). Also the biogas quality was lower, H$_2$S concentration increased from values below 200 ppm to values above 1800 ppm. Loading to the anaerobic reactor was interrupted for two weeks and after the volatile fatty acids concentration decreased below 6000 mg/L, rye silage and maize splits loadings were slowly resumed. Following the initiation of maize silage dosing from the new harvest in about day 300, the reactor operation gradually stabilized and the biogas production almost reached its maximum values. Although the following reactor operation was not quite stable, such severe conditions as after meat and bone meal dosing did not occur.
Another interesting substrate used in the biogas plant was molasses stillage from ethanol production, also called vinasse. At COD of 333,000 mg/L, the TKN concentration was 19,250 mg/L (Table 5), which is quite high. After the meat and bone meal dosing was stopped, the NH$_4$-N concentration in sludge water decreased below 1600 mg/L; however, it increased to almost 4500 mg/L after ca. 200 days of molasses residue loading (between days 500 and 700). Also the volatile fatty acids concentration increased significantly (Figure 15) and pH reached values of above eight (Figure 14). These changes were not abrupt but gradual and the anaerobic biomass adapted to these new conditions; therefore, the changes had no significant effect on the biogas production. Lower biogas production in this time period was caused by the lack of maize silage, as only one third or one half of the designed amount was loaded. After the molasses stillage loadings were stopped, the volatile fatty acids concentration decreased again (Figure 15). In the following season when maize silage was lacking, rye silage or a mixture of oat and peas was used.

Considering the anaerobic reactor operation stability, crude glycerol seems to be a promising co-substrate; it was used for more than two years in the biogas plant. It is a by-product of biofuel production; some of its characteristics are listed in Table 5. As it can be seen from the biogas production (Figure 13), pH (Figure 14), and volatile fatty acids concentration (Figure 15), the use of crude glycerol as a co-substrate with maize silage resulted in the anaerobic reactor stabilization.

To evaluate the specific biogas production from crude glycerol and its contribution to the total biogas production, a stable biogas plant operation period of 141 days was chosen, when only maize silage and crude glycerol were loaded to the anaerobic reactor. The average daily biogas production achieved was 4091.4 m$^3$, at the daily silage loading of 5280.4 kg TS (TS in the silage
used was 42.47%) and the daily crude glycerol loading of 683.7 kg. To determine the specific biogas production from crude glycerol, the value of the specific biogas production from maize silage (0.66 m³ per 1 kg of the maize silage TS, obtained when maize silage was the only substrate) was employed. The average daily amount of biogas produced from maize silage was calculated as 3485 m³ and the average daily amount of biogas produced from crude glycerol was 606.4 m³. The specific biogas production per 1 kg of crude glycerol was 0.887 m³, which corresponds to the specific biogas production of 0.512 m³/kg of COD and is in agreement with the results presented in [31].

Biogas produced from crude glycerol represented 14.82% of the total biogas production, while 11.46% of the total TS, 12.1% of VS and only 5.21% of the total mass of the raw materials loaded. At the electrical power output of the cogeneration unit of 300 kW (electrical power output of the cogeneration unit was increased from 276 kW to 300 kW after an agreement with the producer considering the operation experiences), the daily electric energy production from crude glycerol was 1067 kWh and almost 15% of silage were saved.

Amon et al. [32] studied the influence of various loading doses of crude glycerol on the anaerobic digestion of pig manure, maize silage and maize corms. Co-fermentation effect was observed. It means that methane yield of the basic mixture supplemented with glycerol was higher than the combined methane yields of both substrates if digested separately. The co-fermentation effect was especially high with glycerin additions of 3–6%. They recommend the glycerol content of maximum 6% for a stable reactor operation.

To complete the biogas plant monitoring results obtained for various co-substrates, the course of suspended solids concentration in the anaerobic reactor is provided in Figure 16, which shows a gradual increase of this concentration in the period of more than two years. After crude glycerol started to be added to the reactor, the concentration of suspended solids slightly decreased, which had a positive effect on the reactor mixing.

In our case of long-term crude glycerol loading in the biogas plant, the average glycerol VS addition was 12.1% and the anaerobic digestion process was stable. However, no co-fermentation effect was observed.

Conclusions of the study of various substrates loading on the biogas plant operation:

Co-substrates used in anaerobic digestion of maize silage as the main substrate can have a positive as well as a negative effect on the biogas plant operation.

Uncontrolled meat and bone meal loading resulted in a failure of the anaerobic reactor due to the high nitrogen content in this substrate. Its processing together with the maize silage is possible; however, the loading dose has to be regulated considering the ammonia nitrogen concentration and the pH in the anaerobic reactor.

It has been proved that the inhibitory effect of ammonia nitrogen depends on also the course of its increase. While an abrupt increase of the NH₄-N concentration to approximately 2800 mg/L (loading of meat and bone meal) resulted in an inhibition of the anaerobic processes, gradual increase to almost 4500 mg/L (in case of vinasse loading) showed no negative effect on the process.
Crude glycerol loading of 12.1% of the total loaded VS had a positive and stabilizing effect on the biogas plant operation.

5. Conclusions

The obtained laboratory and full scale results showed that maize silage is a suitable substrate for anaerobic digestion and biogas production. This is confirmed also by thousands of biogas plants in Europe, which use maize silage as the main substrate. Thus, it can be concluded that maize silage as the most widely used substrate for biogas production is a fact of presence. However, biogas production from this substrate is not sustainable nor is the production of the first generation biofuels produced from food commodities (e.g. biodiesel from edible oils, or bioethanol from cereals).

The advantage of using maize silage for biogas production is because of its high yield per hectare and high specific biogas production. The main disadvantage is that maize used for biogas production cannot be used as designed in human or animal nutrition. Such competition deforms also its price and maize silage becomes a scarce commodity. Moreover, mass growing of maize as a monoculture occupies arable land for growing other crops and increases the need for fertilization and plant protection. These negative effects were also presented by the authors from the Karlsruhe Institute of Technology, Germany [33]. The main aims of their project were “Landscaping instead of monoculture” and “Grass is an alternative to silage maize in biogas production.” It is evident that grass hectare yields or specific biogas production cannot compete with maize silage; however, it is a sustainable alternative to maize silage and makes biogas a surplus value of landscaping.
Another alternative is growing energetic crops which are not part of the food chain of humans or animals, for example, sorghum provides interesting hectare yields as well as specific biogas production values [34]. Other such crops include hemp (Cannabis sativa L.) [35] or Chinese silver grass (Miscanthus sinensis Anderss) [36].

Also biologically degradable waste from agriculture and industry as well as municipal waste are suitable substrates for biogas production and are an alternative to maize silage, which has been confirmed by the estimated biogas potential of these substrates in Germany [37]. While the energetic crops biogas potential was estimated to be 46.2% of the total biogas potential, the rest is obtained from various waste materials such as livestock excrements, harvesting residues and by-products of crops processing, municipal waste, sewage sludge, landscaping and industrial wastes.

Although a structure of the substrate mixture used in biogas plants is not sustainable even though its change is inevitable a long-time will pass before maize silage loses its position as the main substrate for biogas production.

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