ANALYSIS OF BIOGAS PRODUCTION FROM GRASS SILAGE, DEPENDING ON ITS QUALITY

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Abstract


This article (work) gives information about production of methane from 11 samples of grass silage. Production was studied in two following levels:

- specific methane production per 1 kg of organic total solids,
- specific methane production per 1 kg of fresh material.

These grass silage samples were at the same time analyzed from the point of view of feeding properties and quality: protein, digestible protein, fiber, acid-detergent fiber (ADF), neutral-detergent fiber (NDF), BNVL, lactic acid, acetic acid, butyric acid, starch values, metabolizable energy, total energy, net energy lactation, net energy for fattening, pH, acidity of the water leachate mg KOH, protein PDIA, protein PDIN, protein PDIE, Na, K, P, Mg, Ca and ash.

Statistic dependence was searched between feeding quality and methane production of tested samples. As statistically significant parameters influencing methane production were: lactic acid, pH, acidity of the water leachate mg KOH and total solids. Change of concentration of remaining parameters had not adequately provable influence on methane production.

Biogas production in the Czech Republic is ensured by the high percentage from maize silage. This work, however, deals with an alternative raw material instead of maize, which could be the grass silage. The area of permanent grassland in the Czech Republic is due to the morphology of the territory 980,000 hectares. In addition to all the border mountains, there is also very large area – Českomoravská vysočina (Czech-Moravian Highlands). Most of these areas are very extensively farmed, especially because of the European Union subsidy policy, and also because of negative trends in the number of cattle. Farmers are not forced to intensively grow the grass. This situation causes the problem that they don't have place to grow it. Assuming an increase in biomass production of grass species composition by replacing the current intense and powerful hybrid while ensuring the grass nutrition in terms of nitrogen, phosphorus and potassium by distribution of biogas fermentation residue, it would be possible to produce such quantities of biomass, which would be able to ensure, through the biogas, production of electrical energy about 2 800 GWh per year, that means 3.5% of total annual electricity production in the Czech Republic.

Production of biogas

Biogas is a product of the bacteria metabolism, that breaks apart their complex organic compounds to the more simple substances and the end product
is a mixture of gases with a predominance of methane CH4, carbon dioxide CO2, hydrogen sulfide H2S, ammonia NH3, water vapor H2O, hydrogen H2 and oxygen O2. The goal of biogas production is to produce gas with highest major portion of methane and as low as possible minor portion of undesirable gas components.

In 1986, Nordberg stated anaerobic fermentation process as a process consisting of 4 stages.

Hydrolysis organic substances (carbohydrates, proteins, fats)

Acidogenesis C2-C6 fatty acids

Acetogenesis C2 acetic acid CH3-COOH

Methanogenesis CH4, CO2, H2S, NH3, H2O, H2, N2

Hydrolysis leads to breakdown of high-molecular compounds (proteins, carbohydrates, fats, cellulose) using enzymes to low molecular weight substances such as simple sugars, fatty acids, amino acids and water. The process of hydrolysis is a slow process and its speed is directly proportional to the enzymes activity such as cellulase, amylase, protease and lipase. Hydrolytic bacteria optimally ferment at pH 4.5–6.

Acidogenesis process is more complex process of fatty acids. Facultative aerobic bacteria consume the last oxygen remains, which creates a strictly anaerobic conditions required for methanogenesis. During this process decreases the pH, i.e. the acidification of the environment. This leads to the production of higher fatty acids Cx-C3 to the level of propionic acid, as well as to the production of simple alcohols (ethanol CH3OH) and especially carbon dioxide, hydrogen, ammonia and hydrogen sulfide. The acetogenesis is a production process of C2 acetic acid CH3-COOH, carbon dioxide CO2 and hydrogen H2. The hallmark of acetogenesis is the sensitivity of bacteria to temperature changes.

Metanogenesis is a process of methane production CH4, carbon dioxide CO2 and water. A 90% of biogas is produced during this phase, while 2/3 are produced from reduction of acetic acid and 1/3 from the fusion of hydrogen and carbon dioxide (Straka et al., 2003).

**MATERIALS AND METHODS**

The definition and parameters of grass silage's feed quality

Quantified quality parameters of silage are total solids, organic total solids, macro components (Ca, P, Na, K, Mg), ash, crude protein, digestible crude protein, fiber, acid-detergent fiber (ADF), neutral-detergent fiber (NDF), lactic acid, acetic acid, butyric acid, acidity of mg KOH leachate water, pH, total energy, metabolizable energy, net energy of lactate, net energy of fattening, starchy food value, and also its own production of methane, measured in cubic meters under normal pressure and temperature conditions per kg of organic dry substance.

The methodology of biogas production determination

The biogas production was measured according VDI 4630 directive – Fermentation of organic substances, characteristics of substrates, sampling, data definitions of substrates and fermentation tests (VDI 4630 – Vergärung organicher Stoff, Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche). Experiments were performed at 38 °C and each substrate was tested in three samples. The resulting output was defined as the average of those three results. Average amount of test substrate was 20 g, the amount of vaccination sludge was 300 g and its total solids was 4%. The content of ammonia nitrogen was less than 800 mg/kg of vaccination sludge. The test lasted for 21 days. In parallel there was the fermentation test of the own vaccination sludge to determine the biogas production of the substrate.

Optional measurement of biogas production:

1. detecting methane production (m3) under normal pressure and temperature standard conditions from the unit of organic substrate weight (kg)
2. detecting biogas production (m3) from unit of the organic substrate weight (kg)
3. detecting biogas production (m3) from unit of the dry substance weight (kg)
4. detecting biogas production (m3) from unit of the substrate weight (kg).

To obtain accurate results, the produced biogas was calculated on the volume (m3) of methane under normal conditions of the pressure and temperature (0 °C, 1.01325 × 105 Pa) and production was related to organic dry substance.

Further results will be presented in terms of fermentation tests:

1. Specific methane production in 10⁻³ m³ per 1 kg of organic total solids (10⁻³ m³/kg oTS)
2. Specific methane production in 10⁻³ m³ per 1 kg of fresh material (10⁻³ m³/kg FM).

The values measured in m³ of biogas production were in all other evaluations converted to the volume (m³) under normal pressure and temperature conditions. Specific methane production is then determined by calculating the representation of methane in the biogas.

**RESULTS**

In chart (Fig. 1) are all considered samples. Specific methane utilization percentage based on organic dry substance of the sample ranged between 293.86 and 436.23 × 10⁻³ m³/kg oTS. The difference between these values is 48%. When we assessed the specific methane utilization percentage of fresh material (i.e. in TS in which the sample was assessed and collected), then the utilization percentage ranged between 61.63 and 151.71 × 10⁻³ m³/kg of FM.
Analysis of biogas production from grass silage, depending on its quality

1: Specific methane production in $10^{-3}\text{m}^3$ per 1 kg of organic total solids ($10^{-3}\text{m}^3/\text{kg oTS}$)

2: Specific methane production in $10^{-3}\text{m}^3$ per 1 kg of fresh material ($10^{-3}\text{m}^3/\text{kg FM}$)

<table>
<thead>
<tr>
<th>Locality</th>
<th>Specific methane production $10^{-3}\text{m}^3$/kg fresh material</th>
<th>Specific methane production $10^{-3}\text{m}^3$/kg organic total solids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dolní Dobrouč</td>
<td>148.08</td>
<td>330.16</td>
</tr>
<tr>
<td>Dlouhá Třebová</td>
<td>112.05</td>
<td>405.70</td>
</tr>
<tr>
<td>Veké Opatovice</td>
<td>88.87</td>
<td>331.96</td>
</tr>
<tr>
<td>Vraclav</td>
<td>61.63</td>
<td>317.35</td>
</tr>
<tr>
<td>Sloupnice</td>
<td>97.80</td>
<td>397.54</td>
</tr>
<tr>
<td>Dolní Újezd</td>
<td>96.59</td>
<td>376.57</td>
</tr>
<tr>
<td>Kunčina</td>
<td>151.71</td>
<td>293.86</td>
</tr>
<tr>
<td>Kouty</td>
<td>126.03</td>
<td>436.23</td>
</tr>
<tr>
<td>Chabičovice</td>
<td>64.47</td>
<td>333.54</td>
</tr>
<tr>
<td>Věžovatá Pláně</td>
<td>69.12</td>
<td>336.68</td>
</tr>
<tr>
<td>Budišov</td>
<td>93.50</td>
<td>344.29</td>
</tr>
</tbody>
</table>
Here is the difference 246%. For this reason, the most accurate method of gas production measurement is to measure methane production within the 1 kg oTS. As there were total of 27 considered dependencies (protein, digestible protein, fiber, acid-detergent fiber (ADF), neutral-detergent fiber (NDF), BNVL, lactic acid, acetic acid, butyric acid, starch values, metabolizable energy, total energy, net energy lactation, net energy for fattening, pH, acidity of the water leachate mg KOH, protein PDIA, protein PDIN, protein PDIE, Na, K, P, Mg, Ca and ash), it's not possible to give all results in this article. For this reason we present only dependencies, which were confirmed for their statistical significance. The first dependence was the dependence of methane production on lactic acid in grass silage.

The concentration of lactic acid proved to be a sensitive parameter. This dependence is very clear and increase the concentration of lactic acid is in direct proportion to the growth of methane production from grass silage. Differences in concentrations of samples were very wide and ranged (Vraclav) from 0.13% in total solids (TS) with methane production 337.35 × 10⁻³ m³/kg of organic total solids (oTS) to 3.66% in TS with production of methane 436.23 × 10⁻³ m³/kg oTS. The sample with the lowest methane production is 293.86 × 10⁻³ m³/kg of organic total solids shows a very low concentration of lactic acid 0.69% in total solids. The determination coefficient (value of reliability – R²) reached the value of 0.4603. Regression line has the form: y = 21.459x + 315.83. Parameter β₁ (slope of straight line) is 21,459 and therefore is positive and has a growing line shape. Parameter β₀ is 315.83. To review the overall suitability of the model we use the F-test to test the model as a whole. It will be determined if the the p-value is less than the chosen level of significance 0.05. 

\[
F = 7.67, \quad p-value = 0.022
\]

It is true that p-value < 0.05 (95% confidence level)

In terms of statistics, the model is significant (conclusive) and shows us the influence of the growth of lactic acid content on the growth of methane production.

Another dependence was the dependence of methane production to the acidity of the water leachate mg KOH in grass silage.

The acidity of the water extraction parameter also reacts sensitively to the increase of its concentration on methane production increase. The lowest value was 702 mg KOH in a sample from Věžovaté Pláně and the highest was 2 415 mg of KOH in a sample from the Dolní Dobrouč. Samples with the lowest production of methane from Kunčina with 293.86 × 10⁻³ m³/kg oTS had the second lowest acidity of the water leachate mg KOH with value 755 mg KOH. On the contrary the sample from Kouty with the production of 436.23 × 10⁻³ m³/kg of organic substance had the second highest acidity of the water leachate mg KOH with a value of 2 160 mg of KOH.

The determination coefficient (value of reliability – R²) reached the value of 0.540757. Regression line has the form: y = 0.046 x +285.414. The parameter β₁ (slope of straight line) is 0.0463 and therefore is positive and has a growing line shape. The parameter β₀ is 285.4144. To review the overall suitability of the model we use the F-test to test the model as a whole. It will be determined if the the p-value is less than the chosen level of significance 0.05.

\[
F = 10.59746913, \quad p-value = 0.009911771
\]

It is true that p-value < 0.05 (95% confidence level)

In terms of statistics, the model is significant (conclusive) and shows the influence of the growth...
of the acidity of the water leachate mg KOH in grass silage content on the growth of methane production.

The pH value of silage is one of the most easily identifiable parameter. The pH value ranged between 4.00 and 5.30. You can tell that the sample was acidic, thus higher production of methane. The sample of the methane production from Kunčina was $293.86 \times 10^{-3}$ m$^3$/kg of organic substance and reached the lowest acidity with a pH of 5.30. On the contrary sample from Kouty with the production of methane $436.23 \times 10^{-3}$ m$^3$/kg of organic substance reached value of 4.00.

The determination coefficient (value of reliability – $R^2$) reached the value of 0.620764.

Regression line has the form: $y = -68.817x + 674.526$. The parameter $\beta_1$ (slope of straight line) is 68.817 and therefore is negative and has a decreasing line shape. The parameter $\beta_0$ is 674.52616. To review the overall suitability of the model we use the F-test to test the model as a whole. It will be determined if the $p$-value is less than the chosen level of significance 0.05.

$F = 14.73196721$

$p$-value = 0.003977321.

It is true that $p$-value < 0.05 (95% confidence level)

In terms of statistics, the model is significant (conclusive) and shows the influence of the decrease of pH on the growth of methane production.

The last and even the most conclusive test was methane production test, depending on % total solids of the substrate. Methane production ranged from $61.63 \times 10^{-3}$ m$^3$/kg of fresh matter to $151.71 \times 10^{-3}$ m$^3$/kg fresh matter. Differences were high and directly proportional to substrate total solids.
The methane production for 1 kg of fresh substance was also statistically evaluated. The determination coefficient (value of reliability – $R^2$) reached the value of 0.770028.

Regression line has the form: $y = 2.8247 \times + 10.138$. Parameter $\beta_1$ (slope of straight line) is 2.8247 and therefore is positive and has a growing line shape. The parameter $\beta_0$ is 10.13769. To review the overall suitability of the model we use the F-test to test the model as a whole. It will be determined if the p-value is less than the chosen level of significance 0.05.

$F = 30.1352462$

$p$-value $= 0.000385357$.

**It is true that p-value < 0.05 (95% confidence level)**

In terms of statistics, the model is significant (conclusive) and shows the influence of the growth of the content of total solids on the growth of methane production.

**DISCUSSION**

The thesis assessed 11 samples of grass silage. In terms of stock-feeding quality they achieved different levels. One sample achieved excellent quality, 4 samples were successful, 3 were less successful, but within the livestock sector still consumable, and two were unsuccessful but conditionally consumable. One sample was even failed and insanitary. Even though, these materials have their foundation in the biogas production.

In terms of statistical evaluation, only four assessed parameters showed statistical significance. One parameter was the energy parameter – lactic acid. The energy contained in simple sugars produced by photosynthesis in the grass is being stabilized at the level of lactic acid. Monosaccharide or their lactic fermentation product produced by lactic acid, is the major carrier of energy in the grass. Therefore, the content of lactic acid in the production of methane is so important, which was also confirmed by the result of statistical analysis with confirmed statistical significance.

Other parameters are to define a biological long-term stability of silage. These parameters are namely pH and acidity of the water extract. The parameters can be partially described as equivalents or as parameters that are against each other in direct proportion. The results could be characterized as the results, which confirm that the reduction of biological stability of grass silage causes a significant decrease in methane production. The more stable is the silage in long term, the higher production of methane (biogas) can be expected.

The last statistically significant parameter was % TS of silage. This parameter is logical, because total solids hold all power in the form of sugars (mono-, di-and polysaccharides to the level of cellulose and hemicelluloses), fat or protein. Therefore, in terms of profitability of grass silage is to maximize the TS yield per hectare of grassland, because the yield of TS is in direct proportion to production of biogas or methane. In the future it will mean to increase the yield per hectare of grass by applying hybrid varieties of grasses in the form of permanent grassland revivals. Intensification will certainly include production of both annual and perennial forage crops grown on arable land. And not only by right choice of an appropriate variety, but also by intensive nutrition with nitrogen but other nutrients. This would be ensured by delivery of fermented rest back to grassland. The content of nutrients in fermented rest is circa 4.5 kg of nitrogen, which is circa 3–3.5 kg in the form of ammonia, phosphorus 2.0 kg and 4.0 kg of potassium. The above values show that nutrition of grassland should be ensured.

Although the other parameters show clear dependences such as energy, namely the total energy...
and metabolizable energy, they also show less net energy of lactation and fattening clean energy, but as evaluated they were not statistically significant. Methane production reacted quite sensibly on the concentration of acetic acid, but even though this dependence could not be statistically confirmed. Response to the methane production was also in BNVL parameter. But even this was not statistically confirmed. The content of any protein, whether it was crude protein, digestible crude protein PDIA, PDIE or PDIN had not any greater influence on methane production and also the dependence between the content of these parameters and methane production was not confirmed.

Also the content of macromolecules, i.e. calcium, sodium, phosphorus, magnesium and potassium, is not in direct correlation to the methane production.

**CONCLUSION**

The aim of this work was to verify if there is necessary to scrupulously observe the quality of input substrates as it's necessary for high production dairy cows. Given that bacteria do not work nearly as intensely as ciliates in the rumen of cows, this goal is not required, but desirable. Cows then may be a suitable model to show us possible direction of biogas research for next few years. If the dairy cow weighing 600 kg receives 20 kg TS per day and the capacity of the digestive tract is 180 liters, then load of the digestive "fermenter" will be 111 kg oTS per m³ and per day. If these parameters are assessed in terms of current construction of biogas plants, then we are talking about loading the fermenter with 5 kg oTS per m³ and per day. Some of the biogas plants (e.g. Kouty) are currently at 7.5 kg oTS/m³/day. In Germany we operated continuously biogas plant with a load of 12 kg oTS/m³/day, and at the same time we also made experiments to load 20 kg oTS/m³/day. The limiting parameter is mainly maintaining optimum TS in fermenter, i.e. adequate water supply to methanegenese bacteria, but preferably in the form of physiological water in the substrate (i.e. lower silage TS and haylage), sufficiently intense movement of the fermented material in the fermenter (sufficiently sized mixing) and last but not least well defined nutrition and micronutrients, especially iron, cobalt, molybdenum and nickel.

**SUMMARY**

Aim of this work (project) was to estimate if feeding quality of grass silage directly influences methane production. Usually is grass silage quality defined by following parameters: protein, digestible protein, fiber, acid-detergent fiber (ADF), neutral-detergent fiber (NDF), BNVL, lactic acid, acetic acid, butyric acid, starch values, total energy, net energy, net energy lactation, net energy for fattening, pH, acidity of the water leachate KOH, protein PDIA, protein PDIN, protein PDIE, Na, K, P, Mg, Ca and ash. Aim was to determine which of these parameters feeds forward to methane production and is possible to prove statistical dependence.

Statistical dependence from the set of 27 feeding samples properties was proved only for 4 following parameters. lactic acid (value of reliability $R^2 = 0.4603$), pH (value of reliability $R^2 = 0.620764$), acidity of the water leachate mg KOH (value of reliability $R^2 = 0.540757$) and total solids (value of reliability $R^2 = 0.770028$). Change of concentration of remaining parameters had not adequately provable influence on methane production.

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