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MONITORING OF THE BIOLOGICAL ACTIVITY OF A BIOGAS PLANT WITH ENZYME ACTIVITY MEASUREMENTS

ABSTRACT

The biochemical processes of biogas production in a perfectly homogenized reactor take place paralelly and simultaneously. Therefore, there is not any sole parameter capable of characterizing the entire process on its own. Together with the Biogas Plant of Pellérd, Tettye Forrásház Ltd. we searched for new ways to complement quality assurance. Due to the variability of parameters in a biogas plant, occasionally the equilibrium of biochemical processes unravels and fermentational acid production or methane production becomes predominant. Enzyme activity measurements can be utilized to characterize degradation processes. Dehydrogenase enzyme activity (DHA) was examined. Samples from anaerobic biogas fermentors were measured using the unique photometric method developed by L. Karaffa et al. 2,3,5-triphenyl-tetrazolium chloride was used as electron acceptor. During the examined period dehydrogenase enzyme activity of the biogas plant (operating mainly on sewage sludge) varied between 5,44-16,66 mg triphenyl formazan / cm^3 sludge * 24 h mg formazan/g sludge/ 24 hours.

Comparing our results with the usual quality assurance parameters a tight correlation was revealed between the measured DHA results and the amount of volatile organic acids (VOA). In co-fermentational conditions, the intensity of hydrolytic processes could be characterized by DHA results.

INTRODUCTION AND AIM

The biochemical processes of biogas production in a perfectly homogenized reactor take place parallelly and simultaneously. Therefore, no sole parameter is capable of characterizing the entire process on its own. Monitoring the changes in the fermenter is indispensable for sustaining a functioning system. Monitoring usually involves continuous sampling. Classical quality assurance parameters of anaerobic degradation involve temperature, pH, alkalinity, volatile organic acid content, organic feeding rate, biogas yield and gas quality. These physical and chemical parameters determine whether the system works in optimal conditions. In case of diverging from the optimum, intervention might be necessary to ensure ideal circumstances.

Classical quality assurance parameters only inform us about the living conditions of bacteria and whether the prerequisites for an ideal environment are met. Among these

parameters, only biogas yield and gas quality provide information about the biological processes taking place inside the fermenter.

The aim of our research was to find and apply a new method capable of describing the different aspects of biological processes, including speed. Enzyme activity measurements were the focus of our research. Enzyme activity values characterize the fermentation by reflecting the metabolism of microorganisms responsible for degradation.

The examined biogas plant's quality assurance and laboratory measurements were analyzed to draw conclusions and find correlations. In the next step, information obtained from the plant was compared to the measured enzyme activities. Our aims were to find correlations between classical quality assurance parameters and the measured enzyme activity values and gain more insight into the biological processes of fermentation, the overall activity of microorganisms and the speed of degradation.

Continuous monitoring is essential to control the complex processes taking place inside the fermenters. Monitoring usually involves measuring chemical and physical parameters that inform us about the possible divergences from the microorganisms' ideal parameters and the quantity and quality of the produced end-product, biogas.

The classical monitoring parameters of biogas plant operation are temperature, pH, alkalinity, volatile organic acid content, dry matter content, organic matter content, hydraulic retention time, occurrence of toxic matters, produced gas quantity and gas quality.[1]

Another critical aspect is specific gas production. Specific gas production is calculated from the feeding rate and the quantity of gas produced. It represents the quantity of biogas produced from 1 m^3 substrate. The goal of biogas plant operation is to achieve higher specific gas production rates. [2]

Analyzing operational parameters aid us in creating an ideal environment for fermentation. Intervention is possible by inspecting the quantity and quality of the end-product. However, these parameters do not enable the monitoring of biological and biochemical processes taking place inside the fermenter.[3]

Soil enzymes

Soil has an easily measurable enzymatic activity. Soil enzymes are characterized by different origins. The most common soil enzymes are oxidoreductases (dehydrogenases, catalases, monooxidases, peroxidases), transferases, hydrolases (for example phosphatase, amylase, cellulase, invertase, urease, proteinase, peptidase, carboxylesterase, lipase, phytase, etc). [3]

Various methods can be utilized to characterize enzyme activity. We can measure the decrease of the enzyme substrate, the formation of the product or the formation of the product from a substrate analog. Substrate analogs are compounds with a similar configuration to the substrate, however with differing function. Often the measurement is aimed to represent the activity of an enzyme system instead of a single enzyme.

The first step of anaerobic degradation is hydrolysis, that determines the speed of the whole degradation process. Different enzyme activity measurements are applicable to investigate hydrolysis. Dehydrogenase enzyme activity characterizes the total activity of cells. Depending on the substrate used, protease, lipase and cellulase enzyme activities are also worth measuring. Analyzing these enzyme activities can provide useful information about the fermentational processes during quality assurance of operation.[3]

Dehydrogenase enzymes catalyze hydrogen transfer in biological oxidation from the reduced hydrogen donor to the hydrogen acceptor substrate. Dehydrogenase belongs to the group of oxidoreductases and it is reversible and substrate specific. [3]

MATERIALS AND METHODS

Measuring dehydrogenase enzyme activity

Dehydrogenases belong to the group of oxidoreductases and they reflect the whole oxidative activity of the microbiota of soil. The measurement of current dehydrogenase activity of soil is based on using an artificial hydrogen acceptor, 2,3,5-triphenyl tetrazolium chloride. As a result of the process catalyzed by the dehydrogenase enzyme, the hydrogen acceptor reduces to 1,3,5-triphenyl formazan, that is a red chemical not soluble in water. The intensity of its color can be measured after extraction by methyl or ethyl alcohol by a spectrophotometer Current enzyme activity is given in mg formazan/1g soil/24 hours. Dehydrogenase enzyme activity is representative of soil types, it is responsive to soil pollution and it also characterizes the fertility of soil.

Microorganisms produce oxidoreductases that play an essential role in degrading macromolecules. In sewage treatment, the DHA value of sewage sludge is often tested. The determination of sludge DHA differs from the method applied for soils. Incubation time is distinct, and the results are given in the volume of sludge.

The first biochemical step of biogas production is hydrolysis. Therefore, among the new quality assurance parameters DHA measurement successfully represents the efficiency of degradation. [4]

RESULTS AND DISCUSSION

First, we analyzed the daily operational and laboratory parameters of the biogas plant. In the next step we compared these data with the measured enzyme activity values and drew conclusions about the two.

Operational parameters of the biogas plant of Pécs

Tables 1 and 2 contain the mean values of the operational and laboratory parameters from November of 2019. These parameters characterize the average operation of the plant. The values represented are from the Eastern Tower, since the samples for our measurements were also obtained there.

Table 1

Operational values from November of 2019

2019. november						
Dry matter content of dehumidified sewage sludge	**Amount of sludge sent to the tower**	**Fermenter temperature**	**Amount of gas produced**	**Methane content of the gas produced (%)**	**Specific gas production**	**Retention time**
%	**m^3/day**	**°C**	**m^3/day**	**%**	**m^3 gas/m^3 sludge**	**day**
5,72	**185**	**37,59**	**3173,26**	**60,20**	**18,65**	**24,4**

Table 2

Laboratory data from November 2019

2019. november						
Alkalinity of the recirculated sludge	**Volatile acid of recirculated sludge DEGREMONT**	**Volatile acid/alkalinity ratio**	**pH value of recirculated sludge**	**Dry matter content of recirculated sludge**	**Organic matter content of recirculated sludge**	**Organic matter content of the dry content of recirculated**

						sludge
$CaCO_3$	mg/l	-	pH	g/l	g/l	%
3433,45	181,82	0,05	7,37	24,86	16,11	65

Figure 1 represents the close relation between feeding rate and biogas yield. Biogas yield primarily depends on the feeding rate. Changes in produced biogas quantity follow the changes in feeding rate closely, in only one day.

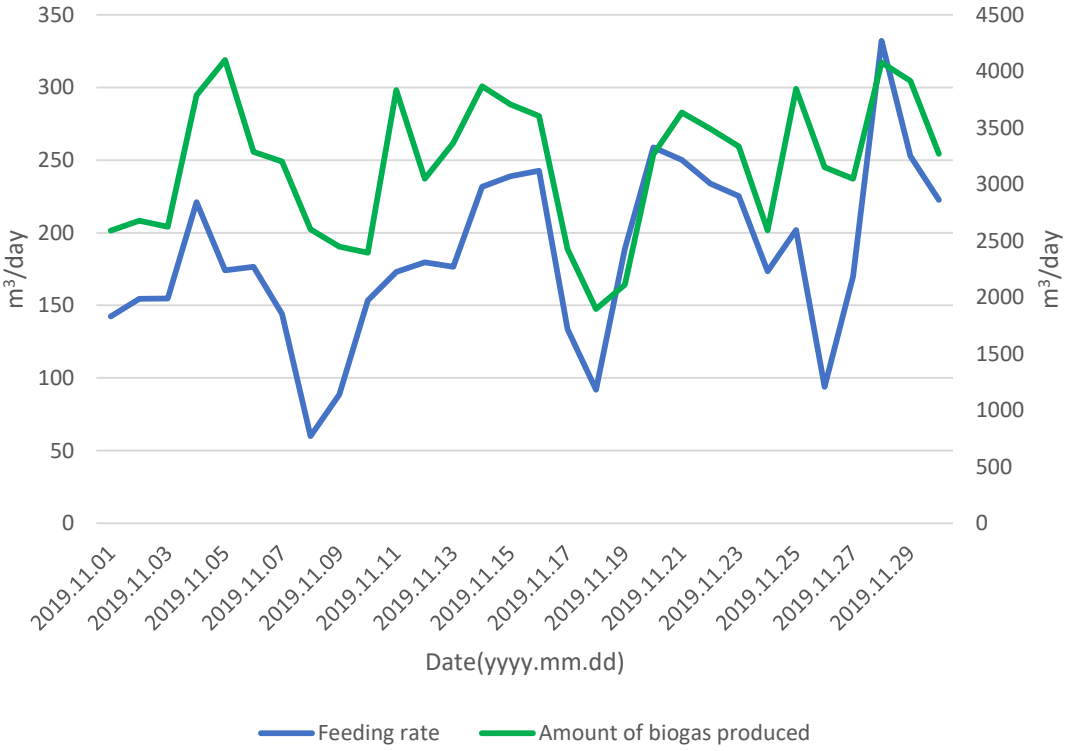

Figure 1

Relation between substrate feeding rate and produced biogas quantity

Figure 2 displays the temperature of the fermenter and the methane content of the produced biogas during our examined time frame. No significant correlation can be observed between these two parameters. However, as it is illustrated in Figure 2, biomethane content of the produced biogas highly depends on the substrate feeding rate. Currently there are too many variable circumstances to establish a link between methane content and the temperature of the fermenter. The connection between temperature and methane yield could be examined if the fermenters were fed with the same substrates at a fixed rate.

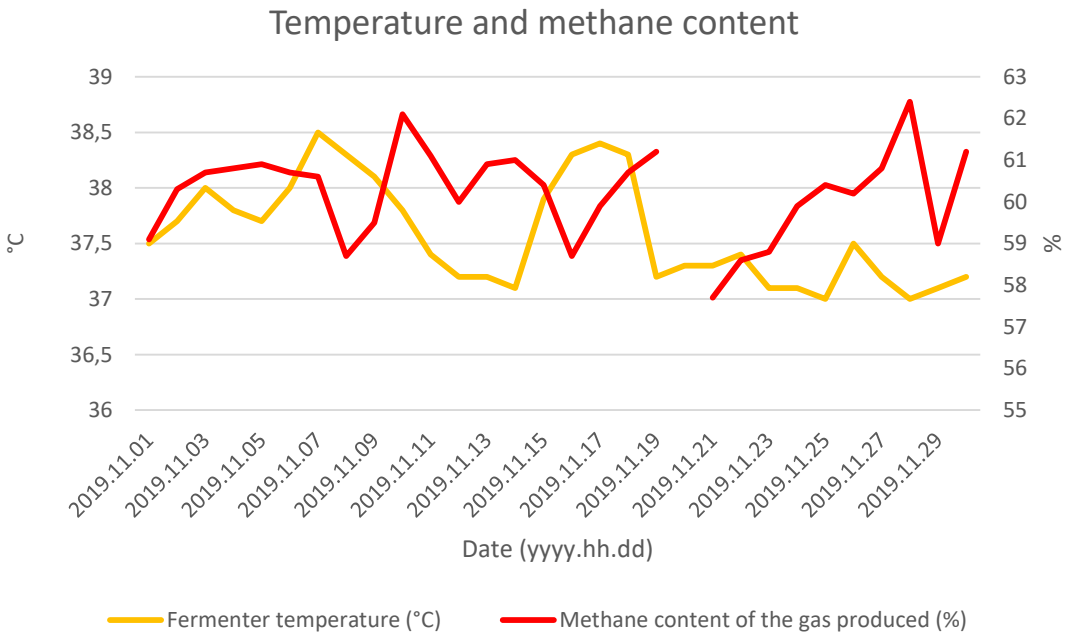

Figure 2

Relation between the temperature of the fermenter and methane content of the produced biogas

The correlation between substrate feeding rate and the methane content of produced biogas is not due to producing higher quality gas from more substrates. The main substrate, sewage sludge from the sewage treatment facility of Pécs, is produced at an approximately constant rate. It is complemented with other external substrates with a varying daily quantity. To counteract this variability, a buffer tank system is operated. Thus, the more external substrates received in any given day, the higher the daily substrate feeding rate is. Meanwhile, with more substrates fed into the fermenter, the proportion of substrates other than sewage sludge also increases. Therefore, the rise in methane content is thanks to the higher co-substrate rate.

We calculated the concentrations of triphenyl formazan in our solutions and measured the absorbances with a spectrophotometer at 485 nm. Ethanol was used as reference. Results are showed in Table 3.

Table 3

Calibration solution series of the 1,3,5 Triphenyl formazan

Calibration solution series		
Composition: solution + ethanol	Concentration (µg/cm^3)	Absorbance (485 nm)
0 cm^3 + 30 cm^3	0	0
1 cm^3 + 29 cm^3	3,33	0,173
2 cm^3 + 28 cm^3	6,67	0,348
5 cm^3 + 25 cm^3	13,7	0,739
10 cm^3 + 20 cm^3	33,3	1,509

From the measured absorbance values and the calculated triphenyl formazan concentrations, we determined the calibration curve (Figure 3). The curve was used to assess our results from sewage sludge:

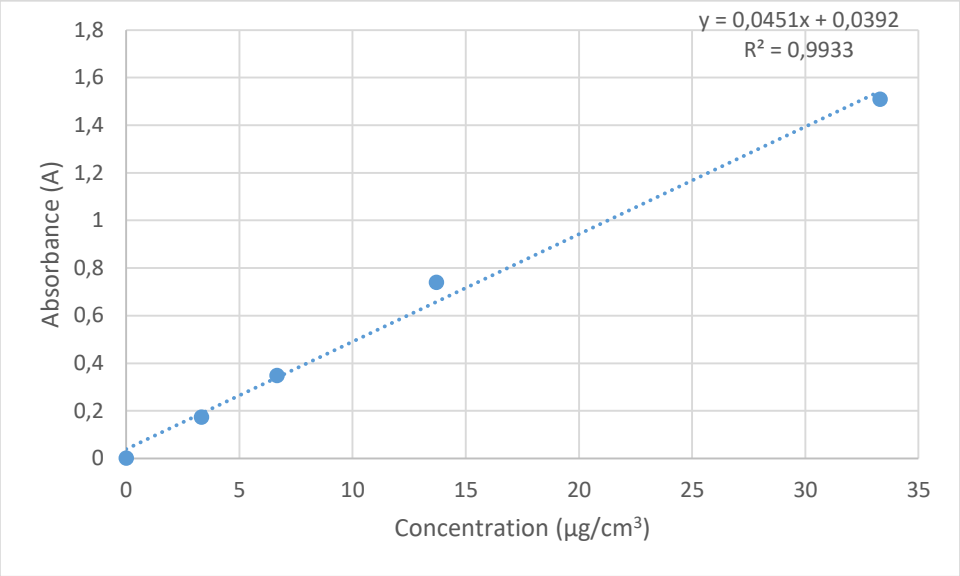

Figure 3

1,3,5 Triphenyl formazan calibration curve

According to the calibration curve, the equation of line is y=0,045x. This equation was used to calculate the triphenyl formazan concentrations of our samples from their absorbances. Table **Hiba! A hivatkozási forrás nem található.**shows our measured absorbances and the calculated triphenyl formazan concentrations.

Table 4

Measured absorbances and calculated triphenyl formazan concentrations

Measurement number	Absorbance						Triphenyl formazan
		1	2	3	Mean	Corrected mean	mg TF / cm³ sludge * 24 h
	0ml (1ml water)	1ml	1ml	1ml			
1	0,036	0,201	0,294	0,364	0,286	0,250	5,44
2	0,036	0,403	0,390	0,437	0,410	0,374	8,13
3	0,036	0,515	0,591	0,552	0,553	0,517	11,23
4	0,036	0,489	0,595	0,492	0,525	0,489	10,64
5	0,034	0,716	0,841	0,731	0,763	0,729	15,84
6	0,035	0,824	0,770	0,810	0,801	0,766	16,66
7	0,047	0,708	0,791	0,741	0,747	0,700	15,21

During our enzyme activity measurements, we had the opportunity to examine a unique period in the operation of the biogas plant. The delivery of external substrates stopped for 6 days due to the maintenance of the receiving unit. The fermenters were only fed with sewage sludge produced in the sewage treatment facility during these 6 days. Results from our measurements during this period are represented in Figure 4.

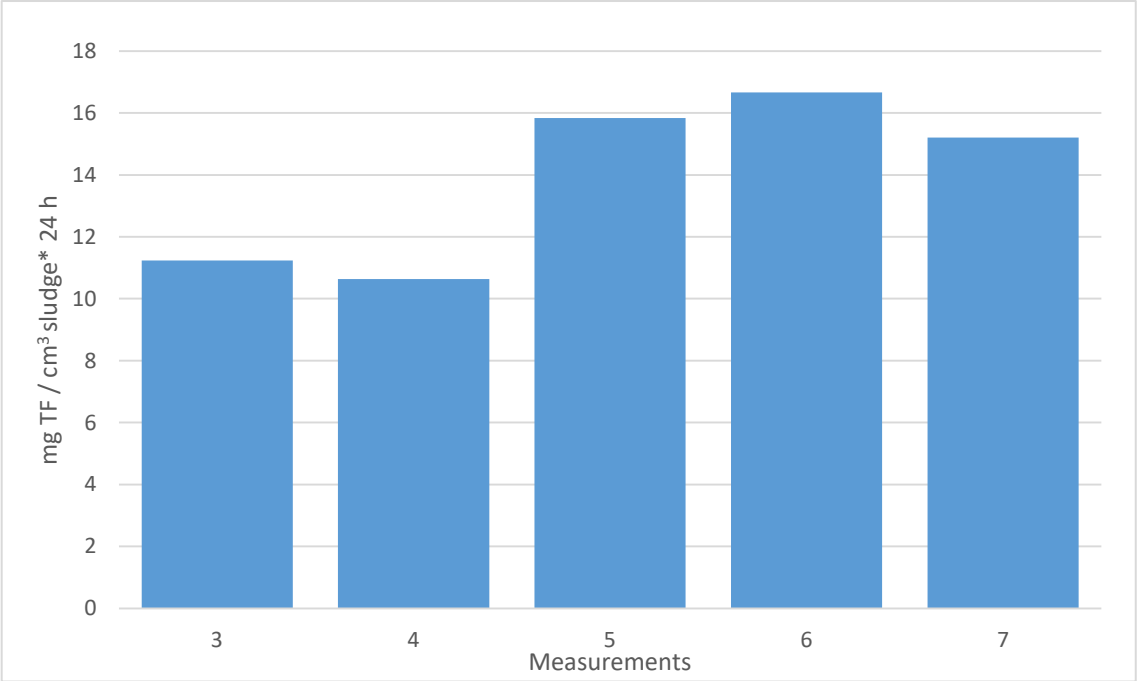

Figure 4

Changes in dehydrogenase enzyme activity due to the suspense of external substrate feeding

Measurement no. 3 was conducted before the suspense of receiving external substrates. After that, only sewage sludge was added to the fermenters. 5 days later, the dehydrogenase enzyme activity decreased by 8.5%, as seen in measurement no. 4. After maintenance, feeding of external substrates continued at a higher rate than usual, since companies tried to dispose of all of the waste materials produced during the suspense at once.

Measurement no. 5 was performed 1 day after the reintroduction of co-substrates at a higher rate than usual. Enzyme activity showed an increase of 41% in 1 day. The escalation of enzyme activity continued for days after. Finally, the microorganisms adapted to the changed circumstances and the enzyme activity decreased to the usual value.

CONCLUSION

We determined that the proper analysis of the relation of methane content and the temperature of fermenter would require feeding the fermenters with the same substrates at a fixed rate, since there are too much variables at the moment to draw proper conclusions.

Dehydrogenase enzyme activity measurements enabled the monitoring of cellular activity of degrading microorganisms inside the fermenters. The cellular activity characterizes the speed of degradation. In the sewage treatment facility of Pécs, the dehydrogenase enzyme activity of the biogas plant (operating mainly on sewage sludge) varied between 5,44-16,66 mg triphenyl formazan / cm^3 sludge * 24 h. Variation occurred mainly due to changes in substrate composition.

Our measurements revealed that the absence of co-substrates causes a decrease in enzyme activity. Upon reintroducing co-substrates into the system, enzyme activity peaked. Changes in biological activity were successfully monitored using enzyme activity measurements, therefore we conclude that enzyme activity measurements can be valuable assets in characterizing microbiological processes during fermentation.

ACKNOWLEDGEMENT

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