Correlation of dissolved hydrogen concentration with VFA/TIC parameter in psychrophilic anaerobic digestion

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Abstract

This paper evaluates the usefulness of measuring the concentration of dissolved hydrogen in an anaerobic fermenter for maintaining the process stability. A laboratory test of two-stage psychrophilic anaerobic digestion of food leftovers from a university canteen was carried out in a vertical reactor with a total working volume of 0.255 m^3 without stirring. In the course of the experiment lasting 1050 days, with an average organic loading of 15.45 kg_{VS} m^{-3} d^{-1} for the 1st stage and 0.657 kg_{VS} m^{-3} d^{-1} for the 2nd stage, a specific biogas production of 0.123 Nm^3 per kilogram of substrate and 0.448 Nm^3 per kilogram of total solids and 0.480 Nm^3 per kilogram of volatile solids, respectively, was achieved. The average methane content in biogas was 55.9 % vol. Slightly higher gas productions were measured in the batch BMP test. The concentration of hydrogen in the mixed biogas from both reactor stages occasionally exceeded 1000 ppm and averaged 134 ppm, the concentration of dissolved hydrogen measured by AMT MS 08 sensor in the overloaded second stage was often 0.10 – 0.23 mg dm^{-3} and correlated with the total concentration of lower fatty acids and with the VFA/TIC parameter. The dissolved hydrogen concentration from AMT instrument was not found to be a reliable timely indicator of overload or process stability.

Keywords: *anaerobic digestion; fermentation; psychrophilic bioreactor; dissolved hydrogen; amperometric sensor*

1. Introduction

Biowaste management is currently a complex and often discussed issue, because many biowaste producers do not know how to properly manage it. In the Czech Republic, in recent years, the annual production of waste from catering establishments has been around 15,000 tons of declared waste, this waste can be disposed of, for example, by composting or anaerobic digestion. According to the Waste Act, every producer of such waste must hand over the waste to an authorized company, which will process it according to available options. For reasons of practicality and speed of disposal, waste was in the past added to livestock or crushed in a kitchen grinder and flushed down the drain. Both options are now prohibited[1].

Catering waste belongs to the group of bio-waste, which is the abbreviated name for biodegradable waste. These are the wastes that are capable of aerobic or anaerobic decomposition. According to Regulation (EC) No. 1774/2002 of the Parliament and of the Council, catering waste can be defined as 'all food waste, including used table oil, originating from restaurants, catering establishments and kitchens, including central kitchens'[2]. Food leftovers from catering establishments are classified in Group 20 (municipal waste) and specifically in Group 20 01 08 - Biodegradable waste from kitchens and catering establishments.

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In canteens and catering establishments, especially those that only heat and serve ready-to-eat products, food leftovers represent the largest part of the waste. How to deal with waste such as plastic, paper or glass is a well-known fact. However, how to properly manage waste such as food scraps is not so clear-cut anymore. Act No. 185/2001 Sb. on Waste and Amendment of Some Other Acts[3] has introduced a very strong instrument for reducing the amount of biodegradable waste going to landfill, and the law implies that material recovery always takes precedence over other uses, such as energy recovery. Waste for which no recovery has been found can be disposed of. Landfilling is then the last method of waste disposal. In the case of anaerobic co-digestion with biogas production, this is a material-energy recovery of biowaste, and it is not always clear in advance whether the method is preferable to material recovery in the form of compost for a given biowaste.

Anaerobic digestion process can take place under thermophilic, mesophilic or psychrophilic conditions. The typical temperature range of psychrophilic digestion is up to 20°C [4]. In the anaerobic system, each new batch of substrate is first dissolved and hydrolyzed, while neither organic acids nor hydrogen are produced to a greater extent. In the second phase, the hydrolysis products are acidified, releasing CO_2 and hydrogen. These changes will most noticeably affect the values of the VFA/TIC parameter and the concentrations of H_2 in liquid and gas. In the next phase - acetogenesis, higher acids are broken down to acetic acid, and CO_2 and H_2 are produced again. A steady state occurs only in case of sufficiently intense methanogenesis, during which both acetic acid and H_2 and CO_2 are consumed[5, 6].

The initial steps of the pathway remain the same in thermophilic, mesophilic and psychrophilic digestion; however, with the operating temperature, other active groups of microorganisms appear in the process. The digestion process consists of events that convert organic compounds into more stable compounds with simultaneous release of biogas, which is a mixture of mostly methane and carbon dioxide. The bacterial hydrolysis breaks down complex insoluble organic matter consisting of carbohydrates, proteins, lipids, and fats. The products are soluble compounds such as simple sugars, amino acids, and fatty acids. This is mediated by various hydrolytic bacteria including members of *Bacteroides*, *Clostridium*, *Streptococcus*, which can produce extra cellular enzymes involved in the degradation and solubilization of complex molecules. In the next phase of acidogenesis, acid-producing bacteria belonging to the genera *Bacillus*, *Lactobacillus and Serratia spp* further degrade the soluble substrates into intermediate products. They consist mainly of heavy fatty acids (VFA) and lower acids such as acetic acid, propionic acid, butyric as well as other short-chain fatty acids, alcohols, H_2 and CO_2. The resulting VFAs and alcohols are further converted into acetic acid, carbon dioxide and hydrogen by acetogenic bacteria during the acetogenesis. Acetate-producing bacteria, including bacteria of the genera *Synthrophomonas* and *Synthrobacter*, are responsible for the conversion of the acid phase to acetogenesis and generate acetate, CO_2, protons and H_2 as the main precursors of methanogenesis. Evidence exists that *Clostridium thermoaceticum* may also reduce protons and, hence, produce H_2 under certain conditions. Thus proton-reducing acetogens may also utilize the acetyl CoA pathway for CO_2 reduction. In the final step, methanogenic archaea, for example *Methanosaeta spp*., they use the products obtained from the previous steps (acetate, CO_2 or methylated compounds) as a substrate for the production of methane-rich biogas [4,7,8].

Food waste in the United States include unconsumed food and food preparation residues from residences, commercial establishments such as restaurants, institutional sources such as school cafeterias, and industrial sources such as factory cafeterias. Zhang et al. measured an average methane yield of food waste at 0.435 m^3 per kg of VS after 28 days of digestion at 50 ± 2 °C. The average CH_4 content was 73 vol. %. The biogas yield was 0.465 m^3 per kg of food waste. The resulting lower heating value was 27.2 MJ m^{-3} in average. About 80% of the methane yield was obtained after the first 10 days of digestion [9].

An example of intensive usage of food leftovers and expired food for biogas production can be found in Austria. Here, after collection and sanitation of food scraps and expired food, the material is anaerobically fermented at mesophilic conditions. With an annual feedstock volume of more than 9,000 tons, approximately 1.8 million cubic meters of biogas is obtained. The economy of the whole plant is due to the high degree of conversion of organic matter during the fermentation process (70 – 75% in the first stage and 90-97% in both stages) [10].

The aim of this article is to verify again the usefulness of measuring the concentration of dissolved hydrogen in an anaerobic fermenter with the aim of maintaining the stability of the anaerobic process. This verification was done for different process conditions than previous than the previous example[11]. We would like to verify whether the dissolved hydrogen concentration correlates with the VFA/TIC ratio, which is a commonly used parameter to assess the stability of the anaerobic process. Dissolved hydrogen monitoring could be a useful tool for monitoring and controlling the anaerobic fermentation process, especially when it comes to maintaining stable conditions for the proper functioning of the anaerobic plant.

Dohányos reports in his research that the concentration of hydrogen in the liquid phase in an anaerobic fermenter is common in the range of 0 – 200 mmol m^{-3}, i.e. 0 – 0.2 mmol dm^{-3} (0 – 0.2 mmol/l or 0 – 0.2 mM or 0.4 mg dm^{-3}). Strong et al. tested a low-cost dissolved H2 probe. VFA accumulation and fermenter failure were observed at dissolved H2 partial pressures below 30 Pa, corresponding to 0.68 ppb. ord-Ruwisch et al. demonstrated that the partial pressure of dissolved hydrogen in an anaerobic bioreactor, within the range of 2 – 8 Pa, exhibits a linear correlation with organic overloading of the process. This parameter is a highly sensitive process indicator, capable of detecting both shock overloads and gradual overloading. In the experimental reactor, when the partial pressure of dissolved H_2 exceeded 6.5 – 7.0 Pa, overloading occurred, leading to the accumulation of organic acids. A computer-based control system, which regulated substrate dosing while maintaining a threshold of 6.5 Pa, ensured stable process operation. These findings were validated in a full-scale 600 m³ reactor, where the critical limit was identified as 7.0 Pa [12,13,14].

In 2001, Björnsson et al. [15] addressed the monitoring of dissolved hydrogen concentration, as it is known to be closely related to VFA accumulation, they addressed the direct measurement of hydrogen in the liquid phase. In this study, a supplementary approach to monitoring dissolved hydrogen was investigated including the transfer of liquid hydrogen through a Teflon membrane and detection in gas phase using a Pd metal oxide semiconductor (Pd-MOS) sensor.

Several abiotic factors have been identified that can serve as early warning indicators, including total or individual volatile fatty acid (VFA) concentration and hydrogen concentration[16]. The concentration of hydrogen affects the degradation of many organic compounds and may be a sensitive guide to the metabolic state of biomass degrading wastes to methane. The use of the concentration of hydrogen in the biogas as a process control index should therefore be evaluated in the industrial context. The results demonstrate that during the operation of the digester the hydrogen concentration in biogas remained fairly constant but following three out of four volumetric shock loads the hydrogen levels rose rapidly before recovering to normal levels within a day. Hydrogen is produced at many stages, and by many bacterial species, during anaerobic digestion and is then consumed by the carbon dioxide-reducing methanogenic bacteria. The capacity of the methanogenic bacteria to remove hydrogen is normally far from being saturated. Increased hydrogen levels inhibit the degradation of volatile fatty acids such as propionic and butyric and can also inhibit acetoclastic methanogenesis by *Methanosarcina spp*. Consequently, in the extreme, accumulation of hydrogen leads to an increase in volatile fatty acid concentrations and acidification within the digester. Measurement of hydrogen levels is therefore appropriate in the development of a process control strategy in anaerobic digestion [17].

2. Materials and Methods

2.1. Inoculum

Digestate or more precisely fermenting biomass suspension from the 1st fermentation stage of the agricultural biogas plant Pustějov II (Zemspol Studénka JSc.) operation close to Ostrava city was used as anaerobic inoculum. The suspension was brought in the morning of the test starting day. The suspension was centrifuged on a small industrial centrifuge CHC-61A (BeHo Ltd., Czech Rep.) at 1200 rpm for 10 minutes. Only the liquid fraction containing a solid particle smaller than approximately 3 mm was further used as inoculum. The anaerobic bioreactor was filled with 0.255 m^3 of inoculum.

2.2. Substrate

The leftover food was obtained from the main canteen of VSB-TU Ostrava. These were food leftovers from plates of various meals. The canteen produces in total at least 30 kg of food leftovers every working day. The material consisted really only of uneaten meals from the canteen as the kitchen processes only semi-finished food and does not produce any serious amount of kitchen biowaste. The leftover food is collected daily in a plastic barrel and this is taken away for disposal by an authorized person under a long-term contract. The leftovers contain all foodstuffs and are not separated according to their origin, i.e., leftovers include bones of cooked meat (chicken only) or also leftover salads and desserts (minimum), see Figure 1.

Figure 1: Leftover food from the university canteen of VSB-TU Ostrava.

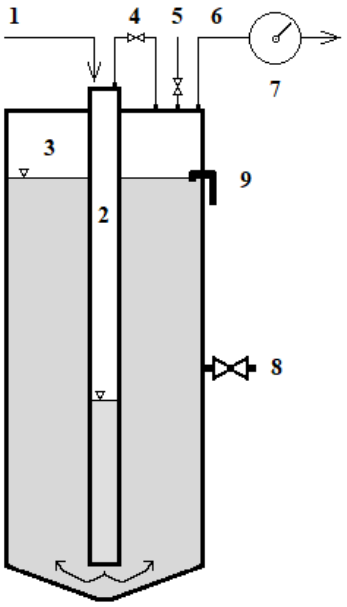
The material was usually only treated by removing large bones and not too forcible mixing with a mortar mixer. It was then stored in a refrigerator at 4 – 6 °C. Occasionally, when the material was too viscous, it was supplemented with a small amount of drinking water. At a later stage, daily batches of material were mixed in a kitchen mixer (25000 rpm) for 30 s. No further adjustments were made. The inoculum and food residue parameters are shown in Table 1. Sampling for analysis was performed only once, at the beginning of the experiment.

Table 1: Inoculum parameters and average parameters of food leftovers.

Parameter	**Symbol, unit**	**Inoculum (start sample)**	**Food leftovers**	**Min**	**Max**	**RSD, %**
pH	pH-H_2O -	7.60	4.63	4.32	5.21	0.5
Total solids (105 °C)	TS, % hm.	4.65	15.59	11.88	19.24	3.7
Volatile solids (Lost on ignition. 550 °C)	VS, $\%_{TS}$	70.1	93.15	91.86	96.95	2.7
Carbon	C, $\%_{TS}$	39.46	46.20	-		
Nitrogen	N, $\%_{TS}$	4.41	2.60			
Ammoniacal nitrogen	N_{NH4+}. $\%_{TS}$	4.03	0.31			
Sulphur	S, $\%_{TS}$	0.63	0.20			
Hydrogen	H, $\%_{TS}$	4.67	6.70			
Oxygen	O, $\%_{TS}$	39.29	40.49			
Ash	CA, $\%_{TS}$	29.90	6.85			
Crude lipids	CL, $\%_{TS}$	1.61	9.08			
Crude fibre	CF, $\%_{TS}$	0.38	1.66			
Crude protein	CP, $\%_{TS}$	1.03	2.36			
Simple carbohydrates	CH, $\%_{TS}$	1.31	2.08			
Starch	ST, $\%_{TS}$	12.34	52.10			
Lignin	LI, $\%_{TS}$	1.94	0.44			
Nitrogenous substances	NC, $\%_{TS}$	8.03	14.63			
Non-nitrogenous extract substances	NFE, $\%_{TS}$	57.86	69.20			

2.3. Psychrophilic two-stage anaerobic digestion of food leftovers

The digestion experiment was carried out in a vertical stainless-steel (AISI 304) bioreactor with a total volume of 0.276 m^3 (see Figure 2). The reactor had two reaction stages. The 1st stage with a working volume of only 0.010 m^3 was formed by axially placed vertical tube with 100 mm internal diameter. This tube was open at the bottom, close to the bottom of the reactor. The 2nd stage was formed by the cylinder with 500 mm internal diameter. The food leftovers were dosed once per day almost every working day. The feeding port was at the top of the 1st stage tube and the digestate overflow was at the level of the batch in the 2nd stage, so the digestion process was semicontinuous. No stirring device was used. There was also no heating device so the fermentation temperature fluctuated slowly in the psychrophilic range (16-26 °C) depending on the actual temperature of the laboratory. The temperature of meat in the batch in the fermenter was 21.3 °C. During the day, the dose of fresh food leftovers hydrolyzed and acidified in the top part of the 1st stage tube and the released CO_2-dominated gas forced slowly the semi-digested suspension down and into the 2nd stage cylinder. In the 2nd stage the process continued mainly with methanization. Some more difficult-to-decompose particles sank to the bottom, where apparently the sludge was strongly anaerobic, and other particles rose to the surface. Spontaneous overflow of the excess volume of digestate occurred continuously through an overflow tube at the surface in 2nd stage. Before daily measuring and feeding gas pressure of both stages had to be equalized though the specialized gas pipeline. This formed mixed sample of biogas. Portable analyzer was used for this mixed biogas composition measurements. Subsequently, the lid of the 1st stage was opened and a new batch of substrate was inserted. Initially, 0.1 kg of food leftovers per day was dosed, but gradually the dose was increased to 2.7 kg per day (occasionally up to 4.4 kg per day). The digestate was sampled at half height of the 2nd stage.



1 – Feeding port

2 – 1st stage reactor tube

3 – 2nd stage reactor cylinder

4 – Pipeline for equalizing biogas pressure

5 – Biogas composition measurement port

6 – Biogas outlet port

7 – Drum-type gas flow meter

8 – Digestate sampling port

9 – Digestate overflow pipe

Figure 2: Two-stage psychrophilic anaerobic bioreactor.

2.4. Analyses

To control the digestion process three parameter of food leftovers were analysed regularly. It was the pH value determined potentiometrically using a WTW 340i with a SenTix 41 probe at 20 °C[18], the total solids content (TS, by drying at 105 °C in an O_2 atmosphere to constant weight, 2.0% RSD) with a KERN DLB 160 3A moisture analyzer with halogen lamp[19] and the volatile solids content (VS, by igniting at 550 °C in O_2 atmosphere to constant weight, 5.0 % RSD) with a LECO TGA 701 thermogravimetric analyzer[20]. These three parameters were analysed also in the digestate samples.

The elemental composition of the solids of inoculum, randomly sampled food leftovers and of the final digestate (C, H, N, S) was determined with a LECO Truspec CHN 628 + S 628 analyzer[20]. The oxygen content was calculated from the other elements according to the ASTM standard[21].

The ratio of the concentrations of total organic acids, primarily less volatile fatty acids (VFA), to residual buffering capacity, represented by total inorganic carbon (TIC), in the digestate was determined using the automatic titrator TIM BIOGAS V02.2 (Hach Lange, Germany), that we recalculated according to the formulas from the study[22]. The concentration of molecular hydrogen dissolved in the suspension was measured using a microamperometric sensor AMT MS 08 (AMT Analysenmesstechnik GmbH, Germany), with a probe range of 0–3.0 mg dm^{-3} and specified for temperatures between 0 – 40 °C. The H_2 sensor was equipped with its own separate temperature sensor and was calibrated daily according to the accompanying documentation. Both sensor probes were placed approximately 30 mm below the surface of the digestate during measurement. After measuring the dissolved hydrogen concentration, a sample of the digestate (approximately 0.9 times the volume of the substrate dose) was taken for analysis, and a new dose of substrate was. The higher heating value of the solid of digestate was determined with a semi-automatic isoperibolic calorimeter LECO AC 600 according to EN 15170[10].

Every working day, volumetric biogas production was recorded according to the conditions in the water-filled drum-type gas flow meter (RITTER TG05, Germany). The biogas composition was analysed using a BIOGAS 5000 portable analyzer (Geotechnical Instruments Ltd., UK) with a dual wavelength IR sensor for measuring methane (CH_4 0 – 100 % vol.) and carbon dioxide (CO_2 0 – 100 % vol.), and an electrochemical sensor for the determination of hydrogen gas (H_2 0 – 1000 ppm), hydrogen sulfide (H_2S 0 – 5000 ppm) and oxygen (O_2 0 – 25 % vol.). Missing data were interpolated linearly.

Other parameters used to describe the anaerobic digestion process are Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT). The OLR parameter indicates the capacity of the anaerobic digestion system to convert organic matter into biogas.

$$OLR\ [kg/m3/day] = \frac{Mass\ of\ Organic\ Substrate\ [kg/day]}{Reactor\ Volume\ [m^3]}$$

The HRT parameter indicates the time the feedstock remains in the fermenter in anaerobic digestion systems.

$$HRT\ [days] = \frac{Volume\ of\ Digester\ [m^3]}{Influent\ Flow\ Rate\ [m^3/day]}$$

2.5. Biochemical methane potencial test

Simultaneously with the long-term experiment in bioreactor two batch tests of biochemical methane potential (BMP) were performed. The aim of the tests was to verify the practically achievable gas yield from food leftovers under different temperature conditions. Both tests passed without reactor stirring. Glass reactors with the total individual volume of 1.2 L topped with ground glass were used. Each reactor was closed with gas measuring burette with the total volume of 1.4 L. Reactors were placed and heated in water bath inside laboratory hood. Burettes stood in the hood. One test was conducted at mesophilic temperature (40 °C ± 0.5 °C), the other at psychrophilic temperature (20 °C ± 2 °C). Each test was performed at three different initial substrate loads, with the overall lowest load used only under psychrophilic conditions and the highest load only under mesophilic conditions. The conversion of the gas volume to normal conditions (0 °C, 101325 Pa) was based on the temperature and barometric pressure in the hood. In each test, two reactors were used to measure endogenous biogas and methane production (inoculum production) and two reactors contained substrate addition. The BMP standard [20] requires that the VS content of the inoculum is higher than 50% TS and the concentration of VS in the inoculum is in the range of 1.5-2.0% by weight. This means setting the total dry matter TS to the level of 3.0% by weight. The mVS-substrate/mVS-inoculum mass ratio should be in the range of 0.3 – 0.5. With a sufficient volume of biogas in the burette (> 150 ml), the composition of this gas was measured. A Biogas5000 portable analyzer (Geotechnical Instruments Ltd., UK) was used. Data from the CH_4 sensor were used to determine methane production. The data from the CO_2 sensor usually needed to be

reduced by 1 – 2 vol. % in order for the sum of volume percentages to be 100%. The theoretical water vapor content corresponding to 2.3 vol. % at 20 °C was included in the sum. Occasional low residual aeration of the sample was subtracted, component contents were corrected. Missing data on biogas volume and composition from weekends were linearly interpolated. The H_2 content was measured to document the low load of the inoculum with acids from the acidification of the organic substances of the substrate. The H_2S content was measured to detect possible inhibition by sulfane or sulfides. The theoretical production of biogas and methane was calculated from the elemental composition of volatile solids according to the Buswell formula modified by Richards, for the case when the released ammonia is dissolved in the suspension and immediately compensated by the carbonate formed from the digestion of produced CO_2 [23].

2.6. Hydrogen concentration correlation

All data were subject to mathematical correlation, specifically VFA/TIC and dissolved hydrogen and hydrogen in biogas data were correlated. The correlation examines the joint variability of two variables and determines whether we can observe a "concurrency" in the data, where higher values of one variable are associated with higher values of the other variable, or, conversely, a "countercurrency," where higher values of one variable are associated with lower values of the other. For this purpose, the concept of covariance is introduced, which measures the strength of the linear relationship between two variables - positive values indicate concurrency in the data, negative values indicate non-continuity, and values close to zero indicate a lack of linear relationship. Therefore, the covariance is standardized with respect to the variances of the variables and in this way the most famous correlation coefficient, the Pearson linear correlation coefficient, is introduced. Pearson's linear correlation coefficient r expresses the degree of linear relationship between two numerical variables. Its values lie in the interval <-1.1>, which greatly facilitates interpretation[24]. Spearman rank-correlation coefficient is a non-parametric correlation coefficient that is robust to outliers and deviations from normality in general, as it, like many other non-parametric methods, works only with the order of the observed values. In contrast to the Pearson correlation coefficient, which describes a linear relationship of the quantities, the Spearman correlation coefficient describes how well the relationship of the quantities and corresponds to a monotonic function, which may of course be nonlinear[25]. The Spearman correlation coefficient describes how well the relationship of the quantities conforms to a monotonic function.

3. Results and discussion

3.1. Process parameters

The anaerobic digestion of food residues was investigated for 1050 days (see Figure 3), with the process being carried out in five phases while maintaining the same process conditions. In the first phase (0 – 45 days), a biogas production of 0.0448 $Nm^3\ d^{-1}$ was recorded with a methane content of 40.3% and an organic loading (OLR) of 8.1 kgVS $m^{-3}d^{-1}$ at a residence time (HRT) of 378 days. The methane production intensity was 0.1 $Nm^3m^{-3}d^{-1}$, with a specific methane production of 0.03 Nm^3kg^{-1}. In the second phase (45 – 210 days), biogas production increased to 0.1147 Nm^3d^{-1} with methane content of 57.1%, while OLR increased to 15.2 kgVS $m^{-3}d^{-1}$at HRT of 205 days. This led to an increase in methane production intensity to 0.3 $Nm^3m^{-3}d^{-1}$and specific methane production to 0.05 Nm^3kg^{-1}. The third phase (210 – 680 days) brought a stabilization of biogas production to 0.1421 Nm^3d^{-1}, methane content was 54.6%, OLR reached 18.3 kgVS $m^{-3}d^{-1}$ and HRT decreased to 178 days. Production intensity remained at 0.3 $Nm^3m^{-3}d^{-1}$and specific methane production increased to 0.06 $Nm^3\ kg^{-1}$. In the fourth phase (680-920 days), biogas production decreased slightly to 0.1369 Nm^3d^{-1}, methane content was 53.8%, while OLR was 17.8 kgVS $m^{-3}d^{-1}$ and HRT decreased to 142 days. Methane production intensity remained at 0.3 $Nm^3m^{-3}d^{-1}$, but specific methane production decreased to 0.04 Nm^3kg^{-1}. In the fifth phase (920 – 1050 days), biogas production decreased to 0.0608 Nm^3d^{-1} while methane content increased to 65.7%. The OLR reached 3.9 kgVS $m^{-3}d^{-1}$ at extended HRT to 399 days, with methane production intensity of 0.2 $Nm^3m^{-3}d^{-1}$ and specific methane production of 0.06 $Nm^3\ kg^{-1}$.

During the 1050 days of the experiment (see Figure 3), the average total solids content of the food leftovers dosed (pH 3.4 – 5.9) was 15.59 wt. %. At an average specific gravity of 1068 kg m^{-3}, the organic content was 93.15 wt.%. At an average organic loading rate (OLR) of 15.45 kg_{VS} m^{-3} d^{-1} the 1st stage worked with an average theoretical retention time (THRT) of 12 days. The 2nd stage worked at OLR of 0.657 kg_{VS} m^{-3} d^{-1} with THRT of 210 days. The reactor as a whole produced 0.123 Nm^3 d^{-1} biogas and 0.069 Nm^3 d^{-1} methane, respectively. The average biogas production per unit mass of wet substrate reached 0.070 Nm^3 kg^{-1}. The CH_4 production per unit mass of total solids input averaged 0.250 Nm^3 kg_{TS}^{-1} and per unit mass of volatile solids input averaged 0.268 Nm^3 kg_{VS}^{-1}. The volatile solids content of the suspension was reduced to 2.40 wt. %, i.e. 84 %, by the process. The average values of the measured and calculated experimental parameters are given in Table 2 and graphically represented in Figure 3 – 5.

Table 2: Average parameters of two-stage psychrophilic anaerobic digestion of food leftovers

Feed parameters	**Mean**	**Min**	**Max**	**RSD, %**
Daily dose weight, kg d^{-1}	1.758			
pH, -	4.63	4.32	5.21	0.5
TS, Wt. %	15.59	11.88	19.24	3.7
	14.54	91.86	96.95	2.7
OLR 1st stage, kg_{VS} m^{-3} d^{-1}	15.452	0.80	61.00	31.4
OLR 2nd stage, kg_{VS} m^{-3} d^{-1}	0.657	0.04	2.82	1.5
THRT 1st stage, d	12	2.4	103.6	55.9
THRT 2nd stage, d	210	59.2	2072.7	1122
Mixed raw biogas parameters				
Daily production, Nm^3 d^{-1}	0.123	0.001	0.287	0.14
CH_4 content, Vol. %	55.9	21.1	77.8	28.6
Digestate parameters				
Temperature, °C	21.30	16.0	26.0	5.0
pH, -	7.43	6.41	8.20	0.9
TS, Wt. %	3.70	2.45	5.00	1.3
VS, Wt. %	2.40	1.38	3.45	1.0

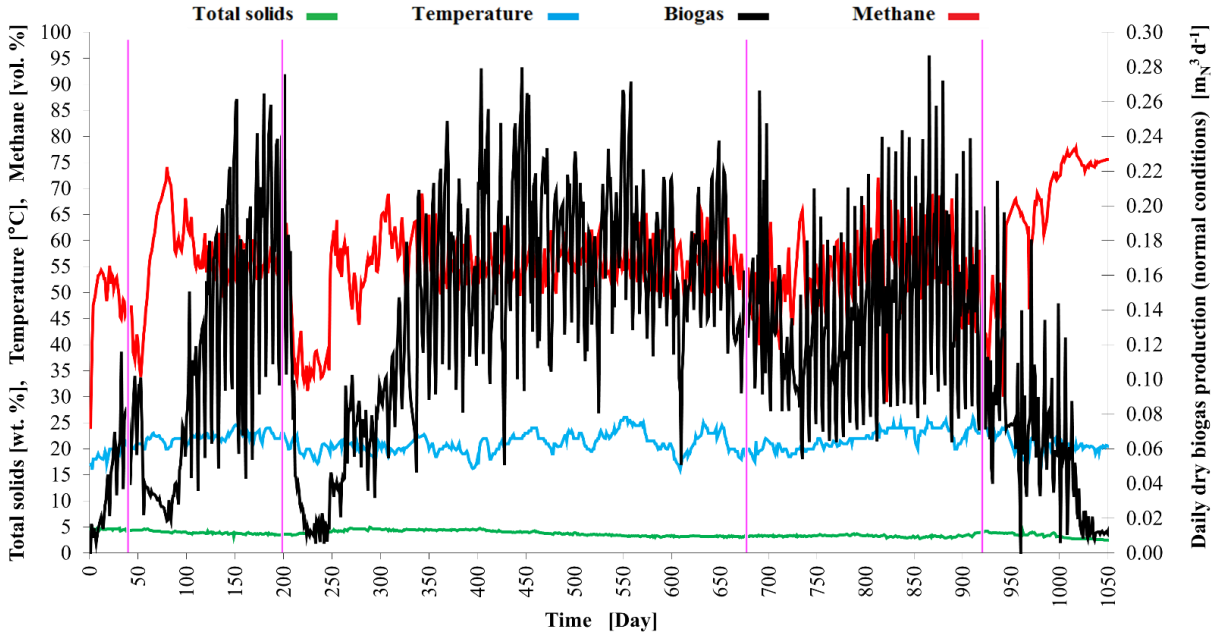


Figure 3: Process temperature, total solids content in the digestate, biogas production and methane content

The curve of daily biogas production in Figure 3 shows that four overload crisis periods occurred during the digestion experiment. The first overload started around the day 30 when the OLR for the 2nd stage increased to 1.2 kg_{VS} m^{-3} d^{-1} and the corresponding THRT shortened under 140 days. Between days 50 – 100 the VFA concentration increased (see Figure 4) and the VFA/TIC ratio was in the interval of lower stability (0.4 0.6). There was high peak of hydrogen in mixed biogas in this period (see Figure 5). This peak was limited by the analyser limit (1000 ppm H_2). The peak of hydrogen concentration dissolved in the 2nd stage slurry (see Figure 4 and 5) did not precede the peak of H_2 in gas or the peat of VFA much. Feeding had to be decreased and a few doses of substrate had to be omitted. After that the digestion process relatively stabilized and biogas and methane production efficiency increased.

The second overload has occurred between the day 200 and 300 when the OLR for the 2nd stage was increased over 2.0 kg_{VS} m^{-3} d^{-1} and the corresponding THRT shortened under 80 days. The VFA concentration increased to similar level like in the first overload period (approx. 8000 mg dm^{-3}) and the maximum of VFA/TIC ratio was 1.0. Highest peak of hydrogen in mixed biogas did not occur soon and was overtaken by the peak of dissolved hydrogen (maximum of 0.21 mg dm^{-3}). This overload period was best signalized by the dissolved hydrogen measurement. During a stable process in the 2nd reaction stage in days 400 600 the concentration of dissolved hydrogen measured by the AMT MS 08 instrument was 0.005 – 0.05 mg dm^{-3}.

The third period of overload has occurred between the day 680 and 800 when the OLR for the 2nd stage was increased over 1.3 kg_{VS} m^{-3} d^{-1} but some doses corresponded to loads of up to 2.5 kg_{VS} m^{-3} d^{-1}. The VFA concentration increased once more to approx. 8000 mg dm^{-3} and the maximum of VFA/TIC ratio was 1.3. Almost all the peaks of hydrogen concentration in mixed biogas were lower (see Figure 5) than these peaks in the period of relative process stability with high methane yield (see days 400 – 600). The rise of dissolved hydrogen concentration was almost continuous (to the maximum of 0.20 mg dm^{-3}) but there were no high peaks at the beginning of overload period that would strongly suggest overloading. This time the better alarm parameter seemed to be the VFA/TIC ratio.

The last overload period started around the day 920. The OLR for the 2nd stage reached 1.8 kg_{VS} m^{-3} d^{-1} in some doses and the corresponding THRT was around 135 days. The VFA concentration went to the extreme (11000 mg dm^{-3}) and the VFA/TIC peak reached 2.5. The only strong peak of hydrogen concentration in mixed biogas come in the day 941 when the VFA/TIC ration was already on the decline. Peak of dissolved hydrogen preceded the peaks of VFA a VFA/TIC by 23 days and had the power to draw attention to the problem. But the dissolved hydrogen concentration was increased from 0.01 mg dm^{-3} to 0.08 mg dm^{-3} long before this overload and yet at this time (days 800 – 850) the reactor reached one of the highest biogas productions with a stable CH_4 content of around 60 vol. %.

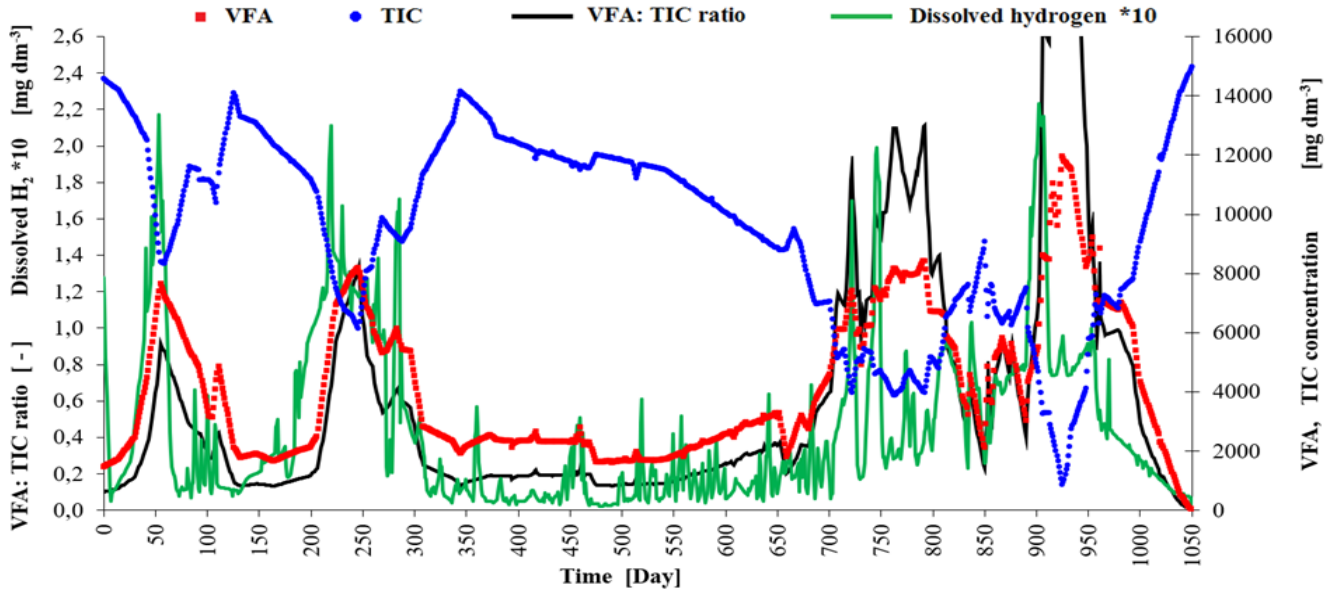


Figure 4: Evolution of the VFA/TIC ratio, its components and dissolved hydrogen concentration

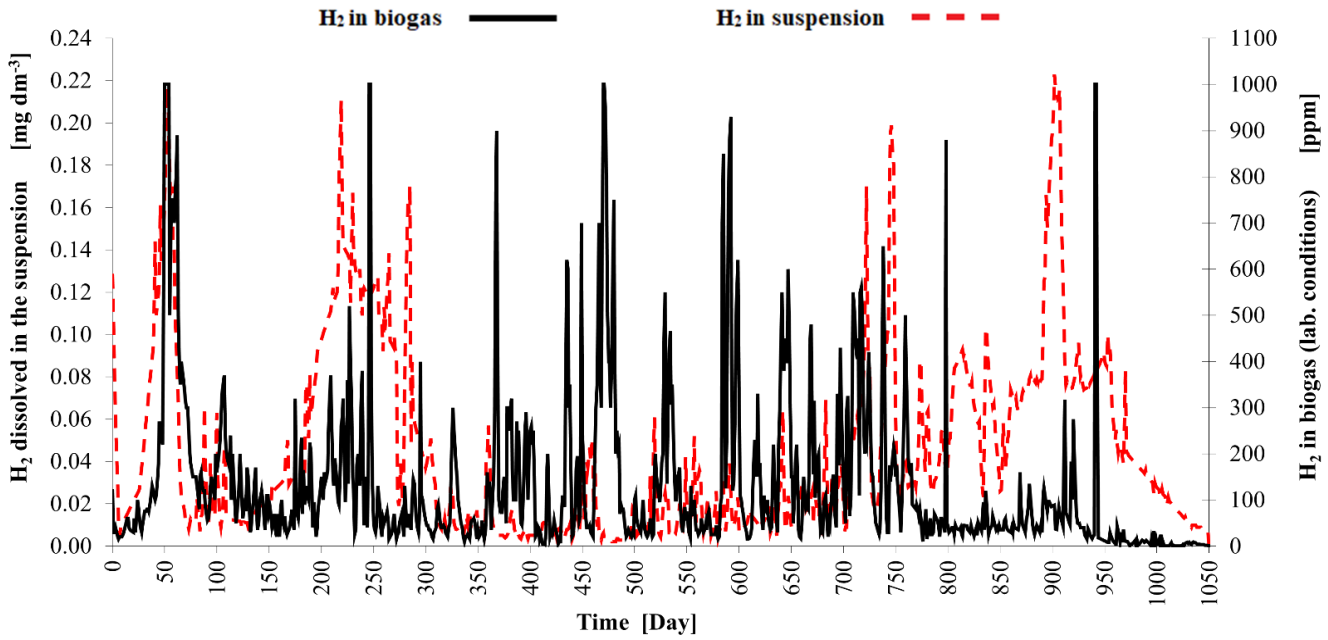


Figure 5: Concentration of H_2 dissolved in the 2nd stage anaerobic slurry and of H_2 in mixed biogas

The Organic Loading Rate values for the 1st and 2nd stages of the process and the dissolved hydrogen concentration are plotted in Figure 6. It is obvious that the OLR curve of the 1st stage is similar to the curve of the dissolved hydrogen curve.

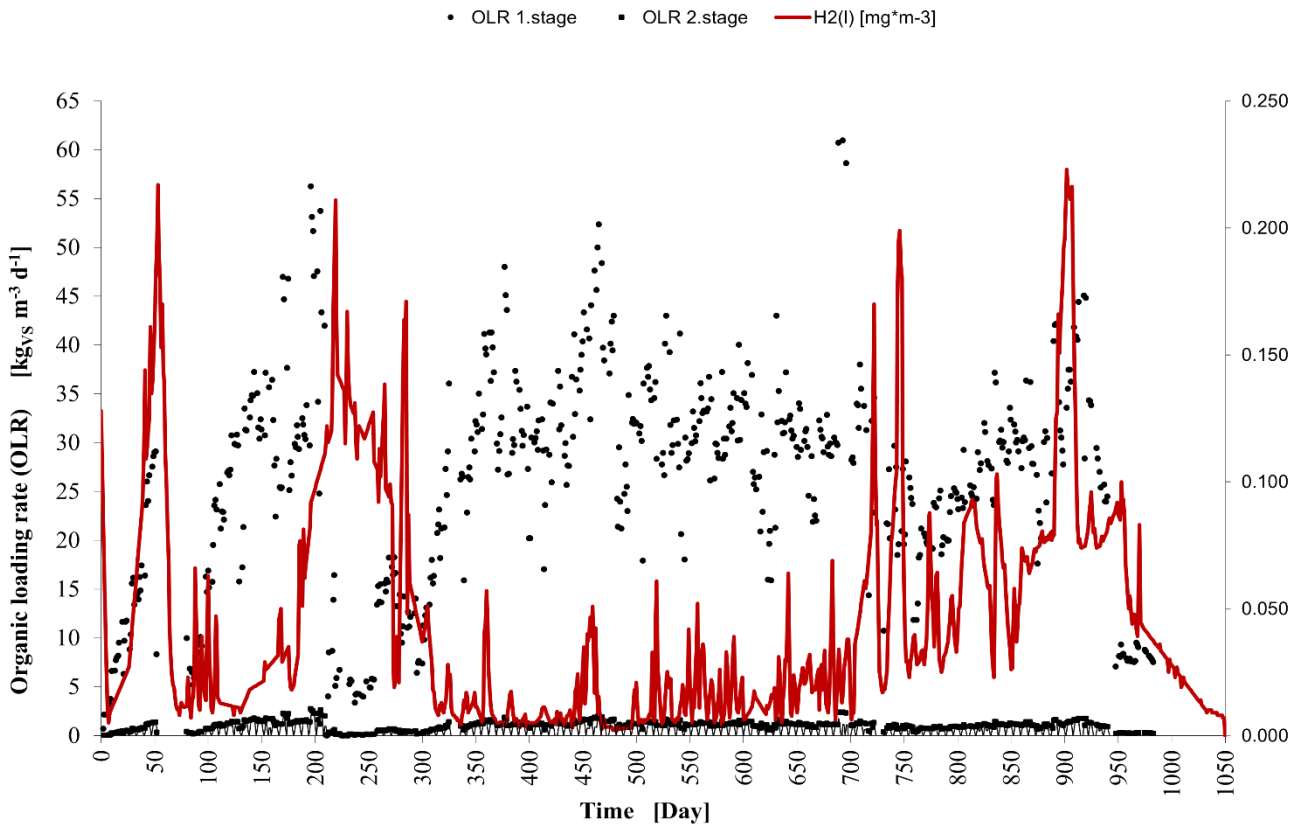


Figure 6: Organic Loading Rate 1st stage, Organic Loading Rate 2nd stage and H_2 dissolved concentration

The H_2S content in mixed biogas increased from 500 ppm over 1000 ppm every time the H_2 content in gas had high peak and this could apparently also cause cross-interference when measuring hydrogen in the liquid and gas phase. The AMT Analysenmesstechnik GmbH stated that their AMT MS 08 instrument has very low interference with H_2S, but sensor poisoning cannot be ruled out during a long-term use in digestate. The only very high peak of H_2S (3800 ppm) occurred during the days 330 – 375. That time the measured content of hydrogen in liquid and gas phase was generally low, VFA/TIC ratio was stable under 0.3 and methane production was increasing quickly so the process was in good balance and inhibition of methanogenesis from H_2S probably did not have a significant effect. pH value in the 2nd stage varied in the range 6.4 – 8.2 and visibly correlated with the course of VFA concentration but the relationship between the change in pH value and the change in biogas or methane production was not clear.¨

3.2. Hydrogen concentration correlation

The correlation analysis between VFA/TIC and the variables H_2 dissolved in suspension and H_2 in biogas was conducted using both Pearson and Spearman correlation coefficients to comprehensively assess the relationships. Figure 7 depicts the results of the cross-correlation analysis which investigates the temporal relationships between VFA/TIC values and both hydrogen concentrations in suspension and biogas at various lags using both Pearson and Spearman correlation methods. We have decided to calculate the correlation coefficients for a 14-days long time span, due to the fact that the trends for all of the correlation coefficients are damped towards the end of this time period thus there would be no meaningful information obtained by calculating the coefficients for higher lag values. The full 1050-day period was used in the cross-correlation analysis, capturing the statistical dependence of these variables across the full timeline. Firstly, no time lag between the correlated parameters was used. The Pearson correlation coefficients for H_2 in suspension and VFA/TIC, as well as H2 in biogas and VFA/TIC, were 0.508 and 0.021, respectively. These values suggest a moderate positive linear relationship between H_2 in suspension and VFA/TIC, while the correlation between H_2 in biogas and VFA/TIC appears to be weak. On the other hand, the Spearman correlation coefficients for H_2 in suspension and VFA/TIC, as well as H_2 in biogas and VFA/TIC, were notably higher at 0.660 and 0.061, respectively. This indicates a stronger monotonic relationship, emphasizing that although the correlation is not strictly linear, there exists a consistent trend in the variables changing together. The discrepancy between the Pearson and Spearman coefficients underscores the importance of considering different aspects of the relationship, as Spearman correlation is less sensitive to outliers and captures nonlinear associations. These results collectively provide a nuanced understanding of the associations between VFA/TIC and the variables H_2 in suspension and H_2 in biogas, shedding light on both linear and monotonic aspects of their relationships.

Our hypothesis was that after introducing a time lag between the measured data of VFA/TIC versus H_2 in gas or VFA/TIC versus H_2 in suspension, a stronger correlation would be revealed. Time lag of 0 to 13 days was tested.

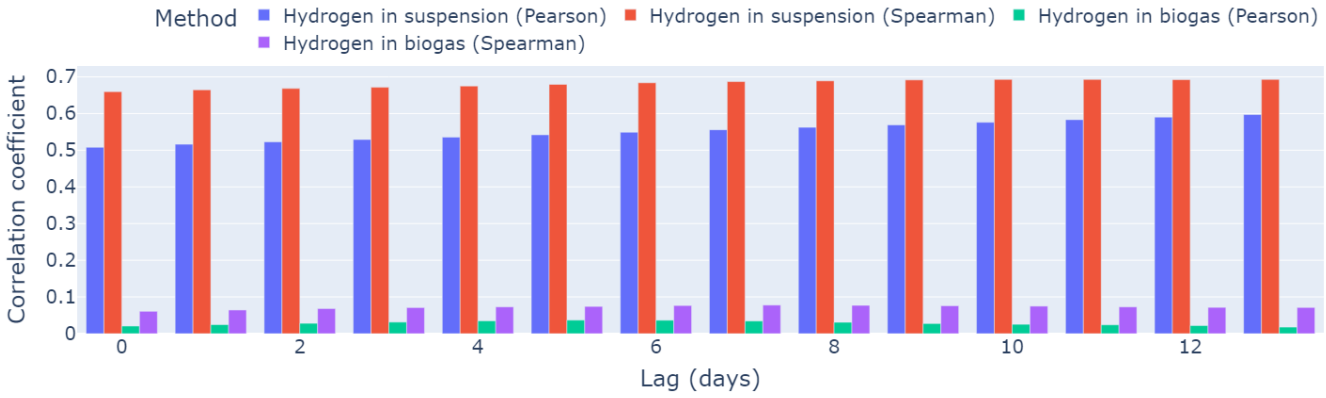


Figure 7: Pearson and Spearman correlation methods

Both Pearson and Spearman coefficients between H_2 in suspension and VFA/TIC show positive relationships across all lags. The Spearman coefficients consistently exceed the Pearson coefficients, emphasizing a stronger monotonic association. The correlations tend to increase slightly with increasing lags. For hydrogen in biogas the Spearman coefficients consistently surpass the Pearson coefficients, however the coefficients values are near-zero, thus the relationship is very weak/non-existent. These results collectively suggest a delayed but persistent positive correlation between hydrogen levels (mainly for hydrogen in suspension) and VFA/TIC, with the Spearman method capturing the overall stronger and more consistent relationship. The increasing trend in correlation with lags suggests a temporal connection between H_2 in suspension and the VFA/TIC ratio in the dataset. While hydrogen production by acidification reactions can in principle be very rapid, especially after hydrolysis of sugars, the decomposition or consumption of acids is highly dependent on the type of acid. For example, propionic acid decomposes very slowly. Consequently, there must be a period when the VFA or VFA/TIC parameter will not correlate with the concentration of dissolved H_2. When individual limiting acid concentrations are exceeded, methanogenic microorganisms are reduced or inhibited, resulting in a further increase in hydrogen concentration.

3.3. Results of BMP tests

The results of both BMP (batch) tests are summarized in the graph in Figure 8 and in the Table 4.

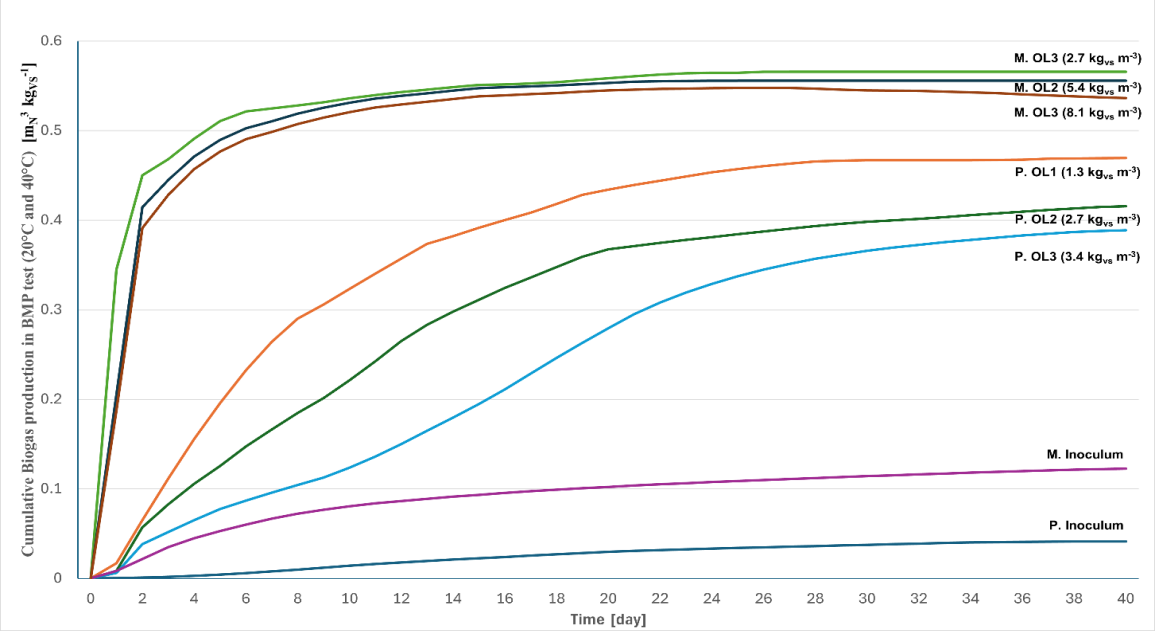


Figure 8: Cumulative biogas production in mesophilic and psychrophilic BMP test

Table 4: Gas production in mesophilic and psychrophilic BMP test of food leftovers

Test (40 days)	Batch temperature	Initial organic load of substrate		Biogas production	Methane production	Methane yield relative to the theoretical maximum
	°C		$kg_{VS}\ m^{-3}$	$Nm^{-3}\ kg_{VS}^{-1}$	$Nm^{-3}\ kg_{VS}^{-1}$	%
Mesophilic	40 ± 0.5	M-OL1	2.7	0.934	0.565	99
		M-OL2	5.4	0.919	0.556	97
		M-OL3	8.1	0.858	0.535	94
Psychrophilic	20 ± 2.0	P-OL1	1.3	0.839	0.470	82
		P-OL2	2.7	0.714	0.416	73
		P-OL3	5.4	0.685	0.389	68

In accordance with general knowledge, both biogas and methane production decreased with increasing load at both temperatures. An infinitesimal load could theoretically give a gas yield of 100%. Psychrophilic conditions made it possible to obtain 74% and 70% of the methane obtainable under mesophilic conditions, respectively, at a load of 2.7 kg and 5.4 $kg_{VS}\ m^{-3}$.

The food waste was subjected to a single-phase mesophilic and a two-phase psychrophilic anaerobic digestion to identify the effect of the two-phase process and validate the efficiency of psychrophilic technology in converting food waste[12,26]. Both digestion methods proved to be exceptionally effective in converting most of the biodegradable material in food waste into biogas. The single-phase mesophilic CSTR reactor demonstrated high performance in methane production. A relatively high concentration of H_2S does not significantly affect the performance of the mesophilic reactor, provided that no other inhibitory or synergistic effects occur. On the other hand, the high amount of H_2S produced during the process startup appears to be responsible for the increased risk of acidification in the early stages of operation of the psychrophilic two-phase reactor. The system achieves stable digestion and balanced operation within a relatively short period. Despite the limiting effect of low operating temperature, biogas production is not low due to the high hydraulic retention time (HRT) characterizing the two-phase process. Two-phase digestion, even under psychrophilic conditions, is significantly efficient in ensuring a high proportion of volatile organic compounds contained in food waste. The low-temperature two-phase system is much more energy-efficient for processing food waste than the single-phase mesophilic process. Therefore, a two-phase anaerobic digester operating under psychrophilic conditions could be an economically viable option for effectively processing food waste[27].

The fact that the psychrophilic process is more suited to a lower load is also evidenced by the lower CH_4 content in the biogas (56 – 58% by volume) versus 60 – 62% by volume in the mesophile. Psychrophilic hydrolysis and acidogenesis are fast enough to easily overwhelm methanogenesis.

Even at the highest load, the psychrophilic biogas production and CH_4 content in the batch test were slightly higher than when using the two-stage bioreactor, but this was apparently mainly due to periods of deliberate overloading of the reactor. It can be said that the data from the batch test and from the reactor agree.

Measurements of the concentration of dissolved hydrogen from the rest of the meals in the psychrophilic conditions of the BMP test showed the production of high peaks only in the first two days of the experiment. This was followed by a significant drop in hydrogen production. At the end of the experiment, production was practically zero. Changes in the concentration of soluble hydrogen are shown in Figure 9.

BMP biogas production from the BMP test had uncertainty of ± 5 % and the methane production had uncertainty of ± 7 %. But because of high heterogeneity of each storage tank of food leftovers the biogas production could easily vary ± 50 %.

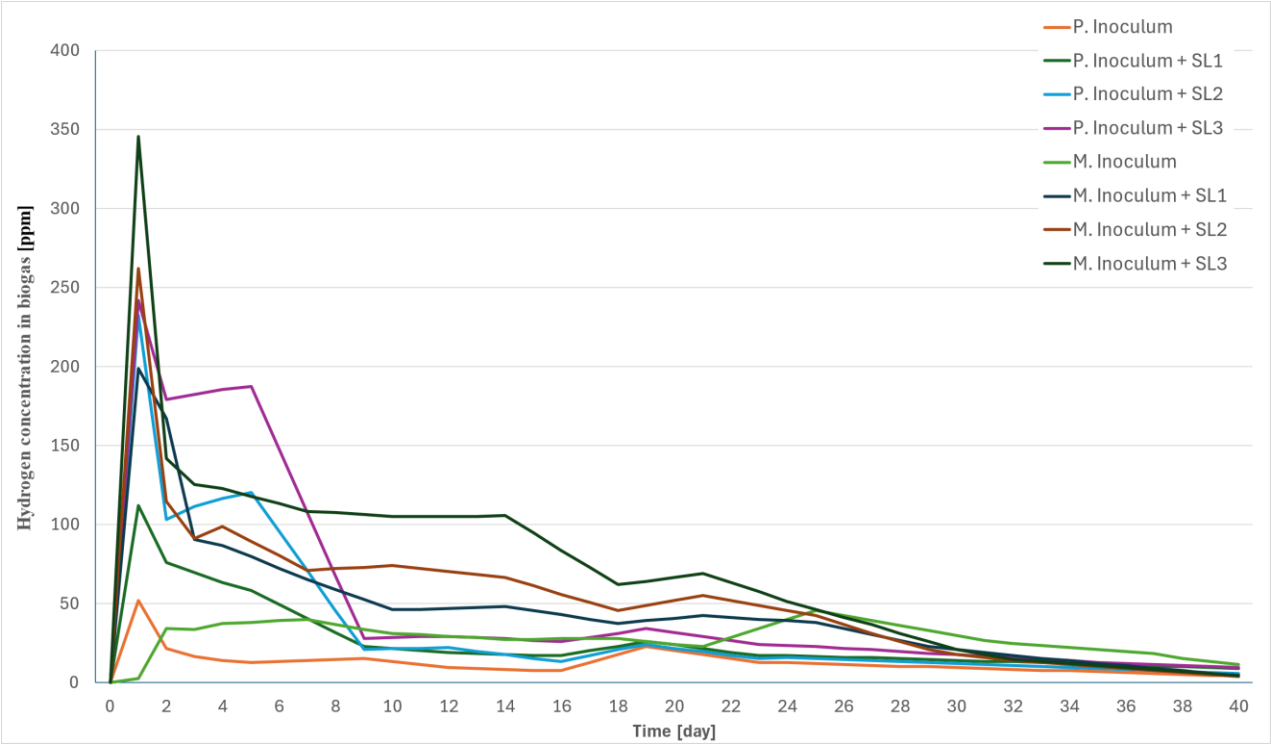


Figure 9: Hydrogen concentration in biogas from mesophilic and psychrophilic BMP test

From the available literature, it has been verified that after the processing of food leftovers or expired food, the gas yield can be described as follows, see Table 5. The production of both biogas and methane in the two-stage reactor under discussion does not deviate from the known limits.

Table 5: Biogas yield in the anaerobic processing of food leftovers

Substrate	Total solids (TS)	Volatile solids (VS)	Biogas yield		CH_4 content	Source
	%	%	$Nm^3\ kg^{-1}$ fresh matter	$Nm^3\ kg_{VS}^{-1}$	vol. %	
Food residues and expired food	9 – 37	80 – 98	0.050 – 0.480	0.200 – 0.500	45 – 65	[28, 29]
Food residues	23	86	0.100	0.220	n.a.	[28, 29]
Food leftovers	15.59	93.65	0.0698	0.597	55.9	This study

4. Conclusions

The anaerobic digestion of food leftovers was tested on a long-term basis in a two-stage bioreactor under the psychrophilic conditions without stirring. The process was overloaded four times with a high amount of substrate in order to trace the dependence between known process stability parameters and dissolved hydrogen concentration. Despite drops to blackouts during overload, the average specific production of methane was interesting for practical use. The monitoring of the VFA/TIC parameter in the digestate from the second processing stage was essential for the detection of approaching overloading. Monitoring the hydrogen concentration in the blended biogas from both stages of the process was not very useful. In only one case did a sharp rise in dissolved hydrogen concentration detect overload before a distinct rise in VFA/TIC. The concentration of dissolved hydrogen measured by the AMT MS 08 instrument during a stable process in the 2nd reaction stage was 0.005 – 0.05 mg dm^{-3}. Overall, it can be summarized that the continuous assessment of process stability according to dissolved hydrogen concentration was useful, but not indispensable. Batch tests confirmed still relatively intense digestion of food leftovers under psychrophilic conditions.

References

1. Odpadové fórum [online]. 2019, 12(3) [cit. 2019-02-21]. ISSN 1212-7779. Available at: http://www.odpadoveforum.cz/upload/pageFiles/3-2011-pdf.pdf
2. Commission Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption.
3. Act No. 185/2001 Sb. on Waste and Amendment of Some Other Acts. In: Prague: Collection of Laws of Czech Republic, 2001. Available at: https://www.mzp.cz/C125750E003B698B/en/used_product_collection/$FILE/Waste%20Act_1852001.pdf
4. Akindolire, M. A., Rama, H., & Roopnarain, A. (2022). Psychrophilic anaerobic digestion: A critical evaluation of microorganisms and enzymes to drive the process. Renewable and Sustainable Energy Reviews. https://doi.org/10.1016/j.rser.2022.112394
5. Gunnerson, Ch. G., Stuckey, D.: Anaerobic Digestion Principles and Practices for Biogas Systems, World Bank Technical Paper Number 49, ISSN 0253-7494
6. Meegoda JN, Li B, Patel K, Wang LB. A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion. Int J Environ Res Public Health. 2018 Oct 11;15(10):2224. doi: 10.3390/ijerph15102224. PMID: 30314318; PMCID: PMC6210450.
7. Upadhyay, J., & Stokes, J. L. (1961). Anaerobic growth of psychrophilic bacteria. Department of Bacteriology and Public Health, Washington State University, Pullman, Washington. Received for publication July 28, 1961. Downloaded from https://journals.asm.org/journal/jb on 13 March 2024 by 2001:718:1001:157:5463:e457:36c9:51d0.
8. Mackie, R. I., & Bryant, M. P. (1990). Acetogenesis and the Rumen: Syntrophic Relationships. Springer New York, NY. DOI: 10.1007/978-1-4615-1777-1, ISBN 978-1-4613-5716-2.
9. Bauer, F. Food scraps become current and heat, Biom [online]. 2019, [cit. 2019-02-22]. Available at: https://biom.cz/cz-bioplyn/zpravy-z-tisku/ze-zbytku-jidla-se-stava-proud-a[1]teplo
10. Zhang, R., El-Mashad, H. M., Hartman, K., Wang, F., Liu, G., Choate, C., & Gamble, P. (2007 Characterization of food waste as raw material for anaerobic digestion. Bioresource Technology, 98(4), 929-935. Available at: https://www.sciencedirect.com/science/article/pii/S0960852406000940
11. Platošová, D., Rusín, J., Platoš, J., Smutná, K., & Buryjan, R. (2021). Case Study of Anaerobic Digestion Process Stability Detected by Dissolved Hydrogen Concentration. Processes, 9(1), 106. https://doi.org/10.3390/pr9010106
12. DOHÁNYOS, Michal: Anaerobic reactor is not a black box - theoretical foundations of anaerobic fermentation. Biom.cz [online]. 2008-11-17 [cited 2025-01-24]. Available from WWW: <https://biom.cz/cz/odborne-clanky/anaerobni-reaktor-neni-cernou-skrinkou-teoreticke-zaklady-anaerobni-fermentace>. ISSN: 1801-2655.
13. Strong, GE and Cord-Ruwisch, R. (1995), In situ dissolved hydrogen probe for monitoring anaerobic fermenters under overload conditions. Biotechnol. Bioeng., 45: 63 – 68. https://doi.org/10.1002/bit.260450109
14. Cord-Ruwisch R, Mercz TI, Hoh CY, Strong GE. Dissolved hydrogen concentration as an on-line control parameter for the automated operation and optimization of anaerobic digesters. Biotechnol Bioeng. 1997 Dec 20;56(6):626 – 34. doi: 10.1002/(SICI)1097-0290(19971220)56:6<626::AID-BIT5>3.0.CO;2-P. PMID: 18642334.
15. Björnsson, L., Murto, M., Jantsch, T. G., & Mattiasson, B. (2001). Evaluation of new methods for the monitoring of alkalinity, dissolved hydrogen and the microbial community in anaerobic digestion. Water Research, 35(12), 2833-2840. https://doi.org/10.1016/S0043-1354(00)00585-6
16. Moeller, L., Zehnsdorf, A. Process disturbances in a full-scale anaerobic digestion bioreactor: overacidification and foaming during biogas production. Energ Sustain Soc 6 , 30 (2016). https://doi.org/10.1186/s13705-016-0095-7
17. Archer, DB, Hilton, MG, Adams, P. a kol. Hydrogen as an index of process control in a pilot scale anaerobic digester. Biotechnol Lett 8, 197-202 (1986). https://doi.org/10.1007/BF01029380

18. EN ISO 10390:2021 Soil, treated biowaste and sludge – Determination of pH.
19. EN 15934:2012 Sludge, treated biowaste, soil and waste - Calculation of dry matter fraction after determination of dry residue or water content.
20. EN 15935:2021 Soil, waste, treated biowaste and sludge - Determination of loss on ignition.
21. ASTM D5373-16 Standard Test Methods for Determination of Carbon, Hydrogen and Nitrogen in Analysis Samples of Coal and Carbon in Analysis Samples of Coal and Coke.
22. Liu, X.; André, L.; Mercier-Huat, M.; Grosmaître, J.-M.; Pauss, A.; Ribeiro, T. Accurate Estimation of Bicarbonate and Acetic Acid Concentrations with Wider Ranges in Anaerobic Media Using Classical FOS/TAC Titration Method. *Appl. Sci.* **2021**, *11*, 11843. https://doi.org/10.3390/app112411843
23. VDI 4630: Vergärung organischer Stoffe – Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche. 1. 11. 2016.
24. Richards, B.K., Cummings, R.J., White, T.E., Jewell, W.J. (1991) Methods for Kinetic Analysis of Methane Fermentation in High Solids Biomass Digesters. Biomass and Bioenergy, 1 (2), 65-73. https://www.sciencedirect.com/science/article/pii/096195349190028B.
25. Pearson, Karl (20 June 1895). "Notes on regression and inheritance in the case of two parents". Proceedings of the Royal Society of London. 58: 240–242. Bibcode:1895RSPS...58..240P, https://www. jstor .org /stable/115794.
26. Spearman, C. (January 1904). "The Proof and Measurement of Association between Two Things" (PDF). The American Journal of Psychology. 15 (1): 72 – 101. doi:10.2307/1412159. JSTOR 1412159.
27. Rusin, J., Chamrádová, K., & Basinas, P. (2021). Two-stage psychrophilic anaerobic digestion of food waste: Comparison to conventional single-stage mesophilic process. *Waste Management, 119*, 172 – 182. https://doi.org/10.1016/j.wasman.2020.09.039
28. Kratochvílová, Z., Habart, J.: Guide to the production and use of biogas (in Czech). Praha, 2009. [cit. 2019-02-21]. Dostupné z: https://biom.cz/upload/6e01d6d4c4835ec93cda508772f3bf6e/pruvodce_vyrobou_vyuzitim_bioplynu.pdf
29. Hlinčík, T.: Non-traditional heating gases – biogas, landfill gas, use of hydrogen as a heating gas (in Czech). VŠCHT, [cit. 2019-02-21].Available at: https://web.vscht.cz/~hlincikt/Z%C3%A1klady%20zpracov%C3%A1n%C3%AD%20a%20vyu%C5%BEit%C3%AD%20uhl%C3%AD%20a%20plynu/13.pdf

Korelace koncentrace rozpuštěného vodíku s parametrem VFA/TIC při psychrofilní anaerobní digesci

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Abstrakt

Tento článek hodnotí užitečnost měření koncentrace rozpuštěného vodíku v anaerobním fermentoru za účelem udržení stability procesu. Laboratorní test dvoustupňové psychrofilní anaerobní digesce zbytků jídla z univerzitní menzy byl proveden ve vertikálním reaktoru o celkovém pracovním objemu 0,255 m^3, tento reartor pracoval bez míchání. V průběhu experimentu trvajícího 1050 dní při průměrném organickém zatížení 15,45 kg_{VS} m^{-3} d^{-1} pro 1. stupeň a 0,657 kg_{VS} m^{-3} d^{-1} pro 2. stupeň byla měrná produkce bioplynu 0,123 Nm^3 na kilogram sušiny a 0,4048 Nm celkem, 0,448 Nm na kilogram organické sušiny. Průměrný obsah metanu v bioplynu byl 55,9 % obj. Mírně vyšší produkce plynu byla naměřena v dávkovém testu BMP. Koncentrace vodíku ve směsném bioplynu z obou stupňů reaktoru občas přesahovala 1000 ppm a průměrně 134 ppm, koncentrace rozpuštěného vodíku měřená senzorem AMT MS 08 v přetíženém druhém stupni byla často 0,10 – 0,23 mg dm^{-3} a korelovala s celkovou koncentrací nižších mastných kyselin a s parametrem VFA/TIC. Nebylo zjištěno, že by koncentrace rozpuštěného vodíku z přístroje AMT byla spolehlivým včasným indikátorem přetížení nebo stability procesu.

Klíčová slova: *anaerobní digesce; fermentace; psychrofilní bioreaktor; rozpuštěný vodík; amperometrický senzor*