Postprint of: Słupek E., Makoś P., Kucharska K., Gębicki J., Mesophilic and thermophilic dark fermentation course analysis using sensor matrices and chromatographic techniques, CHEMICAL PAPERS, Vol. 74 (2020), pp. 1573–1582

Mesophilic and thermophilic dark fermentation course analysis using sensor matrices and chromatographic techniques

Edyta Słupek[*1], Patrycja Makoś[1], Karolina Kucharska[1], Jacek Gębicki[1]

[1] *Gdańsk University of Technology, Faculty of Chemistry, Department of Process Engineering and Chemical Technology, 80-233 Gdańsk, Narutowicza 11/12 street, Poland*

[*]Corresponding author, e-mail: edyta.slupek@pg.edu.pl

Received [Dates will be filled in by the Editorial office]

Abstract

Production of biofuels from biomass is expected to benefit society and the environment. At present bio waste residues processing includes hydrolysis, dark fermentation, photo fermentation, pyrolysis, gasification, and chemical synthesis. As the composition and the chemical structure of organic substances affect the efficiency of mentioned processes, it is believed, that the glucose concentration is a crucial parameter for the evaluation of the efficiency of biological processes. Also, the control of by-products formulated during each stage of biomass processing affects the course of dark fermentation. Therefore model processes regarding mesophilic and thermophilic dark fermentation were carried. Glucose as a sole carbon source was applied as the fermentation broth and Faloye-pretreated activated municipal wastewater sludge was introduced as the source of sporulating microorganisms. Production of hydrogen and methane was controlled by means of sensor matrices. Obtained results are comparable to those obtained using the standard method based on gas chromatography and indicate the suitability of their application for on-line routine analyses of hydrogen and methane during fermentation processes. In addition, the fermentation broth was also examined by means of gas and liquid chromatography in the scope of glucose reduction, and generation of volatile fatty acids and phenols.

Keywords: biogas analysis; chromatographic analysis; hydrogen; methane; dark fermentation; sensor matrices

Introduction

The generation of biofuels from lignocellulosic biomass is a complexed process, including three main stages, i.e. pretreatment, saccharification and fermentation (Kucharska et al. 2018). The yield and the rate of biogas or hydrogen productivity are affected mainly by process parameters i.e. pH of the pulp, temperature, composition, biomass pre-treatment method and digestion time (Gomez et al. 2006; Wang et al. 2009; Chu et al. 2012; Lestinsky et al. 2017). Several reports regarding the application of activated municipal wastewater sludge for dark fermentation processes can be found in the literature (Wu and Chang 2007; Jeppsson et al. 2007; Azbar et al. 2009; Ottaviano et al. 2017). However, the literature lacks complexed experiments related to the comparison between mesophilic and thermophilic dark fermentation course when the same sporulating microorganisms obtained during inoculum pre-treatment from activated sludge were applied (Faloye et al. 2013, 2014).

The authors propose to use glucose based fermentation broths for the evaluation of biofuels efficiency The analysis of efficiency associated with glucose aims to maximize the technological, energy and economic benefits in production processes. Energy efficiency is understood as the ratio of energy obtained from biofuels to the energy consumed in all unit processes (Wu et al. 2007). In order to compare the dark fermentation process course, mesophilic and thermophilic conditions were used for the culture (Ivanova et al. 2009; Yasin et al. 2013). A gas mixture containing hydrogen and carbon dioxide is formed during the dark fermentation process. However, reports regarding methane formulation are also published (Levin et al. 2004; Cheng et al. 2010). According to the literature, several differences in the hydrogen: methane ratio may occur (Lay et al. 1999; Guo et al. 2008; Manish and Banerjee 2008; Wu et al. 2009; Zhu et al. 2010).

Hydrogen and methane can be co-generated via anaerobic digestion (AD), a multi-step process carried out by highly differentiated microorganisms. Anaerobic conditions enable the transformation of organic matter into carbon dioxide and methane or hydrogen. It is found, that several types of microbial populations have specific optimal working conditions regarding pH, temperature, alkalinity, concentration ammonia, sodium and potassium ions, volatile fatty acids or heavy metals presence (Wilkie et al. 2000; Yang et al. 2006).

Biogas is composed of methane (up to 75%), carbon dioxide (up to 40%) and constituents such as ammonia, hydrogen, hydrogen sulfide, and nitrogen. Usually, consortia of highly diversified microorganisms enable the generation of biogas and liquid by-products,

i.e. volatile fatty acids (VFA) and other metabolic products. It is reported that metabolic pathways related to biogas generation are highly complicated (De Gioannis et al. 2013; Veluchamy and Kalamdhad 2017). When hydrogen generation is concerned, different sporulating bacteria capable of glucose conversion to valuable acids, i.e. propionic acid, succinic acid, lactic acid and alcohols, i.e. 2,3-butanediol, ethanol with simultaneous liberation of hydrogen is discussed. However, if glucose fermentation is considered, every liquid by-product may lead to a decrease of the overall hydrogen or methane yield (Lee et al. 2006; Wu et al. 2007; Panagiotopoulos et al. 2010).

Several types of main-gaseous product and liquid by-product formulation during anaerobic digestion are presented in Table 1.

Table 1. Gaseous and liquid products generated during anaerobic digestion on different inoculum and broths.

Carbon source	**Applied microorganisms**	**Main gaseous products**	**Main liquid products**	**References**
Glucose/ model process	Mixed anaerobic microflora	Hydrogen	Butyric acid, acetic acid	(Pan et al. 2010)
Lignocellulosic hydrolysate	Anaerobic bacteria	Methane	Lactic acid, citric acid, acetic acid	(Wong et al. 2014)
Barley straw, corn stover and switch grass	*Clostridium sp.*	Methane-low efficiency	ABE (acetone; butanol; ethanol)	(Qureshi et al. 2010b, a)
Food waste	Sewage sludge	Methane	Acetate, propionate, butyrate, valerate, hexanoic acid	(Cheng et al. 2018)
Waste paper and kitchen waste	*Genera 060F05-B-SD-P93* and *Thermosyntropha*	Methane	Ethanol, propionic acid, lactic acid	(Tan et al. 2019)
Food waste	*Bifidobacterium, Lactobacillus*	Methane	Lactic acid, ethanol, acetic acid, propionic acid, butyric acid, valeric acid	(Feng et al. 2020)
Arthrospira platensis	*Clostridium butyricum, Rhodopseudomonas palustris*	Methane and hydrogen	Ethanol, acetate, propionate, butyrate, isobutyrate, valerate,	(Ding et al. 2017)

			isovalerate, and caproate	
Biodiesel industry residue	Mixed cultures (from activated sludge)	Methane	Butyric acid, ethanol, acetic acid, propionic acid, valeric acid	(Kumar et al. 2015)

When mixed bacterial culture is used in dark fermentation, i.e. bacteria obtained from mixed activated wastewater sludge, hydrogen is generated in the initial stage of the process (Pandu and Joseph 2012). However, methane may occur in the final stage of the fermentation process (Teplyakov et al. 2002). As it can be inferred from the data presented in Table 1, a large number of anaerobic digestion examples concerning various feeds have been published.

The fermentation process is usually monitored by pH, biogas production rate, redox potential, concentration of volatile fatty acids (VFA), total phenolic content (TPC) and gas composition, in order to ensure the correctness of the process. Among these indicators, VFA concentration in fermentation broth, as well as biogas compositions, are widely considered as the two most crucial and direct indicators of the biogas production process due to the fact that the dark fermentation process leads mainly to the formation of VFA followed by gasses production (hydrogen and carbon dioxide) which, in the last step, are transformed into methane. However, the increase in VFA concentration is linked to the methanogenesis inhibition or organic overloading and implies a risk of reducing the efficiency of biogas production (Rosecrance et al. 2013). In addition, several studies have also observed that the formation of phenols may also adversely affect the fermentation process (Fenske et al. 1998; Luo et al. 2002; Per Persson et al. 2002).

For the determination of VFA in fermentation broths, the techniques of fluorescence spectroscopy, near-infrared spectroscopy, titration, high performance liquid chromatography (LC) and gas chromatography (GC) are mainly used. The concentration of phenolic compounds could be analysed using UV-Vis spectroscopy (Madsen et al. 2011), GC and HPLC analysis (Nilvebrant et al. 2001; Quéméneur et al. 2012). The analysis of gas formulated during anaerobic digestion is usually carried using gas chromatography, for the determination of the gas content and composition (Rosales-Colunga et al. 2010).

However, GC measurement has several disadvantages i.e.: manual injections and long-time analysis (Isobe et al. 2011). To analyze the processes occurring during dark fermentation, a sensor matrices consisted of sensors selective for hydrogen, methane, carbon dioxide, hydrogen sulphide and ammonia may be applied (Hoff et al. 2006; Gebicki 2016;

Gebicki and Dymerski 2016). Nowadays, sensor arrays in environmental applications are mainly used for air analysis. This technique belongs to dynamically developing instrumental techniques and it is increasingly applied for monitoring and evaluation of the effectiveness of deodorization of unpleasant odours generated by different fields of human activity (Szulczyński et al. 2017).

However, they can also be used to on-line analysis of the biogas composition. In such cases, the biogas characteristics can be detected using metals oxide based MOS sensors. These sensors should be selective for hydrogen, methane and inorganic compounds, i.e. hydrogen sulphide, ammonia, oxygen, carbon dioxide, as well as organic compounds, toluene, benzene or VFA (e.g. acetic acid, butyric acid). In addition, the sensors should be characterized by good selectivity for a given gas and a lack of sensitivity to the interaction of other gases contained in the mixture (Ponzoni et al. 2017). In addition, the sensor matrices require careful design and testing for which model conditions are used and then perform tests on real samples. Continuous biogas measurements using sensor matrices are possible using the flow configuration of the measurement system.

The paper presents a comprehensive evaluation of the mesophilic and thermophilic dark fermentation processes in model conditions. As a source of carbon, glucose was selected because it may be sole carbons source for most the microorganisms. The fermentation broth was examined by means of gas and liquid chromatography in the scope of glucose reduction as well as generation of dark fermentation by-products (i.e. VFA and TPC). The possibility of using sensory matrices to investigate the composition of biogas was also examined. The results obtained with sensor matrices were compared with gas chromatography. Then the correlation matrices were created to better understand the course of fermentation processes.

Experimental

Materials and Methods

Chemicals

For the purposes of analytical methods, the standard substances: D (+) Glucose (≥99.5% Sigma Aldrich), Sodium Hydroxide (99%, Sigma Aldrich), Dichloromethane (≥99.9%, Sigma Aldrich), Buffered Peptone Water (Biomaxima, Poland), Syringol (99% Sigma Aldrich), Formic Acid (80% POCH), Acetic Acid (>99% Sigma Aldrich), Propionic

Acid (≥99% Sigma Aldrich), Butanoic Acid (≥99% Sigma Aldrich), Isobutanoic Acid (≥99% Sigma Aldrich), were used in the study.

Anaerobic conditions during dark fermentation were created by purging the bioreactor with nitrogen – purity N5 (Linde Gas, Poland).

Hydrogen – purity N 5.5 from a Packard 9400 hydrogen generator (Packard, USA) was used in the gas chromatography. During the analysis of the sensor matrices, N5 purity compressed air was used (Linde Gas, Poland). An eluent consisting of aqueous 0.2% HCOOH (POCH, Poland) was used for the high-performance liquid chromatography analysis.

Dark fermentation

Dark fermentation was carried out in sterile 1200 mL glass bioreactors withworking volume of 1000 mL). The initial fermentation broth was composed of 900 mL of 20 g/L solution of Buffered Peptone Water (Biomaxima, Poland) and 5.5 g/L of glucose (POCH, Poland) as a sole carbon source. Dark fermentation was carried out with the use of activated sludge after Faloye procedure. The Faloye procedure was used for inoculum preparison. The pH of the activated sludge was adjusted to 8.93 with 1 M NaOH solution and further autoclaved (15 minutes, 121 ^{o}C). After autoclaving the pre-treated sludge was thermostated for 20 h at 37 °C with constant stirring to stabilize the culture of microorganisms.

The fermentation broth was adjusted to pH = 7.00 (1 M NaOH) and a constant pH was maintained throughout the process, using Arduino Data Logger. The anaerobic conditions were created by purging the reactor with sterile nitrogen for 20 to 60 min. After establishing anaerobic conditions, inoculations were carried out using 100 mL of activated sludge after the Faloy'e procedure. The fermentation in bioreactors were carried at 35 °C (mesophilic process) and 65 °C (thermophilic process) with magnetic stirring of 150 RPM. Fermentation was carried for 115 hours. Due to exploitation of the carbon source, after 80 hours of the process, 3.0 g of glucose was added to stimulate the further biogas production.

Sensors analysis – gas phase analysis

The biogas samples were analyzed using a self - constructed sensor matrice (SM). The device was equipped with commercial sensors selective for methane and hydrogen manufactured by Figaro Engineering (TGS2611, TGS2600). In the figure 1. it the scheme of the measurement system is shown. A stream of clean air flows through the measuring

chamber at a constant flow rate of 100 mL/min. The flow stream is controlled by an ADM 1000 flow meter (Agilent Technologies, USA). By changing the position of the valve (see Fig. 1 - point 3), the biogas sample was directed to the measurement chamber. The volume of the analyzed sample was 5.0 mL while the time of dosing the sample was equal to 30 s. After this time the clean air was returned to the measurement chamber for the regeneration of the sensors by changing the position of the valve. Signals from the sensors were recorded using an AD (analog – to – digital) converter (Simex SIAi-8). Data analysis were performed using SigmaPlot 11.0 software .

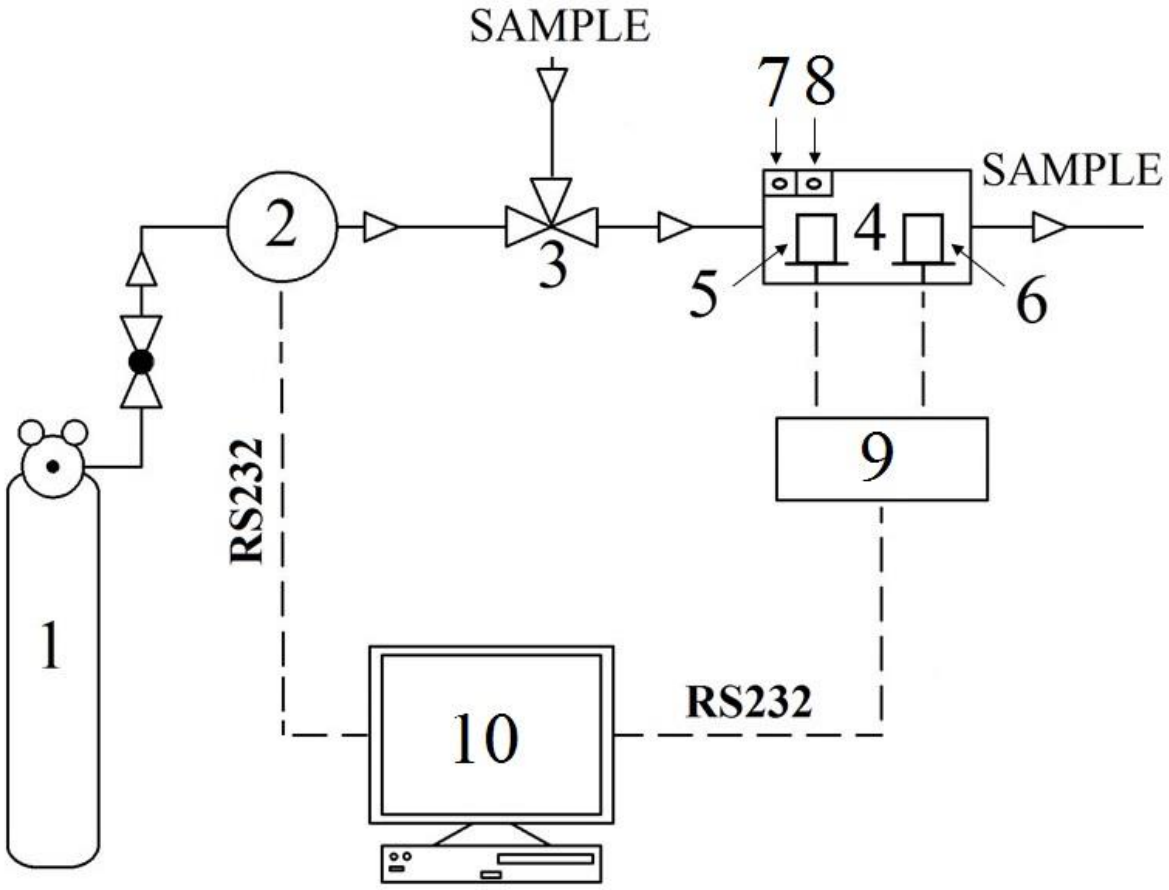

Fig. 1 Measurement system: 1 – air, 2 – flow meter, 3 – valve 4 – sensor chamber, 5 – methane sensor, 6 - hydrogen sensor, 7- temperature sensor, 8- humidity sensor, 9- analog-to-digital converter (ACD Converter), 10 – computer.

Gas chromatography analysis - gas phase analysis

The biogas was also analyzed by means of gas chromatography (Perkin-Elmer AutoSystem XL) with a Porapak Q column (100-120 mesh length 6.5 m, diameter 1/8 inch) and an oven temperature of 60 °C. The following conditions were used during analysis: flame

ionization detector (FID, temperature 220 °C) and thermal conductivity detector (TCD, temperature 100 °C). Nitrogen with a flow of 30 mL/min was used as the carrier gas. The volume of the analyzed sample was 0.5 mL. The total analysis time was 12 minutes. During the analysis, Turbochrom software was applied.

Gas chromatographic analysis - fermentation broth analysis

Samples from dark fermentation process were taken to determine the changes in the concentration of individual and total content of volatile fatty acids and phenols. During the fermentation process, 2 mL samples were collected and stored frozen in the temperature of -18 ºC. For analysis the samples melted and centrifuged (Hitachi EBA 8S) for 5 min at 3000 RPM, an initial removal of the solid phase was realised. The aqueous phase (1.0 mL) was filtered through a 0.45 μm hydrophilic-cellulose filter (Hahnemühle FineArt HmbH, Germany) and transferred to a 1.5 mL vial. 10 μL of hydrochloric acid was added to the sample to adjust the pH to 2.0 and then 300 μL of dichloromethane (DCM) was added. The sample was shaken vigorously in the vial for 1 minute and then centrifuged (3000 RPM) for 5 minutes for liquid-liquid extraction. The obtained organic phase was transferred in a volume of 150 μL by means of an automatic pipette into 2.0 mL vials. The extracted sample (1 μL) was introduced into GC-FID. Individual VFA were analyzed by gas chromatography (Varian CP 3800) with a DB-WAX column (30 m x 0.53 mm x 1.0 μm). The following chromatographic conditions were used: oven temperature 100 °C (5 min) – ramped at 10 °C/min to 250 °C (10 min); injection port temperature 280 °C; injection volume 1 μL; injection mode: split 1:20; FID detector temperature 200 °C; carrier gas N5 nitrogen (flow 1 mL/min). During the analysis System Control software - Varian Star was used.

High-performance liquid chromatography analysis - fermentation broth analysis

For the determination of the glucose and TPC content in fermentation broth, liquid chromatography was applied. The filtered sample (50 μL) of fermentation broth was directly introduced into the HPLC system. The analysis was provided by means of liquid chromatograph (Merck - Hitachi, Germany) equipped with a pump L-7100 with the so-called low-pressure gradient system was applied. The Shodex SH1011, (7 μm, 8 x 300 mm) column was used. It was thermostated by means of the ACS thermostat. The system had two detectors connected in series: Spectrophotometric (L-7450 - Merck - Hitachi, Germany) in the UV-VIS range using photodiode (DAD) and differential refractometric sensors (RID - RI Detector 2100 - Knauer, Germany). In addition, the apparatus had a valve to change the direction of the

mobile phase flow in the back-flush column (Merck, Germany), controlled manually. HSM software was used to record and process the results.

In the HPLC studies, the eluent used was: H_2O + 0.2% HCOOH at a flow rate of 1.2 mL/min. The temperature of the thermostated column was 60 °C. The total analysis time was 30 minutes. HPLC analysis was carried out of 50 μL sample. After 7.6 minutes, a back-flush was used to elute the TPC that were determined relative to the syringol standard. The total TPC content was determined with reference to the syringol calibration curve (TPC standard) in the range from 0.9 to 6.5 mg/mL according to previous work (Słupek et al. 2018).

Results and discussion

The objective of this paper is to present the application of sensor matrices as an alternative method for gas analysis. To analyze the products obtained during mesophilic and thermophilic dark fermentation, a sensor matrices consisted of sensors selective for hydrogen and methane were constructed.

The changes in the composition of gases generated during dark fermentation are presented in Fig. 2 and Fig. 3. Significantly higher concentrations of hydrogen and methane were obtained for fermentation under mesophilic conditions compared to thermophilic conditions. The highest concentrations of hydrogen and methane were obtained in the range of 40 to 45 hours of the mesophilic process and in the range of 20 to 24 hours of the thermophilic process. In both processes, the concentration of methane produced remains constant, while the hydrogen concentration changes significantly during the process. In order to effectively use the activated sludge after the Faloye process, 3.0 g of glucose was added for stimulation of the microspheres of bacteria responsible for methanogenesis. After 85 h of both processes, the termination of hydrogen production is visible, while methane production remains at a constant level.

It was noticed, that the application of sensor matrices allows to obtain an on-line gas analysis and with its application it is possible to obtain two different streams during anaerobic digestion. In the first stage of the process the main gaseous products are hydrogen and carbon dioxide, while after 80 hours of the process, the second stage starts and methane is formed. It can be assumed that methanogens consume the acids generated by hydrogenogenic bacteria in the first stage of the process. Separation of the streams may allow decreasing the costs related to the separation of biohydrogen from biomethane. In order to accurately determine the end of the stage production biohydrogen and the start of the stage production biomethane, on-line gas analysis is necessary.

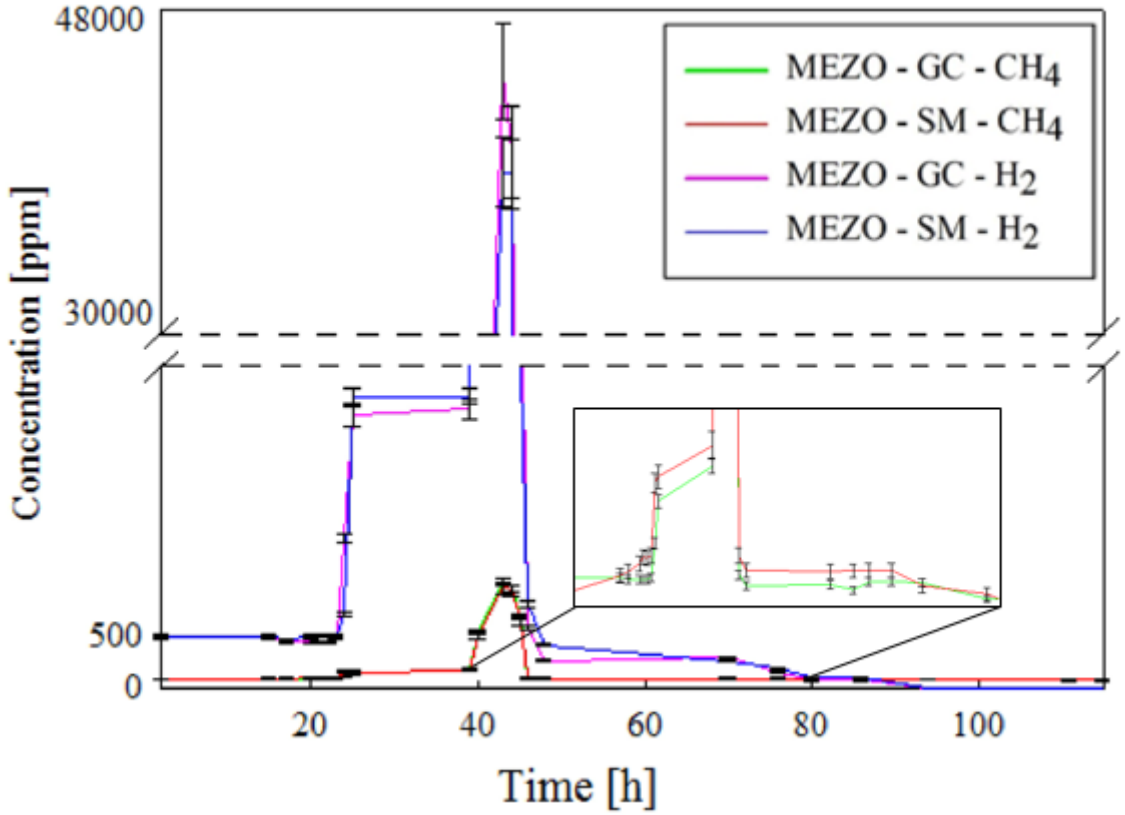

Fig. 2 Changes in the gas composition (hydrogen and methane) occurring during dark fermentation with respect to the mesophilic process determined by means of gas chromatography (GC – green and violet line) and sensor matrices (SM – red and blue line) (n=3).

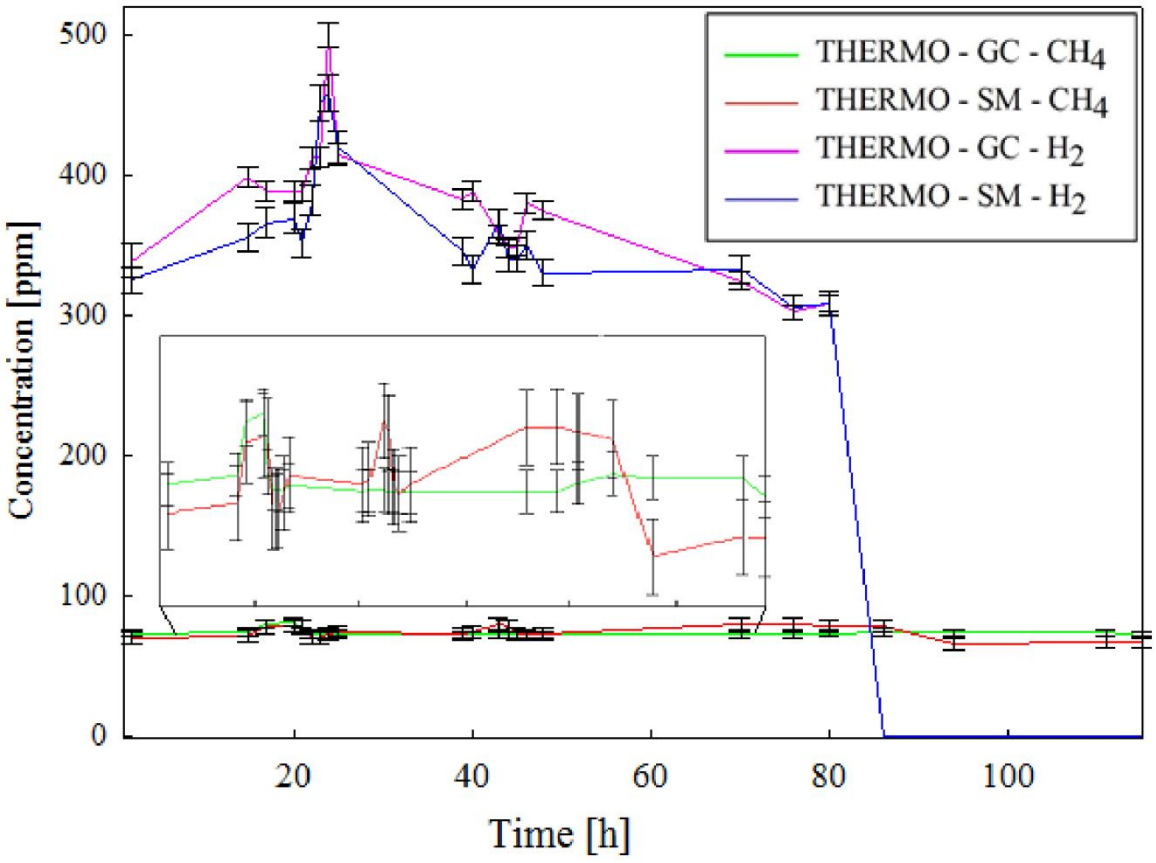

Fig. 3 Changes in gas composition (hydrogen and methane) occurring during dark fermentation with respect to the thermophilic process done by means of gas chromatography (GC – green and violet line) and sensor matrices (SM – red and blue line) (n=3).

The results obtained by commercially available selective sensors for methane and hydrogen were used and the results were compared with the results obtained during GC analysis. It can be concluded that the results obtained using sensor matrices (see blue and red line, Fig. 2 and Fig. 3) correspond with the gas chromatography results (see violet and green line, Fig. 2 and Fig. 3).

The repeatability of the analytical procedure for sensor matrices and gas chromatography was determined by means of the standard deviation value (RSD) obtained as a result of three analysis operations of the reference gas sample at 1000 ppm methane and hydrogen. As a result of comparison of repeatability of both analytical procedures, RSD = 2.82% (methane) and RSD = 3.54% (hydrogen) were obtained for sensor matrices, RSD = 1.59% (methane) and RSD = 1.81% (hydrogen) for gas chromatography. In the real process, the minimum and maximal concentration differences of the resulting biogas were found between the results obtained from sensor matrices and GC. The lowest difference for the

methane concentration in the mesophilic process was 0.35 ppm, while the highest 52.66 ppm. In the thermophilic process, the lowest difference was 0.20 ppm and the highest difference was 6.52 ppm. The calculations were also made for hydrogen concentration where the lowest differences of 9.47 ppm were obtained in the mesophilic process, while the highest - 1053.46 ppm. In the thermophilic process, the lowest concentration was 0.85 ppm, while the highest difference was 56.15 ppm. The average standard deviation between the obtained results from the sensor matrix and GC, which was at the RSD level = 3.89% (methane) and RSD = 8.95% (hydrogen) was calculated. In addition, the results were analyzed by analysis of variance (ANOVA) (Table S1-S4). In ANNOVA, the values of statistical parameter p were used as criteria at a 95% confidence level. All the obtained results using both methods (GC and SM) were found to be statistically insignificant due to the p-value higher than 0.05. For the thermophilic process, the p-value was 0.68 and 0.74 for methane and hydrogen respectively and for the mesophilic process, the p-value was 0.99 and 0.97 for methane and hydrogen respectively. The obtained differences in the values are acceptable and indicate the usefulness of sensor matrices in the on-line control of the dark fermentation process. However, the correctness of the results obtained by sensors matrices should be periodically checked using gas chromatography.

Biogas production is a sensitive process because there are strong correlations of many factors (such as substrate concentration, composition of fermentation broth, temperature and pH value) that affect the efficiency of the production of biohydrogen and biomethane. These additional parameters were also monitored and controlled throughout the dark fermentation. Total glucose concentration used as the sole carbon source in the initial fermentation broth was set at 5.5 g/L for each of the processes (mesophilic and thermophilic). Hence, the glucose content for each analyzed process corresponds proportionally with the data presented in Fig. 4 as well as Tables S5 and S5. One way dark fermentation can occur is the conversion of glucose to hydrogen and acetic acid (Eq. 1). This reaction occurs spontaneously with a maximum theoretical production of 4 moles of hydrogen per mole of glucose as soon as acetic acid is one of VFA. In addition, other VFAs may be formed in fermentation processes such propionic acid (Eq. 2), which reduce the efficiency of the process (Manish and Banerjee 2008; Luo et al. 2010).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1)$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad (2)$$

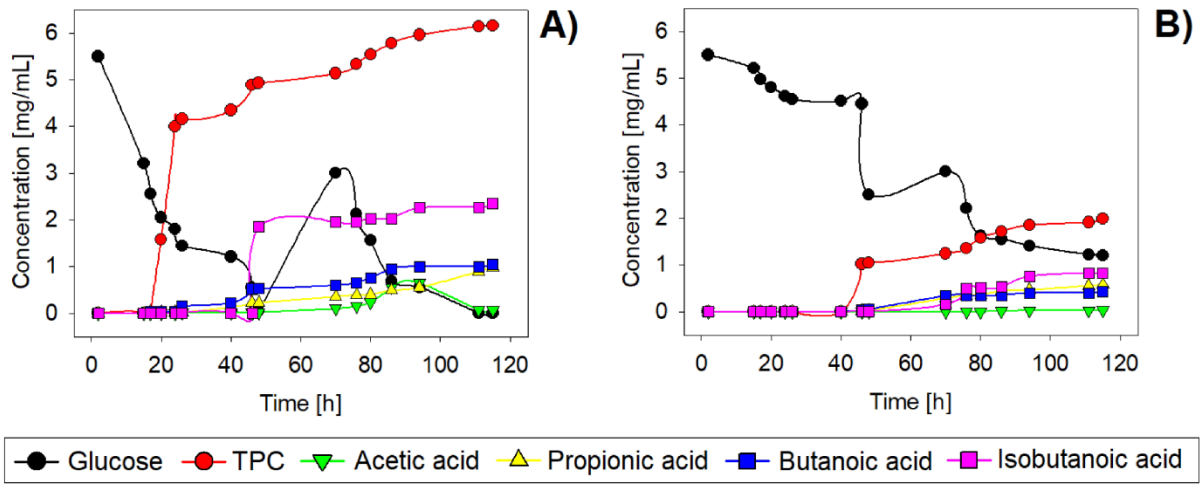

Fig. 4 Changes in the glucose, total phenolic compound (TPC) and selected volatile fatty acids (VFA) concentration in fermentation broth during dark fermentation A) mesophilic process, B) thermophilic process.

The production of biogas under anaerobic conditions in the digester requires the joint action of many populations of microorganisms that have been extracted from the activated sludge. In the fermentation processes, it can be observed that microorganisms not only produce biogas but also VFA (acidogenesis stage). In the next fermentation stage (acetogenesis) propionic, butanoic, and isobutanoic acids are converted to octanoic acid and phenolic compounds (Fig. 4). In the mesophilic process, VFA accumulated much faster in the fermentation chamber, than in thermophilic conditions. It may be a consequence of a much faster loss of glucose in the mesophilic process, which led to faster biogas production but also resulted in the formation of more inhibitors of dark fermentation. Previous studies showed that the optimal pH in terms of biohydrogen production is within a range of 5.0–7.0 which favors the activity of the hydrogenases and is also suitable for microbial development in dark fermentation (Li and Fang 2007; Szulczyński et al. 2019). During the fermentation process, growth of the bacteria that contribute to the formation of volatile organic acids, resulting in decrease in pH. However, after a few days of the process of reaching an increase in pH due to the conversion of organic acids to methane after multiplication of methanogens. Rapid pH changes can adversely affect stability and efficiency process. Therefore, the process was carried out with pH control (Cieślik et al. 2016).

Based on the data presented in Tables S5 and S6, a correlation matrix for the formation of hydrogen and methane, as well as for glucose, one of VFAs and TPC was

prepared. The correlation matrix was created by means of R Studio software (Fig. 5 and Fig. 6) (RStudio 2016; RCore 2018).

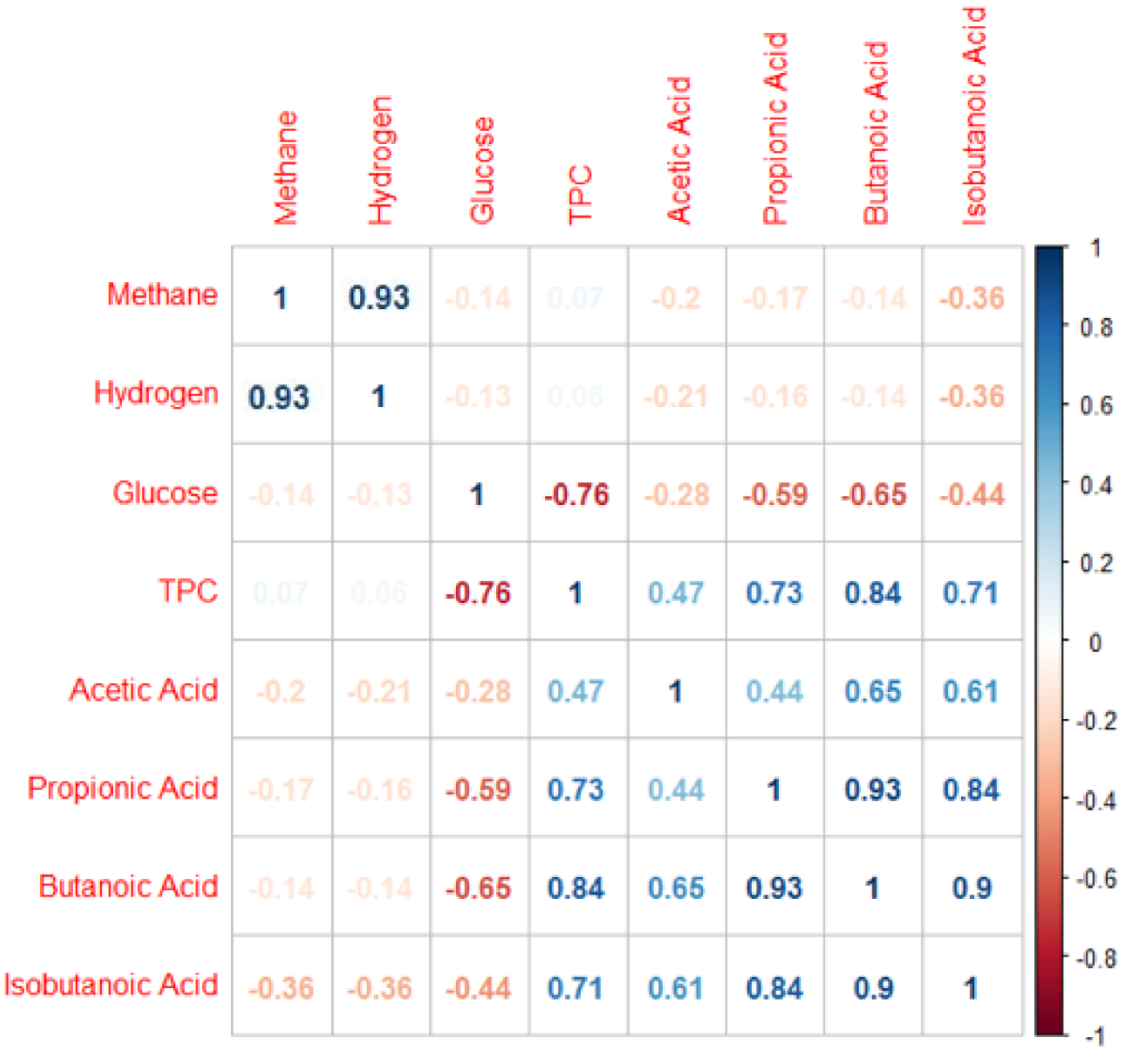

Fig. 5 Correlation matrix for the formation of hydrogen and methane, but also for glucose, individual inhibitors and total phenolic compound (TPC) - mesophilic process.

Fig. 6 Correlation matrix for the formation of hydrogen and methane, but also for glucose, individual inhibitors and total phenolic compound (TPC) - thermophilic process.

Correlation analysis consists in examining whether two variables (expressed in numbers) are significantly related to each other. The calculated determination ratio varies from -1 to 1. A positive correlation appears when the increase in the value of one variable corresponds with the increase in the value of the second variable, while negative correlation occurs when the increase in the value of one variable corresponds with the decrease in the value of the second variable. A value of (0) means a total lack of correlation between the two factors (Zhu et al. 2017). Unexpectedly, it was found that the production of hydrogen and methane is negatively correlated with the concentration of glucose in the growth medium (see Fig.5) - the mesophilic process, with respect to the increase of the glucose concentration. However, there is a positive correlation between hydrogen and methane generation and the

concentration of glucose during the process carried out in thermophilic conditions. Thermophilic process course corresponds with the tendencies presented in the literature, for diversified biofuels generation (Wilkie et al. 2000; Eskicioglu et al. 2011; Cieślik et al. 2016; Łukajtis et al. 2018). The authors suppose, that these untypical results are related with the sudden changes in the broth composition, i.e a significant decrease in glucose concentration after 46 hours of the process, due to glucose supplementation. In the mesophilic process the decrease in glucose concentration is observed after 15 h of the process. In addition, a strong positive correlation is observed for methane and hydrogen generation, which indicates the simultaneous formation of both gases under mesophilic and thermophilic conditions. During the dark fermentation process organic compounds break down into small molecules which are substrates for the hydrogen generation by hydrogenogenes, which are also able to generate acetic acid. In the first stage of biogas production, hydrogen, methane, and TPC are produced. The formulated acetic acid is used by methanogenes to produce methane (Bateni et al. 2017). Therefore, for this stage of the process, it may be crucial to consider acetic acid as a second carbon source, besides glucose in order to provide conclusions regarding chemometrical analysis of the processes. Higher concentrations of acetic acid are obtained during mesophilic process and therefore, its effect on the mesophilic process (Fig.4a) course is noticeable. Preparing a procedure for carbon balance in the system may be a required step to be considered in further research. Methane and hydrogen productivity correlation during the thermophilic process is lower in comparison with the mesophilic process. The correlation matrices for the mesophilic and thermophilic process demonstrate a strong negative correlation of glucose concentration with TPC and VFA concentration during hydrogenogenesis. Decreasing glucose concentration and an increase in TPC concentration result in a decrease in biogas productivity.

Conclusions

The paper presents the use of chromatographic techniques and sensor matrices for the monitoring of hydrogen and methane production during the dark fermentation process carried out under mesophilic and thermophilic conditions (model conditions). In order to understand the changes occurring during the whole dark fermentation process, gas phase (methane, hydrogen) studies and fermentation broth (glucose, VFA, TPC) studies were carried out. In the first stage of dark fermentation, the production of hydrogen was mainly observed. The

second stage was initiated, which consisted of redirecting the process to methanisation. The results indicate significantly higher concentrations of hydrogen and methane during the dark fermentation process under mesophilic conditions than in the process under thermophilic conditions. The concentration of biogas (methane and hydrogen) is closely related to the content of glucose in the nutrient solution. In the mesophilic process, a significant decrease in glucose concentration was observed. Microorganisms in the first stage of the fermentation process, convert glucose to biogas, and after 17 hours to VFA, while after 20 h other fermentation inhibitors (TPC) was also created. Similarly, in the thermophilic process, initially, glucose is converted by bacteria into gases, in turn, both phenolic compounds and VFA are formed after 46 hours of the process. In both mesophilic and thermophilic processes, the decrease in the production efficiency of hydrogen and methane is associated with an increase in the concentration of fermentation inhibitors (VFA and TPC). Microorganisms cease to produce both hydrogen and methane after consumption of glucose.

Correlation of factors enabled also the selection of significant variables that should be controlled on-line during processes carried out in actual real conditions. The most important parameters – concentration of methane and hydrogen was monitored on-line during fermentation processes by sensor matrices. The results obtained from sensor matrices are comparable to those obtained with gas chromatography coupled with a TCD and FID. The results indicate suitability of sensors matrices for on-line routine analyses of hydrogen and methane during fermentation processes. Moreover, sensor matrices based analysis enables finding the point at which the hydrogen generating bacteria culture is terminated and the fermentation process tends to redirect to the anaerobic digestion and the production of methane. Hydrogen and methane production using one process allows a better use of the potential of bacteria contained in the activated sludge, and also significantly reduces the cost of biogas production compared to individual processes. In addition, the use of sensor matrices allows immediate correction of the fermentation broth composition, which allows to improve the efficiency of biogas production. The use of GC techniques in "off-line" or "in-line" mode results in a long delay in the results obtained, which prevents immediate action to correct the process or eliminate potential system failures.

In the case of biogas production, i.e. from landfills, the obtained biogas stream contains much more pollutants (i.e. hydrogen sulphide, ammonia, carbon dioxide, volatile organic compounds) which can affect the process of dark fermentation and the operation and correctness of results obtained from sensor matrices. Therefore, in the future, it is planned to

create sensor matrices in which additional temperature, humidity, and selective pollution sensors will be considered.

Author Contributions: Edyta Słupek, Karolina Kucharska conceived and designed the experiments. Edyta Słupek, Patrycja Makoś carried the experiments. Edyta Słupek, Patrycja Makoś, Karolina Kucharska and Jacek Gębicki wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

Azbar N, Dokgöz FT, Keskin T, et al (2009) Comparative Evaluation of Bio-Hydrogen Production From Cheese Whey Wastewater Under Thermophilic and Mesophilic Anaerobic Conditions. International Journal of Green Energy 6:192–200. doi: 10.1080/15435070902785027

Bateni H, Saraeian A, Able C (2017) A comprehensive review on biodiesel purification and upgrading. Biofuel Research Journal 4:668–690. doi: 10.18331/brj2017.4.3.5

Cheng H, Hiro Y, Hojo T, Li YY (2018) Upgrading methane fermentation of food waste by using a hollow fiber type anaerobic membrane bioreactor. Bioresource Technology 267:386–394. doi: 10.1016/j.biortech.2018.07.045

Cheng J, Xie B, Zhou J, et al (2010) Cogeneration of H2 and CH4 from water hyacinth by two-step anaerobic fermentation. International Journal of Hydrogen Energy. doi: 10.1016/j.ijhydene.2009.07.012

Chu CFC-F, Xu K-QKQK-Q, Li YYY-Y, Inamori Y (2012) Hydrogen and methane potential based on the nature of food waste materials in a two-stage thermophilic fermentation process. International Journal of Hydrogen Energy 37:10611–10618. doi: 10.1016/j.ijhydene.2012.04.048

Cieślik M, Dach J, Lewicki A, et al (2016) Methane fermentation of the maize straw silage under meso- and thermophilic conditions. Energy 115:1495–1502. doi: 10.1016/j.energy.2016.06.070

De Gioannis G, Muntoni A, Polettini A, Pomi R (2013) A review of dark fermentative hydrogen production from biodegradable municipal waste fractions. Waste Management 33:1345–1361. doi: 10.1016/j.wasman.2013.02.019

Ding L, Cheng J, Lu H, et al (2017) Three-stage gaseous biofuel production combining dark hydrogen, photo hydrogen, and methane fermentation using wet Arthrospira platensis cultivated under high CO2 and sodium stress. Energy Conversion and Management 148:394–404. doi: 10.1016/j.enconman.2017.05.079

Eskicioglu C, Kennedy KJ, Marin J, Strehler B (2011) Anaerobic digestion of whole stillage from dry-grind corn ethanol plant under mesophilic and thermophilic conditions. Bioresource Technology 102:1079–1086. doi: 10.1016/j.biortech.2010.08.061

Faloye FD, Gueguim Kana EB, Schmidt S (2013) Optimization of hybrid inoculum development techniques for biohydrogen production and preliminary scale up. International Journal of Hydrogen Energy 38:11765–11773. doi: 10.1016/j.ijhydene.2013.06.129

Faloye FD, Gueguim Kana EB, Schmidt S (2014) Optimization of biohydrogen inoculum development via a hybrid pH and microwave treatment technique - Semi pilot scale production assessment. International Journal of Hydrogen Energy 39:5607–5616. doi: 10.1016/j.ijhydene.2014.01.163

Feng K, Li H, Deng Z, et al (2020) Effect of pre-fermentation types on the potential of methane production and energy recovery from food waste. Renewable Energy 146:1588–1595. doi: 10.1016/j.renene.2019.07.127

Fenske JJ, Griffin DA, Penner MH (1998) Comparison of aromatic monomers in lignocellulosic biomass prehydrolysates. Journal of Industrial Microbiology and Biotechnology 20:364–368. doi: 10.1038/sj.jim.2900543

Gebicki J (2016) Trends in Analytical Chemistry Application of electrochemical sensors and sensor matrixes for measurement of odorous chemical compounds. Trends in Analytical Chemistry 77:1–13. doi: 10.1016/j.trac.2015.10.005

Gebicki J, Dymerski T (2016) Application of Chemical Sensors and Sensor Matrixes to Air Quality Evaluation. Elsevier Ltd

Gomez X, Moran A, Cuetos MJ, et al (2006) The production of hydrogen by dark fermentation of municipal solid wastes and slaughterhouse waste: A two-phase process. Journal of Power Sources 157:727–732. doi: 10.1016/j.jpowsour.2006.01.006

Guo WQ, Ren NQ, Wang XJ, et al (2008) Biohydrogen production from ethanol-type fermentation of molasses in an expanded granular sludge bed (EGSB) reactor. International Journal of Hydrogen Energy. doi: 10.1016/j.ijhydene.2008.05.033

Hoff SJ, Bundy DS, Nelson MA, et al (2006) Emissions of ammonia, hydrogen sulfide, and odor before, during, and after slurry removal from a deep-pit swine finisher. Journal of

503 the Air and Waste Management Association 56:581–590. doi:
504 10.1080/10473289.2006.10464472
505 Isobe K, Koba K, Ueda S, et al (2011) A simple and rapid GC/MS method for the
506 simultaneous determination of gaseous metabolites. Journal of Microbiological Methods
507 84:46–51. doi: 10.1016/j.mimet.2010.10.009
508 Ivanova G, Rákhely G, Kovács KL (2009) Thermophilic biohydrogen production from energy
509 plants by Caldicellulosiruptor saccharolyticus and comparison with related studies.
510 International Journal of Hydrogen Energy 34:3659–3670. doi:
511 10.1016/j.ijhydene.2009.02.082
512 Jeppsson U, Pons M-N, Nopens I, et al (2007) Benchmark simulation model no 2: general
513 protocol and exploratory case studies. Water Science & Technology 56:67. doi:
514 10.2166/wst.2007.604
515 Kucharska K, Hołowacz I, Konopacka-Łyskawa D, et al (2018) Key issues in modeling and
516 optimization of lignocellulosic biomass fermentative conversion to gaseous biofuels.
517 Renewable Energy 129:384–408. doi: 10.1016/j.renene.2018.06.018
518 Kumar G, Sen B, Sivagurunathan P, Lin CY (2015) Comparative evaluation of hydrogen
519 fermentation of de-oiled Jatropha waste hydrolyzates. International Journal of Hydrogen
520 Energy 40:10766–10774. doi: 10.1016/j.ijhydene.2015.06.118
521 Lay JJ, Lee YJ, Noike T (1999) Feasibility of biological hydrogen production from organic
522 fraction of municipal solid waste. Water Research 33:2579–2586. doi: Doi
523 10.1016/S0043-1354(98)00483-7
524 Lee KS, Lo YC, Lin PJ, Chang JS (2006) Improving biohydrogen production in a carrier-
525 induced granular sludge bed by altering physical configuration and agitation pattern of
526 the bioreactor. International Journal of Hydrogen Energy 31:1648–1657. doi:
527 10.1016/j.ijhydene.2005.12.020
528 Lestinsky P, Grycova B, Pryszcz A, et al (2017) Hydrogen production from microwave
529 catalytic pyrolysis of spruce sawdust. Journal of Analytical and Applied Pyrolysis
530 124:175–179. doi: 10.1016/j.jaap.2017.02.008
531 Levin DB, Pitt L, Love M (2004) Biohydrogen production: Prospects and limitations to
532 practical application. International Journal of Hydrogen Energy 29:173–185. doi:
533 10.1016/S0360-3199(03)00094-6
534 Li C, Fang HHP (2007) Fermentative hydrogen production from wastewater and solid wastes
535 by mixed cultures. Critical Reviews in Environmental Science and Technology 37:1–39.
536 doi: 10.1080/10643380600729071

Łukajtis R, Rybarczyk P, Kucharska K, et al (2018) Optimization of saccharification conditions of lignocellulosic biomass under alkaline pre-treatment and enzymatic hydrolysis. Energies. doi: 10.3390/en11040886

Luo C, Brink DL, Blanch HW (2002) Identification of potential fermentation inhibitors in conversion of hybrid poplar hydrolyzate to ethanol. Biomass and Bioenergy 22:125–138. doi: 10.1016/S0961-9534(01)00061-7

Luo G, Xie L, Zou Z, et al (2010) Anaerobic treatment of cassava stillage for hydrogen and methane production in continuously stirred tank reactor (CSTR) under high organic loading rate (OLR). International Journal of Hydrogen Energy 35:11733–11737. doi: 10.1016/j.ijhydene.2010.08.033

Madsen M, Holm-Nielsen JB, Esbensen KH (2011) Monitoring of anaerobic digestion processes: A review perspective. Renewable and Sustainable Energy Reviews 15:3141–3155. doi: 10.1016/j.rser.2011.04.026

Manish S, Banerjee R (2008) Comparison of biohydrogen production processes. International Journal of Hydrogen Energy 33:279–286. doi: 10.1016/j.ijhydene.2007.07.026

Nilvebrant NO, Reimann A, Larsson S, Jönsson LJ (2001) Detoxification of lignocellulose hydrolysates with ion-exchange resins. Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology 91–93:35–49. doi: 10.1385/ABAB:91-93:1-9:35

Ottaviano LM, Ramos LR, Botta LS, et al (2017) Continuous thermophilic hydrogen production from cheese whey powder solution in an anaerobic fluidized bed reactor: Effect of hydraulic retention time and initial substrate concentration. International Journal of Hydrogen Energy. doi: 10.1016/j.ijhydene.2016.11.168

Pan C, Zhang S, Fan Y, Hou H (2010) Bioconversion of corncob to hydrogen using anaerobic mixed microflora. International Journal of Hydrogen Energy. doi: 10.1016/j.ijhydene.2009.04.023

Panagiotopoulos IA, Bakker RR, De Vrije T, et al (2010) Pretreatment of sweet sorghum bagasse for hydrogen production by Caldicellulosiruptor saccharolyticus. International Journal of Hydrogen Energy 35:7738–7747. doi: 10.1016/j.ijhydene.2010.05.075

Pandu K, Joseph S (2012) Comparisons and Limitations of Biohydrogen Production Processes: a Review. International Journal of Advances in Engineering & Technology 2:2231–1963

Per Persson †, Jessica Andersson †, Lo Gorton †, et al (2002) Effect of Different Forms of Alkali Treatment on Specific Fermentation Inhibitors and on the Fermentability of

571 Lignocellulose Hydrolysates for Production of Fuel Ethanol. Journal of Agricultural and
572 Food Chemistry 50:5318–5325. doi: 10.1021/jf025565o
573 Ponzoni A, Baratto C, Cattabiani N, et al (2017) Smetal oxide gas sensors, a survey of
574 selectivity issues addressed at the SENSOR lab, Brescia (Italy). Sensors (Switzerland)
575 17:. doi: 10.3390/s17040714
576 Quéméneur M, Hamelin J, Barakat A, et al (2012) Inhibition of fermentative hydrogen
577 production by lignocellulose-derived compounds in mixed cultures. International Journal
578 of Hydrogen Energy 37:3150–3159. doi: 10.1016/j.ijhydene.2011.11.033
579 Qureshi N, Saha BC, Dien B, et al (2010a) Production of butanol (a biofuel) from agricultural
580 residues: Part I - Use of barley straw hydrolysate. Biomass and Bioenergy 34:566–71.
581 doi: 10.1016/j.biombioe.2009.12.024
582 Qureshi N, Saha BC, Hector RE, et al (2010b) Production of butanol (a biofuel) from
583 agricultural residues: Part II - Use of corn stover and switchgrass hydrolysates. Biomass
584 and Bioenergy 32:176–83. doi: 10.1016/j.biombioe.2009.12.023
585 RCore (2018) R Core, Team, https://www.r-project.org/
586 Rosales-Colunga LM, González-García R, De León Rodríguez A (2010) Estimation of
587 hydrogen production in genetically modified E. coli fermentations using an artificial
588 neural network. International Journal of Hydrogen Energy 35:13186–13192. doi:
589 10.1016/j.ijhydene.2010.08.137
590 Rosecrance JC, Paulsen R, Gilkey D, et al (2013) Control of mixing step in the bread
591 production with weak wheat flour and sourdough. Journal of Agricultural … XLIV:10–
592 13. doi: 10.4081/jae.2013.(s1)
593 RStudio (2016) RStudio Team, Version: 3.5.2, http://www.rstudio.com/
594 Słupek E, Makoś P, Kamiński M (2018) CAMERA SEPARATORIA Volume 10 , Number 2
595 / 2018 , pp . 52-63 Metodyka oznaczania sumarycznej zawartości inhibitorów
596 fermentacji ciemnej oraz monocukrów w brzeczkach fermentacyjnych techniką HP LC-
597 RID-UV-VIS / DAD Methodology for determining the total conte. 10:52–63
598 Szulczyński B, Kucharska K, Kamiński M (2019) Laboratory bioreactor with pH control
599 system for investigations of hydrogen production in the dark fermentation process.
600 Aparatura Badawcza i Dydaktyczna 39–46
601 Szulczyński B, Wasilewski T, Wojnowski W, et al (2017) Different ways to apply a
602 measurement instrument of E-nose type to evaluate ambient air quality with respect to
603 odour nuisance in a vicinity of municipal processing plants. Sensors (Switzerland) 17:.
604 doi: 10.3390/s17112671

605 Tan L, Nishimura H, Wang YF, et al (2019) Effect of organic loading rate on thermophilic
606 methane fermentation of stillage eluted from ethanol fermentation of waste paper and
607 kitchen waste. Journal of Bioscience and Bioengineering 127:582–588. doi:
608 10.1016/j.jbiosc.2018.10.006
609 Teplyakov V V., Gassanova LG, Sostina EG, et al (2002) Lab-scale bioreactor integrated with
610 active membrane system for hydrogen production: Experience and prospects.
611 International Journal of Hydrogen Energy 27:1149–1155. doi: 10.1016/S0360-
612 3199(02)00093-9
613 Veluchamy C, Kalamdhad AS (2017) Enhanced methane production and its kinetics model of
614 thermally pretreated lignocellulose waste material. Bioresour Technol 241:1–9. doi:
615 10.1016/j.biortech.2017.05.068
616 Wang B, Wan W, Wang J (2009) Effect of ammonia concentration on fermentative hydrogen
617 production by mixed cultures. Bioresource Technology 100:1211–1213. doi:
618 10.1016/j.biortech.2008.08.018
619 Wilkie AC, Riedesel KJ, Owens JM (2000) Stillage characterization and anaerobic treatment
620 of ethanol stillage from conventional and cellulosic feedstocks. Biomass and Bioenergy
621 19:63–102. doi: 10.1016/S0961-9534(00)00017-9
622 Wong YM, Wu TY, Juan JC (2014) A review of sustainable hydrogen production using seed
623 sludge via dark fermentation. Renewable and Sustainable Energy Reviews 34:471–482.
624 doi: 10.1016/j.rser.2014.03.008
625 Wu KJ, Chang CF, Chang JS (2007) Simultaneous production of biohydrogen and bioethanol
626 with fluidized-bed and packed-bed bioreactors containing immobilized anaerobic sludge.
627 Process Biochemistry 42:1165–1171. doi: 10.1016/j.procbio.2007.05.012
628 Wu KJ, Chang JS (2007) Batch and continuous fermentative production of hydrogen with
629 anaerobic sludge entrapped in a composite polymeric matrix. Process Biochemistry
630 42:279–284. doi: 10.1016/j.procbio.2006.07.021
631 Wu X, Zhu J, Dong C, et al (2009) Continuous biohydrogen production from liquid swine
632 manure supplemented with glucose using an anaerobic sequencing batch reactor.
633 International Journal of Hydrogen Energy 34:6636–6645. doi:
634 10.1016/j.ijhydene.2009.06.058
635 Yang H, Shao P, Lu T, et al (2006) Continuous bio-hydrogen production from citric acid
636 wastewater via facultative anaerobic bacteria. International Journal of Hydrogen Energy
637 31:1306–1313. doi: 10.1016/j.ijhydene.2005.11.018
638 Yasin NHM, Mumtaz T, Hassan MA, Abd Rahman N (2013) Food waste and food processing

639 waste for biohydrogen production: A review. Journal of Environmental Management
640 130:375–385. doi: 10.1016/j.jenvman.2013.09.009
641 Zhu G-F, Wu P, Wei Q-S, et al (2010) Biohydrogen production from purified terephthalic
642 acid (PTA) processing wastewater by anaerobic fermentation using mixed microbial
643 communities. International Journal of Hydrogen Energy 35:8350–8356. doi:
644 10.1016/j.ijhydene.2009.12.003
645 Zhu Q, Liu Q, Qin SJ (2017) Concurrent Monitoring and Diagnosis of Process and Quality
646 Faults with Canonical Correlation Analysis. IFAC-PapersOnLine 50:7999–8004. doi:
647 10.1016/j.ifacol.2017.08.1222
648
649
650

Electronic Supplementary Material

Mesophilic and thermophilic dark fermentation course analysis using sensor matrices and chromatographic techniques

Edyta Słupek[*1]. Patrycja Makoś[1]. Karolina Kucharska[1]. Jacek Gębicki[1]

[1] *Gdańsk University of Technology. Faculty of Chemistry. Department of Process Engineering and Chemical Technology. 80-233 Gdańsk. Narutowicza 11/12 street. Poland*

[*]Corresponding author. e-mail: edyta.slupek@pg.edu.pl

Table S1 Analysis of variance (ANOVA) for the methane in thermophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	2.109149	1	2.109149	0.16484	0.686707	4.061706
Within groups	562.9854	44	12.79512			
Total	565.0945	45				

Table S2 Analysis of variance (ANOVA) for the hydrogen in thermophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	2390.174	1	2390.174	0.108959	0.742899	4.061706
Within groups	965205.1	44	21936.48			
Total	967595.3	45				

Table S3 Analysis of variance (ANOVA) for the methane in mesophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	4.28611	1	4.28611	5.97E-05	0.99387	4.061706
Within groups	3158609	44	71786.58			
Total	3158614	45				

Table S4 Analysis of variance (ANOVA) for the hydrogen in mesophilic process.

Source of variation	SS	df	MS	F	p-value	F-critical
Between groups	154985.6	1	154985.6	0.001415	0.970167	4.061706
Within groups	4.82E+09	44	1.1E+08			
Total	4.82E+09	45				

Table S5 Changes in the glucose. total phenolic compounds (TPC) and selected volatile fatty acids (VFA) concentration in fermentation broth during dark fermentation - mesophilic process

Time	Glucose	TPC	Acetic Acid	Propionic Acid	Butanoic Acid	Isobutanoic Acid
[h]				[mg/mL]		
2	5.50	<LOD	<LOD	<LOD	<LOD	<LOD
15	3.21	<LOD	<LOD	<LOD	<LOD	<LOD
17	2.56	<LOD	<LOD	0.020	0.034	<LOD
20	2.05	1.582	<LOD	0.020	0.031	<LOD
24	1.80	3.991	<LOD	0.022	0.031	<LOD
26	1.44	4.160	0.011	0.022	0.147	<LOD
40	1.21	4.350	0.012	0.123	0.222	<LOD
46	0.55	4.888	0.015	0.228	0.525	<LOD
48	0.05	4.932	0.015	0.228	0.529	1.845
70	3.00	5.136	0.095	0.358	0.598	1.948
76	2.12	5.340	0.150	0.390	0.658	1.955
80	1.56	5.545	0.240	0.399	0.758	2.020
86	0.68	5.786	0.550	0.490	0.950	2.029
94	0.55	5.958	0.650	0.555	0.999	2.255
111	<LOD	6.145	0.071	0.898	1.001	2.268
115	<LOD	6.15	0.074	0.969	1.041	2.345

LOD – limit of detection;

LOD = 0.01 mg/mL. RSD = 2.15% - values calculated for a concentration of 2.5 mg/mL (glucose). LOD = 0.073 mg/mL. RSD = 1.23% - values calculated for a concentration of 3.6 mg/mL (TPC). LOD = 0.001 – 0.003 mg/mL. RSD = 2.13% - values calculated for a concentration of 5 μg/mL (organic acids)

Table S6 Changes in the glucose. total phenolic compounds (TPC) and selected volatile fatty acids (VFA) concentration in fermentation broth during dark fermentation- thermophilic process

Time	Glucose	TPC	Acetic Acid	Propionic Acid	Butanoic Acid	Isobutanoic Acid
[h]				[mg/mL]		
2	5.50	<LOD	<LOD	<LOD	<LOD	<LOD
15	5.21	<LOD	<LOD	<LOD	<LOD	<LOD
17	4.97	<LOD	<LOD	<LOD	<LOD	<LOD
20	4.80	<LOD	<LOD	<LOD	<LOD	<LOD
24	4.61	<LOD	<LOD	<LOD	<LOD	<LOD
26	4.55	<LOD	<LOD	<LOD	<LOD	<LOD
40	4.51	<LOD	<LOD	<LOD	<LOD	<LOD
46	4.45	1.021	<LOD	0.020	0.030	<LOD
48	2.50	1.044	<LOD	0.020	0.049	<LOD
70	3.00	1.245	<LOD	0.289	0.339	0.154
76	2.21	1.355	<LOD	0.350	0.340	0.495
80	1.62	1.579	<LOD	0.359	0.339	0.513
86	1.55	1.714	0.010	0.450	0.349	0.526
94	1.41	1.849	0.020	0.468	0.398	0.759
111	1.22	1.912	0.021	0.555	0.400	0.815
115	1.20	1.985	0.030	0.581	0.434	0.828

LOD – limit of detection;

LOD = 0.01 mg/mL. RSD = 2.15% - values calculated for a concentration of 2.5 mg/mL (glucose). LOD = 0.073 mg/mL. RSD = 1.23% - values calculated for a concentration of 3.6 mg/mL (TPC). LOD = 0.001 – 0.003 mg/mL. RSD = 2.13% - values calculated for a concentration of 5 μg/mL (organic acids)

Table S7 Changes in the glucose. total phenolic compounds (TPC) and selected volatile fatty acids (VFA) concentration in fermentation broth during dark fermentation A) mesophilic process. B) thermophilic process.

Time	Mesophilic Process				Thermophilic Process			
	GC-H_2	MS-H_2	GC-CH_4	MS-CH_4	GC-H_2	MS-H_2	GC-CH_4	MS-CH_4
[h]	[mg /L]*							
2	0.043	0.042	0.061	0.054	0.030	0.029	0.052	0.050
15	0.043	0.042	0.061	0.062	0.036	0.032	0.053	0.051
17	0.038	0.040	0.060	0.064	0.035	0.033	0.057	0.056
20	0.039	0.043	0.060	0.068	0.035	0.033	0.058	0.056
21	0.038	0.043	0.060	0.070	0.035	0.031	0.053	0.056
22	0.038	0.044	0.060	0.070	0.037	0.034	0.052	0.050
23	0.043	0.044	0.061	0.071	0.037	0.040	0.052	0.050
24	0.124	0.061	0.077	0.105	0.045	0.041	0.052	0.052
25	0.225	0.241	0.096	0.108	0.037	0.038	0.052	0.053
39	0.231	0.241	0.112	0.122	0.034	0.031	0.052	0.052
40	1.649	1.743	0.372	0.341	0.035	0.030	0.052	0.053
43	3.327	3.074	0.697	0.661	0.032	0.033	0.052	0.057
44	3.126	3.072	0.658	0.646	0.031	0.030	0.052	0.056
45	0.350	0.308	0.478	0.440	0.031	0.030	0.052	0.052
46	0.050	0.069	0.062	0.071	0.034	0.031	0.052	0.052
48	0.023	0.035	0.057	0.064	0.033	0.029	0.052	0.052
70	0.025	0.023	0.058	0.064	0.029	0.030	0.052	0.057
76	0.013	0.015	0.055	0.064	0.027	0.027	0.052	0.057
80	0.006	0.008	0.059	0.064	0.028	0.028	0.052	0.056
86	0.006	0.008	0.059	0.064	0.000	0.000	0.053	0.056
94	0.000	0.000	0.059	0.057	0.000	0.000	0.053	0.047
111	0.000	0.000	0.051	0.054	0.000	0.000	0.053	0.048
115	0.000	0.000	0.051	0.051	0.000	0.000	0.051	0.048

LOD – limit of detection; RSD = 2.82% and LOD = values calculated for a concentration of 1000 mg/mL CH_4 for MS; RSD = 3.54% and LOD = 0.001 mg/L values calculated for a concentration of 1000 mg/mL H_2 for MS, RSD = 1.59% and LOD = 0.002 mg/L values calculated for a concentration of 1000 mg/mL CH_4 for GC; RSD = 1.81% and LOD = 0.001 mg/L values calculated for a concentration of 1000 mg/mL H_2 for GC

*mg – (H_2 or CH_4) / L (total gas phase)