Chemical and Process Engineering: New Frontiers, 2024, 44(2), e63
DOI: 10.24425/cpe.2024.149458

# The influence of inoculum source and pretreatment on biohydrogen production in the dark fermentation process

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Presented at
14th Polish Scientific Conference "Advances in Bioreactor Engineering", 25–27 September 2023, Konopnica, Poland.

**Article info:**
Received: 15 September 2023
Revised: 22 December 2023
Accepted: 24 January 2024

**Abstract**

The production of biohydrogen from food waste (FW) by dark fermentation (DF) is a promising technology for commercialisation, as it is both a clean fuel and a suitable means of sustainable waste management. The described experiments compared the biohydrogen production yields obtained after the use of inoculum from two different sources: digested sludge from the wastewater treatment plant (WWTP) in Lodz and sludge from the anaerobic treatment of dairy industry wastewater (DIW) (unconcentrated and double-concentrated). In addition, the effect of different temperatures (70, 90 and 121 °C) of inoculum pretreatment on the biohydrogen production in DF was tested. The process was carried out batchwise at 37 °C. The highest yield of hydrogen production was obtained after the inoculum pretreatment at 70 °C. In addition, a higher amount of hydrogen could be obtained by using sludge from the WWTP as the inoculum (96 cm$^3$ $H_2/g_{TVSFW}$) than unthickened sludge from the DIW (85 cm$^3$ $H_2/g_{TVSFW}$). However, after thickening the sludge from the dairy industry, and at the same time balancing the dry matter of both sludges, the hydrogen production potential was comparable for both sludges (for the WWTP sludge – 96 and for the DIW sludge – 93 cm$^3$ $H_2/g_{TVSFW}$). The kinetics of hydrogen production was described by modified Gompertz equation, which showed a good fit (determination coefficient $R^2$ between 0.909 and 0.999) to the experimental data.

**Keywords**

dark fermentation, hydrogen, inoculum, pretreatment, food waste

## 1. INTRODUCTION

Waste generation is an inevitable aspect of the human life. One of the biggest waste streams are the kitchen waste. According to the EUROSTAT data in 2020 each Europe Union citizen generated 70 kg of the kitchen waste (EUROSTAT, 2021). Taking into account also primary production, manufacture of food products and beverages, restaurant and food services and distribution of food the total amount of food waste per one person grows up to 127 kg. It can be estimated that in 2021 the inhabitants of the European Union produced almost 57 million tons of food waste of which more than 31 million tons was kitchen waste (in 2021 the European Union had 447 million inhabitants, (EUROSTAT, 2023)).

Nowadays, the waste management priority is to meet standards of circular economy. The most favorable method of final management of organic waste is its use as fertilizer. However, the fertilizer must comply with the strict criteria of Regulation (EU) 2019/1009 of the European Parliament and of the Council of June 5, 2019. laying down rules on the making EU fertilising products available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. In organic waste, the carbon/nitrogen ratio is so high that it is possible to simultaneously use part of the organic carbon to produce biofuel and increase the nitrogen content for fertilization purposes. The most commonly used method of recovering energy from waste is methane fermentation (Tamasiga et al., 2022). However, there are many attempts to explore methods for greater energy recovery. Currently, biohydrogen production is gaining more and more interest (Ubando et al., 2022).

Biohydrogen can be derived from the organic waste by various methods – biological dark fermentation, thermochemical gasification and the use of microbial electrolysis cells (Tian et al., 2019). Among them hydrogen production through the dark fermentation (DF) appears to be the most favorable, as it is pollution free, has the ability to use variety of organic substrates, ensures high production rate in comparison to other biological methods (such as photo-fermentation or biophotolysis) and requires less energy than gasification (Srivastava et al., 2021). It should be also emphasized that the production of biohydrogen from kitchen/food waste is not only the method for the waste management but also means to produce energy from a renewable source (Tian et al., 2019).

Although the investigations on the DF have been conducted for decades there is still a need for the further process optimization. One of the important aspects is a type of the microbial community that is responsible for the hydrogen production. The process can be carried out by either pure culture or mixed culture (MC). The use of MC as inoculum has a primary advantage because the bioreactor can operate under non-sterile conditions. MC is most often derived from an anaerobic digester (Bhurat et al., 2021; Massanet-Nicolau et al., 2008; Rafieenia et al., 2018b). As a result it contains not only hydrogen producing strains but also hydrogen consumers (such as methanogenic archaea) and lactic acid bacteria that compete for the substrates with hydrogen producers (Rafieenia et al., 2018a). Hydrogenotrophic methanogens (Zhu and Béland, 2006) and lactic acid bacteria (Noike et al., 2002) can be inhibited by heat pretreatment, but too high temperature and treatment time can also destroy the spore forming hydrogen producers (Rafieenia et al., 2018a). In the literature various pretreatment temperatures may be found – between 70 and 110 °C (Bhurat et al., 2021; Luo et al., 2022; Massanet-Nicolau et al., 2008; Slezak et al., 2017) but optimisation of inoculum pretreatment temperatures for studies on complex substrates is lacking. Such studies were conducted most often on glucose as a substrate (Bundhoo et al., 2015). An additional aspect of the inoculum, which has not been investigated previously, is a source of anaerobic sludge. Although sludge of different origins was used (Bhurat et al., 2021; de Sá et al., 2013; Massanet-Nicolau et al., 2008; Slezak et al., 2017), there was no research on the influence of the different inoculum source on biohydrogen yield.

Therefore, the aim of the study was to investigate the effect of different inoculum pretreatment temperatures, as well as different inoculum sources, on biohydrogen production in the DF process.

## 2. METHODOLOGY

### 2.1. Inoculum and substrate

The experiments described here compare the efficiency of biohydrogen production obtained after the application of inoculum from two different sources: digested sludge from the Łódź wastewater treatment plant (WWTP) and sludge from the anaerobic treatment of wastewater from the dairy industry (DIW). The WWTP treats municipal wastewater from the city of Łódź and neighboring cities and municipalities: Konstantynów Łódzki, Ksawerów, Pabianice, Nowosolna. The designed capacity of WWTP Łódź is 1 026 260 PE (population equivalent), while the actual load is 878 692 PE. The treatment plant operates using the activated sludge method in a system similar to MUCT with chemical support for the removal of phosphorus compounds. The primary and surplus activated sludges are digested under mesophilic conditions (Zakład Wodociągów i Kanalizacji w Łodzi, 2022).

As a substrate selectively collected and then shredded food waste from households consisting mainly of vegetable waste was used. There was no waste of animal origin among them. The waste was ground to solid particles less than 3 mm and stored at minus 20 °C. Tables 1–3 show the basic properties of food waste and inocula.

Table 1. Characteristics of substrate (food waste) used in the experiments.

| | Food waste |
|---|---|
| pH (–) | $4.83 \pm 0.09$ |
| TS ($g/g_{pre}$) | $0.16 \pm 0.01$ |
| VS ($g/g_{FW}$) | $0.15 \pm 0.005$ |
| C (%TS) | $45.48 \pm 0.19$ |
| N (%TS) | $1.83 \pm 0.01$ |
| H (%TS) | $6.13 \pm 0.06$ |
| C/N | $24.85 \pm 0.13$ |

### 2.2. Experimental set-up and procedure

The dark fermentation process was carried out in 1 dm$^3$ glass bottles in a thermostatic shaker (New Brunswick Scientific, EXCELLA E24R, St Albans, UK) in batch mode. The process temperature was 37 °C, the shaking speed 140 rpm. The reaction mixtures consisted of anaerobic sludge (500 cm$^3$), food waste (110 g wet weight) and water added to 1 dm$^3$.

Biogas production was measured using the displacement method. The bottle containing the reaction mixture was connected, by gas-tight tubes, to a graduated (100 cm$^3$ accuracy) 1 dm$^3$ Duran glass bottle filled with brine, then to an ungraded glass bottle.

The pH, total solids (TS), total volatile solids (VS) were measured for the mixture at the beginning and at the end of the dark fermentation process. The sample was then centrifuged at 10 000 rpm for 10 min and volatile fatty acids (VFA), ammonium nitrogen (AN) as well as carbon and total nitrogen content (TOC and TN) of the liquid were measured. An elementary analysis of solids (C, H, N, S content) was also performed.

The inoculum pretreatment process, both for the sludge from the WWTP and the DIW sludge, was carried out at temperatures of 70, 90 and 121 °C in a steam sterilizer – SMS ASL 60 Mc. As the VS of the DIW sludge were half that of the WWTP sludge one experiment with the thickened DIW sludge was also tested – the sludge pretreated at 70 °C was thickened twice by centrifuging and the supernatant liquid poured off. The experiments were carried out in duplicate.

### 2.3. Analytical Methods

TS, VS and pH were determined according to Standard Methods (Eaton, 2005). C, H, N, S content was measured by elemental analyzer NA 2500 (CE Instruments, The Old Barn, UK). The composition of the fermentation gas was determined by SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA, USA) equipped with TCD detector and two chromatographic columns: 5A molecular sieve mesh 80/100

Table 2. Characteristics of the sludge from WWTP in Lodz used in the experiments.

| | WWTP in Lodz | | | |
|---|---|---|---|---|
| | without thermal pretreatment | 70 °C | 90 °C | 121 °C |
| pH (–) | $7.39 \pm 0.03$ | $7.52 \pm 0.05$ | $7.87 \pm 0.01$ | $9.01 \pm 0.08$ |
| TS (g/dm$^3$) | $28.81 \pm 0.64$ | $34.34 \pm 0.08$ | $31.99 \pm 0.32$ | $37.02 \pm 1.66$ |
| VS (g/dm$^3$) | $18.31 \pm 0.50$ | $21.96 \pm 0.00$ | $19.70 \pm 0.27$ | $22.32 \pm 0.23$ |
| C (%TS) | $34.52 \pm 0.08$ | $34.87 \pm 0.03$ | $33.16 \pm 0.08$ | $24.93 \pm 0.11$ |
| N (%TS) | $5.17 \pm 0.00$ | $5.03 \pm 0.00$ | $4.83 \pm 0.01$ | $5.14 \pm 0.01$ |
| H (%TS) | $4.81 \pm 0.06$ | $4.71 \pm 0.00$ | $4.54 \pm 0.05$ | $4.10 \pm 0.02$ |
| C/N | $6.68 \pm 0.01$ | $6.93 \pm 0.01$ | $6.87 \pm 0.03$ | $4.85 \pm 0.02$ |

Table 3. Characteristics of the sludge from DIW used in the experiments.

| | DIW | | | | |
|---|---|---|---|---|---|
| | without thermal pretreatment | 70 °C | 70 °C thickened | 90 °C | 121 °C |
| pH (–) | $7.00 \pm 0.02$ | $6.90 \pm 0.08$ | $7.60 \pm 0.03$ | $7.15 \pm 0.07$ | $9.12 \pm 0.06$ |
| TS (g/dm$^3$) | $14.57 \pm 0.18$ | $10.51 \pm 0.06$ | $25.15 \pm 2.08$ | $19.42 \pm 0.62$ | $7.52 \pm 0.01$ |
| VS (g/dm$^3$) | $11.13 \pm 0.16$ | $7.86 \pm 0.15$ | $19.35 \pm 1.72$ | $14.88 \pm 0.48$ | $5.42 \pm 0.23$ |
| C (%TS) | $40.27 \pm 0.06$ | $39.96 \pm 0.00$ | $39.76 \pm 0.14$ | $39.57 \pm 0.00$ | $36.51 \pm 0.02$ |
| N (%TS) | $7.64 \pm 0.00$ | $7.37 \pm 0.01$ | $7.27 \pm 0.02$ | $7.05 \pm 0.00$ | $6.36 \pm 0.03$ |
| H (%TS) | $5.68 \pm 0.01$ | $5.64 \pm 0.04$ | $5.68 \pm 0.07$ | $5.62 \pm 0.13$ | $5.12 \pm 0.01$ |
| C/N | $5.27 \pm 0.01$ | $5.42 \pm 0.01$ | $5.47 \pm 0.01$ | $5.61 \pm 0.00$ | $5.74 \pm 0.01$ |

and a column packed with Silica Gel No. 8 (1 m length each). The temperature of the column oven and detector was equal to 60 and 150 °C, respectively. The content of VFA and AN were determined using steam distillation method (Buchi Distillation Unit B-324) followed by titration. The AN measurement was carried out in accordance with PN-ISO 5664:2002, VFA measurement according to PN-C-04616-04:1975. TOC and TN were determined by the TOC-TN analyzer – IL550 by Lachat Instruments. The TOC measurement was carried out in accordance with DIN EN 1484 and ISO 8245, TN measurement according to DIN-EN 12260. The analyses were performed in two repetitions.

The Gompertz equation modified by Zwietering and co-workers (Tjørve and Tjørve, 2017) was adopted to fit the hydrogen production curve:

$$H = H_P \cdot \exp\left\{-\exp\left[\frac{e \cdot R_M}{H_P} \cdot (\lambda - t) + 1\right]\right\} \qquad (1)$$

where $H$ is the cumulative hydrogen production (cm$^3$ $H_2$/$g_{TVSFW}$) at a given time $t$, $H_P$ the hydrogen production potential (cm$^3$ $H_2$/$g_{TVSFW}$), $R_M$ the maximum hydrogen production rate (cm$^3$ $H_2$/(h $g_{TVSFW}$)), $\lambda$ the lag phase (h) and $e = 2.7(1828)$. The Origin 2017 was used to fit experimental data with Equation (1).

## 3. RESULTS AND DISCUSSION

### 3.1. Changes of pH

As can be seen in Figure 1 the pH values in all reactors were within the neutral pH range (between 6 and 8). Only the mixture with the WWTP sludge pretreated at 121 °C slightly exceeded this range reaching a value of 8.2. Furthermore, the pH values were higher in reactors with the WWTP sludge, than those with the DIW sludge (Figure 1).

At the end of the process, after 52 h, the pH values in all reactors were in the acidic pH range (about 4–5, Figure 1). Similarly to the beginning of the process, mixtures in reactors with the WWTP sludge had higher pH values than those with DIW sludge. After examining the AN content in the supernatant samples, it was found to be an order of magnitude

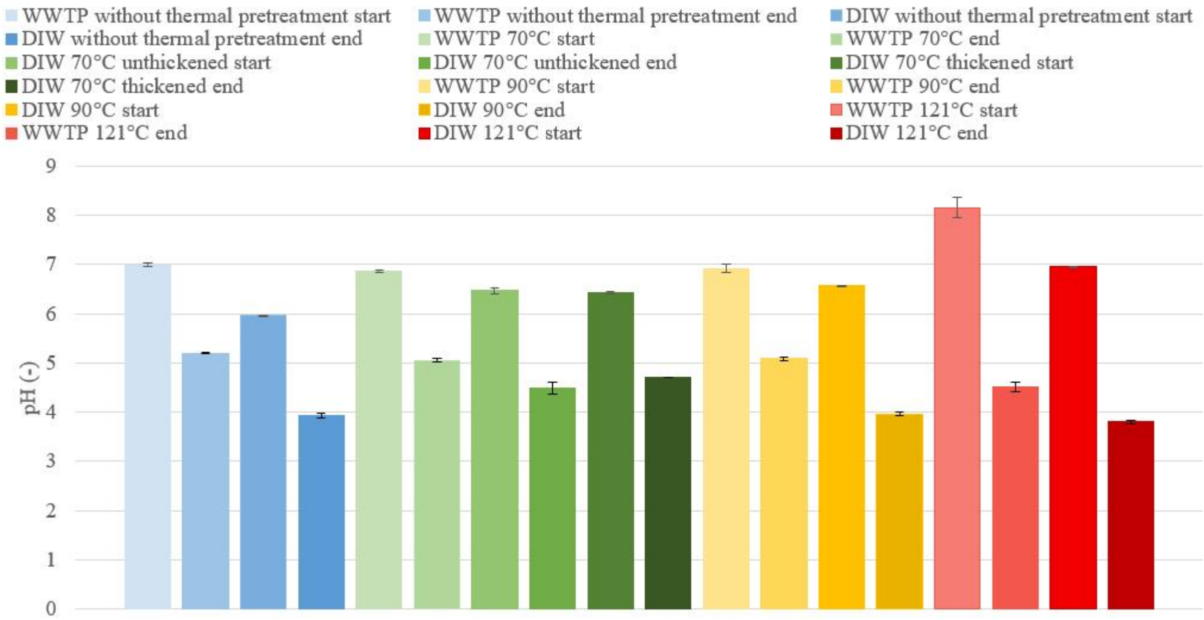


Figure 1. pH at the beginning and the end of processes (after 52 h).

less for the DIW sludge (40–70 $mg_{N-NH4+}/dm^3$) than for WWTP sludge (380–580 $mg_{N-NH4+}/dm^3$). The AN in the sludge results in a higher buffering capacity of the solution. As it is less of it in the DIW sludge, the pH drops to lower values at the end of the process than in the WWTP sludge. It is also possible that the pH drops faster, resulting in less fermentation gas production when using DIW sludge.

In all series, the initial pH was in the range of 6–8, and the final pH was in the range of 4–5. Similar results were obtained by (Arain et al., 2018). With an initial pH of 7, their study resulted in pH between 3.8 and 6.5 at the end of the process.

The mentioned drop in pH values during the process is related to the production of VFA, which acidifies the reaction mixture. The pH dropped to a value at which hydrogen and VFA production can be inhibited. Low pH (below 5) limits the bacteria's ability to maintain intracellular pH at an appropriate level (Łukajtis et al., 2018).

The initial heat treatment of the inoculum at 70 °C and 90 °C did not have a significant effect on the pH values either at the beginning or at the end of the dark fermentation process. However, after initial thermal treatment of the inoculum at the temperature of 121 °C, there was a significant increase in pH at the beginning of the process, and at the end of the process, the pH value was lower than in the other series. The amount of AN in the liquid from WWTP sludge decreased after thermal pretreatment – the higher pretreatment temperature the lower AN concentration (untreated 580 $mg_{N-NH4+}/dm^3$, heated at 70 °C 541 $mg_{N-NH4+}/dm^3$, at 90 °C 442 $mg_{N-NH4+}/dm^3$, at 121 °C 382 $mg_{N-NH4+}/dm^3$). The smallest amount of AN resulted in a lower final pH than the other mixtures (Figure 2), despite the highest initial pH in the mixture with the WWTP sludge heated at 121 °C (Figure 1).

## 3.2. Changes of volatile fatty acids (VFA)

Figure 2 shows the amount of VFA at the beginning of the process. In the reactors with the inoculum without thermal treatment, the amount of VFA in reaction mixtures was almost the same for both sludges and amounted to 400 $mg_{acetate}/dm^3$. Heat treatment of the WWTP sludge at temperatures of 70 and 90 °C increased the amount of VFA to over 600 $mg_{acetate}/dm^3$ and about 550 $mg_{acetate}/dm^3$, respectively. On the contrary, the usage of 121 °C resulted in a VFA decrease to about 360 $mg_{acetate}/dm^3$. Thermal pretreatment of unthickened DIW sludge by 70 °C did not affect the amount of VFA. Thermal treatment at higher temperatures (90 and 121 °C) of unthickened, as well as thickened DIW sludge at 70 °C, resulted in a decrease in the amount of VFA to approximately 350, 180 and 320 $mg_{acetate}/dm^3$, respectively.

In all cases, the values of VFA at the end of the process (after 52 h) are higher than at the beginning of the process which proves that acidogenesis took place in all reactors. In the tests with WWTP sludge, higher amounts of VFA were produced than in the samples with DIW sludge (Figure 2).

In the case of the WWTP sludge, the thermal pretreatment at temperatures of 70 and 90 °C had no significant effect on the amount of VFA produced during the subsequent DF process. In the control and in the 70 and 90 °C trials, the amount of VFA was in the range of 4500–5000 $mg_{acetate}/dm^3$. Pretreatment in 121 °C led to severe decrease of VFA production in comparison to other variants – only 1200 $mg_{CH3COOH}/dm^3$ at the end of the process.

In the case of DIW sludge, the amount of VFA produced in the control sample without sludge heating was 1200 $mg_{acetate}/dm^3$ – significantly lower than that obtained for the WWTP sludge. Although the heat treatment of the

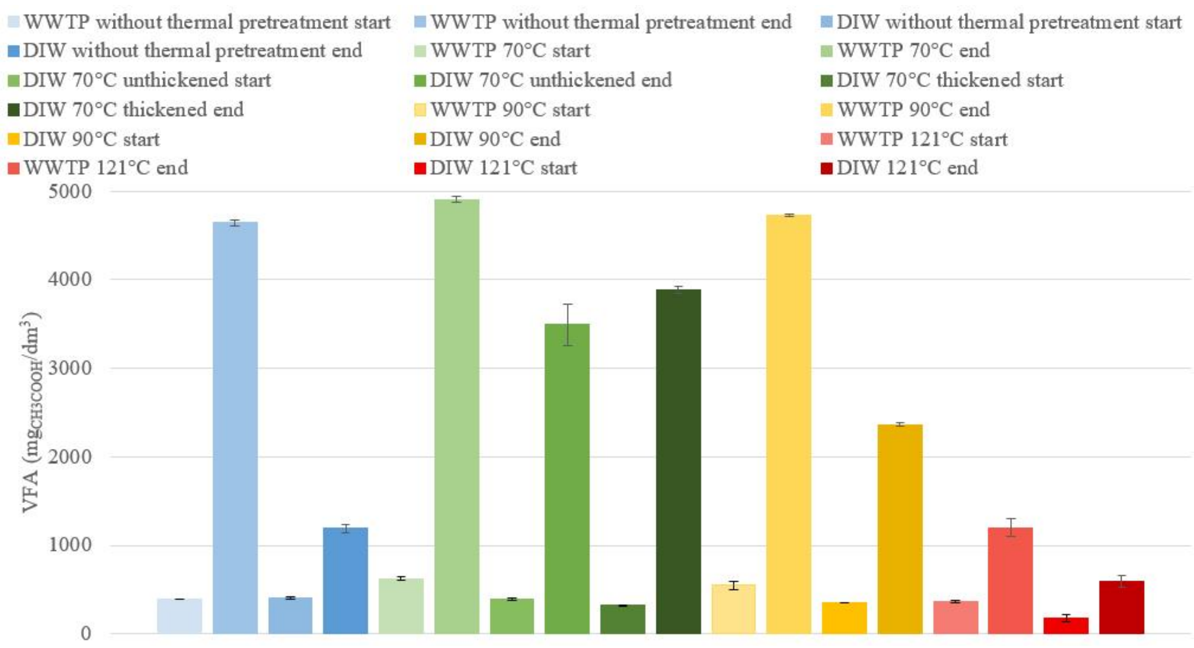


Figure 2. VFA at the beginning and the end of the process (after 52 h).

inoculum at 70 and 90 °C resulted in an increase in the amount of VFA produced (up to 3900 $mg_{acetate}/dm^3$ and 2400 $mg_{acetate}/dm^3$, respectively), the obtained values were still lower in comparison to WWTP sludge (Figure 2).

The VFA amounts produced in the experiments were in the lower range than observed previously (Lay et al., 2003) – up to 20 $g_{scetate}/dm^3$ for vegetable waste.

## 3.3. Composition of fermentation gas

Analysing the composition of fermentation gas (Tables 4 and 5), it is found that hydrogen producers from the WWTP sludge were able to produce hydrogen even without pretreatment (34.10% v/v), while in the DIW sludge only acid producers were active (neither hydrogen nor methane production, high concentration of nitrogen, Table 5). In all variants, low oxygen concentration is observed (up to 1.2% maximum), which shows anaerobic conditions. Significant concentrations of nitrogen confirmed that gas production after sludge pretreatment in 121 °C was negligible (the reaction bottles were purged with nitrogen at the beginning of the process and that nitrogen was not replaced by other gases). The main gas components in series with sludges pretreated in 70 °C and 90 °C were hydrogen and carbon dioxide – values ranged between 43 and 48% v/v.

Table 4. Composition of fermentation gas in series with WWTP sludge.

| | WWTP | | | |
|---|---|---|---|---|
| | without thermal pretreatment | 70 °C | 90 °C | 121 °C |
| $H_2$ (%v/v) | 34.10 | 43.91 | 42.60 | 22.42 |
| $CO_2$ (%v/v) | 58.91 | 45.56 | 48.04 | 43.81 |
| $O_2$ (%v/v) | 0.68 | 0.89 | 0.26 | 0.54 |
| $N_2$ (%v/v) | 2.45 | 4.28 | 1.30 | 22.88 |
| $CH_4$ (%v/v) | 0.44 | 0.02 | 0.04 | 0.00 |

Table 5. Composition of fermentation gas in series with DIW sludge.

| | DIW | | | | |
|---|---|---|---|---|---|
| | without thermal pretreatment | 70 °C unthickened | 70 °C thickened | 90 °C | 121 °C |
| $H_2$ (%v/v) | 0.72 | 45.86 | 46.78 | 43.23 | 0.00 |
| $CO_2$ (%v/v) | 33.48 | 46.62 | 46.42 | 45.47 | 51.19 |
| $O_2$ (%v/v) | 0.88 | 0.27 | 0.32 | 0.27 | 1.21 |
| $N_2$ (%v/v) | 36.61 | 1.54 | 1.94 | 1.29 | 35.53 |
| $CH_4$ (%v/v) | 0.95 | 0.04 | 0.04 | 0.00 | 0.00 |

## 3.4. Changes in hydrogen productivity

Figure 5 shows the hydrogen production per gram of VS of FW (cm$^3$ $H_2/g_{TVSFW}$) during the DF process. In all reactors with significant hydrogen productivity, the highest hydrogen production rate was observed during the first day of the process (Figure 3). After about 2 days, hydrogen production for these series was completely stopped. For the series in which the WWTP sludge was used after treatment at 121 °C, no hydrogen production was observed at the beginning of the process. After about 12 hours, there was a slight production, which stopped after about a day. For unheated DIW sludge and after treatment at 121 °C, no hydrogen production was observed.

The results obtained from the regression analysis of the modified Gompertz model (Table 6, Figure 4) showed a good fit to the experimental data – it is confirmed by the high value of coefficient of determination, $R^2$ above 0.983 for pretreated sludges. An increase in the pretreatment temperature from 70 to 90 °C resulted in a delay in hydrogen production (longer lag phase, Table 6). At the temperature of 70 °C lag phase lasted 3.12 h (DIW sludge) and 4.32 h (WWTP sludge), while for 90 °C it lasted 5.28 h (both sludges). In turn, the lack of pretreatment caused an even longer lag phase and a much lower hydrogen production efficiency (WWTP sludge) or virtually no production (DIW sludge).

The hydrogen production potential with WWTP sludge increased from 17.15 cm$^3$ $H_2/g_{TVSFW}$ (unheated) to 96.17 cm$^3$ $H_2/g_{TVSFW}$ after pretreatment at 70 °C. Further increase of pretreatment temperature led to a lower hydrogen yield – 85.71 cm$^3$ $H_2/g_{TVSFW}$ after 90 °C and 4.26 cm$^3$ $H_2/g_{TVSFW}$ after 121 °C. It is probable that temperature higher than 70 °C caused removal not only of hydrogen consumers but some of its producers. The DIW sludge without pretreatment had negligible hydrogen productivity (0.11 cm$^3$ $H_2/g_{TVSFW}$). The most probable lactic acid bacteria outcompete hydrogen producers – there was observed VFA production (Figure 2). The DIW sludge pretreatment at 70 °C led to hydrogen productivity equal to 79.67 cm$^3$ $H_2/g_{TVSFW}$ comparable to WWTP after

Table 6. Estimated parameters of modified Gompertz model from the experimental data.

| | $H_P$ [cm$^3$ $H_2/g_{TVSF}$ | $R_{max}$ [cm$^3$ $H_2$/ ($h \cdot g_{TVSFW}$)] | $\lambda$ [h] | $R^2$ [–] |
|---|---|---|---|---|
| WWTP | | | | |
| without thermal pretreatment | 17.15 | 2.20 | 4.80 | 0.966 |
| 70 °C | 96.17 | 13.49 | 4.32 | 0.995 |
| 90 °C | 85.71 | 8.47 | 5.28 | 0.998 |
| 121 °C | 4.26 | 0.35 | 15.12 | 0.999 |
| DIW | | | | |
| without thermal pretreatment | 0.11 | 0.01 | 0.24 | 0.909 |
| 70 °C | 79.67 | 10.24 | 3.12 | 0.983 |
| 70 °C thickened | 93.09 | 13.97 | 3.84 | 0.996 |
| 90 °C | 48.51 | 11.65 | 5.28 | 0.997 |

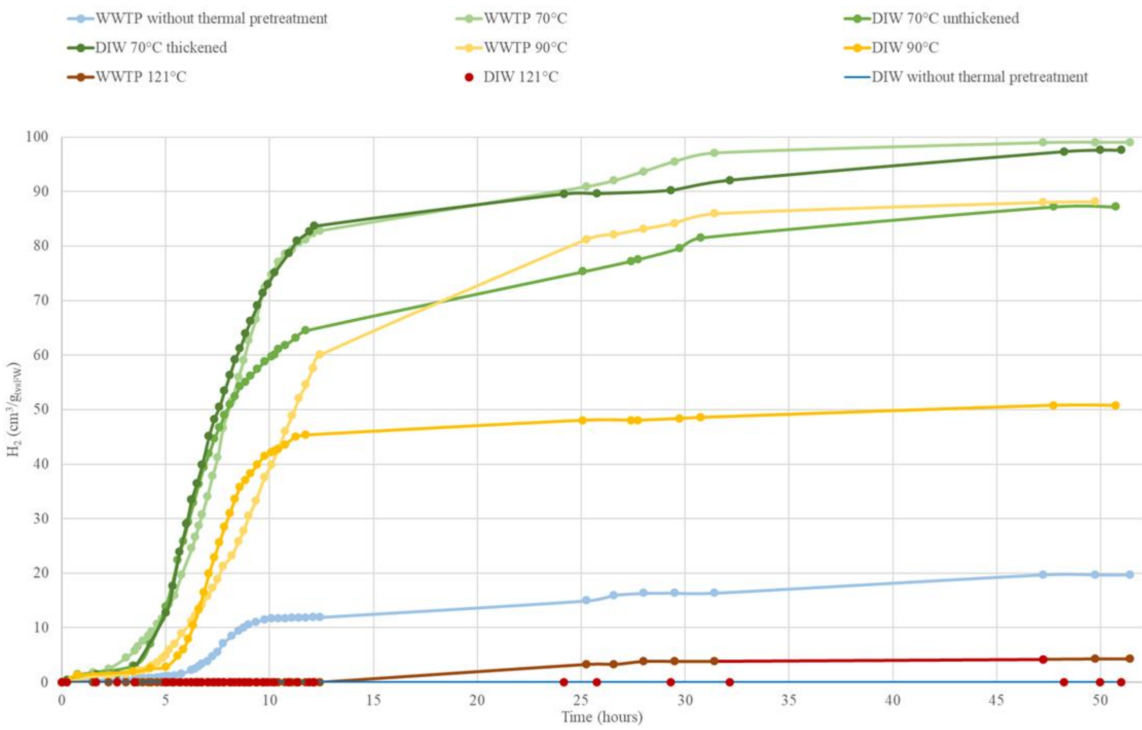


Figure 3. Changes in production of $H_2$ in time.

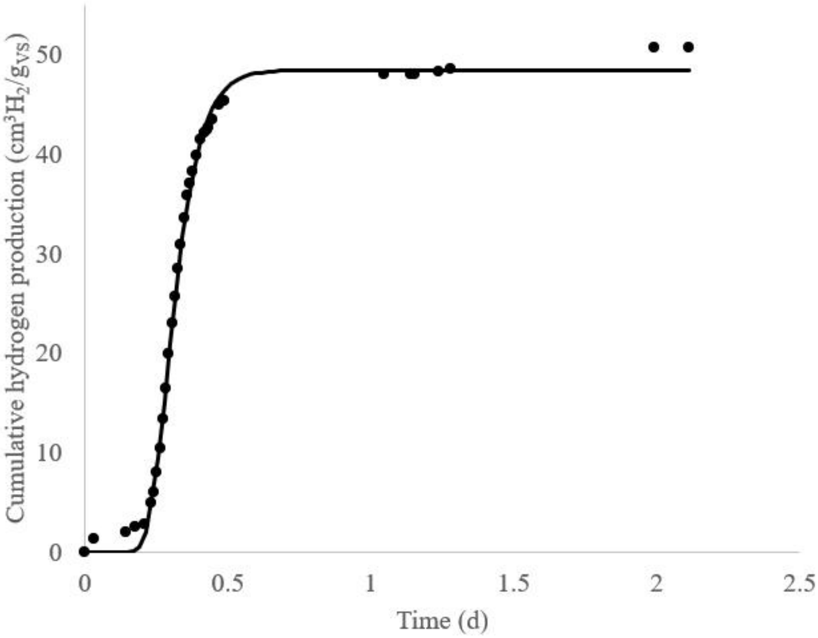


Figure 4. The example of cumulative hydrogen production curve fitting by modified Gompertz model – DIW sludge after pretreatment at 90 °C.

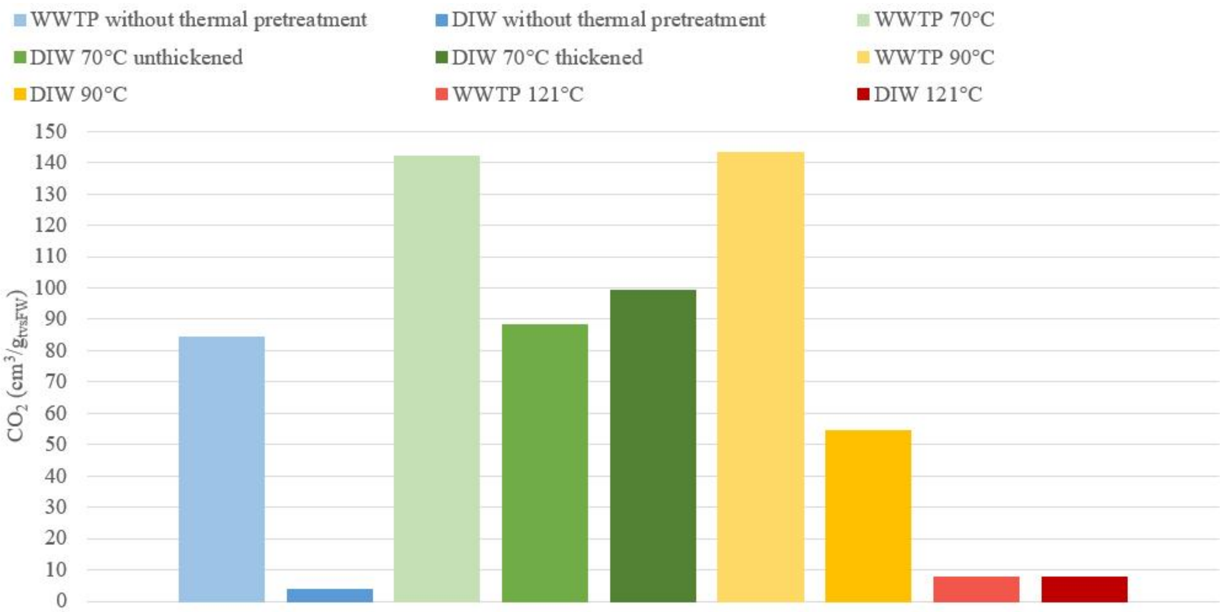


Figure 5. Carbon dioxide yield.

pretreatment at a temperature of 90 °C. However, additional thickening of the DIW sludge resulted in the hydrogen yield equal to 93.09 cm$^3$ $H_2/g_{TVSFW}$, which was a value similar to that obtained for the WWTP sludge. It can be concluded that pretreatment at a temperature of 70 °C effectively removes hydrogen consumers as well as the lactic acid bacteria ensuring expansion of hydrogen producing spore-forming bacteria. A further increase of the temperature of DIW sludge pretreatment to 90 °C resulted in a more significant decrease of hydrogen productivity (to 48.51 cm$^3$ $H_2/g_{TVSFW}$) than in the case of sludge pretreatment from the WWTP.

For comparison, in other similar studies, a maximum of 70–72 cm$^3$ $H_2/g_{TVSFW}$ hydrogen was obtained (Alibardi and Cossu, 2015; Jayalakshmi et al., 2009). The higher hydrogen production values obtained in this research result from the pretreatment used.

## 3.5. Carbon dioxide yield

As can be seen in Figure 5 the WWTP sludge led to higher carbon dioxide yield in the DF process than the DIW sludge. Pretreatment of both inocula at 70 and 90 °C caused the increase of carbon dioxide production. In the case of the WWTP sludge both previously mentioned temperatures resulted in 140 cm$^3$ $CO_2/g_{TVSFW}$, while the $CO_2$ yield for unheated sludge amounted to 85 cm$^3$ $CO_2/g_{TVSFW}$). However, the pretreatment of the DIW sludge at 70 °C resulted in a more significant growth of $CO_2$ production – from 5 cm$^3$ $CO_2/g_{TVSFW}$ for unheated sludge to 90 cm$^3$ $CO_2/g_{TVSFW}$ (unthickened sludge) and 100 cm$^3$ $CO_2/g_{TVSFW}$ (thickened one). A higher pretreatment temperature (90 °C) resulted in 55 cm$^3$ $CO_2/g_{TVSFW}$ $CO_2$ yield. The observed $CO_2$ production after pretreatment of both inocula at 121 °C was the same for the both sludges – less than 10 cm$^3$ $CO_2/g_{TVSFW}$. It could be concluded that after pretreatment at 121 °C only the indigenous microflora of FW led to hydrolysis and acetogenesis with low $CO_2$ production.

### 3.6. Carbon balance

A carbon balance was also performed – presented in Tables 7 and 8. It is assumed that carbon from the substrate (solid and liquid phase) has been converted into soluble carbon compounds in the liquid phase, then to the gas phase (mainly $CO_2$). The amount of carbon in the solid phase decreased after the dark fermentation process for most of the series (except for the series with the WWTP sludge pretreated at 121 °C). The difference in carbon content in the solid phase after the fermentation process compared to its amount at the beginning was 9 to 11% lower for the unheated sludge, 7–10% for the sludge heated at 70 °C, 9–18% for sludge heated at 90 °C, and for sludges heated at 121 ° C the differences were negligible. The biggest errors in the carbon balance calculations were found for the series in which the DIW sludge was heated at 70 °C. Most likely, the agglomerates formed by sludge flocs after such pretreatment were very inhomogeneous which affected the accuracy of performed TS analysis, especially in the case of the twice concentrated sludge.

The ratio of carbon in VFA to TOC in the liquid phase at the beginning of the fermentation processes was not higher than 0.05. Such a low ratio was due to the dominance of high molecular weight compounds of VFA. At the end of the DF process after inoculum pretreatment at 70 and 90 °C, an increase in this ratio to about 0.4 was observed. It confirms conversion of high molecular weight compounds to simpler ones (e.g. VFA).

## 4. CONCLUSIONS

The presented results showed that the process of initial thermal treatment of the inoculum strongly affects the efficiency of hydrogen production in the dark fermentation process. The use of inoculum heating at 70 °C resulted in the inhibition

Table 7. Carbon balance for series with sludge from WWTP in Lodz.

| | WWTP in Lodz | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | without thermal pretreatment | | 70 °C | | 90 °C | | 121 °C | |
| | Start | End | Start | End | Start | End | Start | End |
| Solid phase carbon (gC/dm$^3$) | 11.693 | 10.637 | 11.012 | 9.560 | 11.146 | 9.379 | 10.884 | 10.894 |
| Liquid phase carbon (gC/dm$^3$) | 3.053 | 4.305 | 4.993 | 4.659 | 5.240 | 5.152 | 5.715 | 5.167 |
| Gas phase carbon (gC/dm$^3$) | | 0.728 | | 1.224 | | 1.237 | | 0.067 |
| Total carbon (gC/dm$^3$) | 14.746 | 15.670 | 16.005 | 15.443 | 16.386 | 15.768 | 16.599 | 16.128 |
| Balance error (%) | 6.265 | | –3.510 | | –3.770 | | –2.839 | |

Table 8. Carbon balance for series with sludge from DIW.

| | DIW | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | without thermal pretreatment | | 70 °C | | 70 °C thickened | | 90 °C | | 121 °C | |
| | Start | End | Start | End | Start | End | Start | End | Start | End |
| Solid phase carbon (gC/dm$^3$) | 9.585 | 8.496 | 8.352 | 5.883 | 11.008 | 6.547 | 6.024 | 4.403 | 5.860 | 5.724 |
| Liquid phase carbon (gC/dm$^3$) | 2.958 | 4.092 | 5.094 | 4.611 | 4.874 | 3.680 | 2.985 | 4.138 | 3.614 | 4.170 |
| Gas phase carbon (gC/dm$^3$) | | 0.067 | | 0.543 | | 0.883 | | 0.397 | | 0.027 |
| Total carbon (gC/dm$^3$) | 12.543 | 12.656 | 13.446 | 11.037 | 15.882 | 11.110 | 9.009 | 8.938 | 9.474 | 9.921 |
| Balance error (%) | 0.901 | | –17.918 | | –30.049 | | –0.784 | | 4.715 | |

of hydrogen consumers and, consequently, an increase in biohydrogen production (80–90% compared to the series with unheated inoculum), while further temperature increase to 90 °C led to lower biohydrogen yield rise (by 50–70%), most likely as a result of partial inhibition of hydrogen-producing bacteria. Preliminary heating at 121 °C resulted in the destruction of inoculum microorganisms – both the yield of carbon dioxide and hydrogen were negligible.

Additionally, the temperature of inoculum pretreatment affects biohydrogen productivity to a greater extent than inoculum source. Even though the sludge from dairy industry wastewater treatment without any pretreatment had no hydrogen productivity, it contained ten times less ammonium nitrogen compounds than the sludge from wastewater treatment plant in Lodz, after thickening and pretreatment at optimal temperature (70 °C) it led to the almost the same hydrogen production efficiency – 96 $cm^3$ $H_2/g_{TVSFW}$ versus 93 $cm^3$ $H_2/g_{TVSFW}$. Moreover, the modified Gompertz equation allowed a good fitting to experimental data of the cumulative hydrogen production – the value of the determination coefficient exceeded 0.98 for the sludges after thermal shock.

## ACKNOWLEDGMENTS

*The presented results were obtained as part of the project No. 2021/43/B/ST8/00298 financed by the National Science Centre.*

## REFERENCES

Alibardi L., Cossu R., 2015. Composition variability of the organic fraction of municipal solid waste and effects on hydrogen and methane production potentials. *Waste Manage.*, 36, 147–155. DOI: 10.1016/j.wasman.2014.11.019.

Arain M., Mahar R.B., Sahito A.R., 2018. Biohydrogen Production from Co-Digestion of High Carbohydrate Containing Food Waste and Combined Primary and Secondary Sewage Sludge. *Mehran Univ. Res. J. Eng. Technol.*, 37, 139–148. DOI: 10.22581/muet1982.1801.12.

Bhurat K.S., Banerjee T., Pandey J.K., Bhurat S.S., 2021. A lab fermenter level study on anaerobic hydrogen fermentation using potato peel waste: effect of pH, temperature, and substrate pre-treatment. *J. Mater. Cycles Waste Manage.*, 23, 1617–1625. DOI: 10.1007/s10163-021-01242-3.

Bundhoo M.A.Z., Mohee R., Hassan M.A., 2015. Effects of pre-treatment technologies on dark fermentative biohydrogen production: a review. *J. Environ. Manage.*, 157, 20–48. DOI: 10.1016/j.jenvman.2015.04.006.

de Sá L.R.V., Cammarota M.C., de Oliveira T.C., Oliveira E.M.M., Matos A., Ferreira-Leitão V.S., 2013. Pentoses, hexoses and glycerin as substrates for biohydrogen production: an approach for Brazilian biofuel integration. *Int. J. Hydrogen Energy*, 38, 2986–2997. DOI: 10.1016/j.ijhydene.2012.12.103.

EUROSTAT, 2021. *Food waste: 127 kg per inhabitant in the EU in 2020*. Available at: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220925-2.

EUROSTAT, 2023. *Key figures on the EU in the world – 2023 edition*. Available at: https://ec.europa.eu/eurostat/web/products-key-figures/w/ks-ex-23-001.

Jayalakshmi S., Sukumaran V., Joseph K., 2009. Enhancement of hydrogen production from Kitchen Waste using heat treated anaerobic biogas plant slurry with pH control. *Int. J. Environ. Sustainable Dev.*, 8, 23–35. DOI: 10.1504/IJESD.2009.023710.

Lay J.-J., Fan K.-S., Chang J., Ku C.-H., 2003. Influence of chemical nature of organic wastes on their conversion to hydrogen by heat-shock digested sludge. *Int. J. Hydrogen Energy*, 28, 1361–1367. DOI: 10.1016/S0360-3199(03)00027-2.

Łukajtis R., Hołowacz I., Kucharska K., Glinka M., Rybarczyk P., Przyjazny A., Kamiński M., 2018. Hydrogen production from biomass using dark fermentation. *Renewable Sustainable Energy Rev.*, 91, 665–694. DOI: 10.1016/j.rser.2018.04.043.

Luo L., Sriram S., Johnravindar D., Louis Philippe Martin T., Wong J.W.C., Pradhan N., 2022. Effect of inoculum pretreatment on the microbial and metabolic dynamics of food waste dark fermentation. *Bioresour. Technol.*, 358, 127404. DOI: 10.1016/j.biortech.2022.127404.

Massanet-Nicolau J., Dinsdale R., Guwy A., 2008. Hydrogen production from sewage sludge using mixed microflora inoculum: effect of pH and enzymatic pretreatment. *Bioresour. Technol.*, 99, 6325–6331. DOI: 10.1016/j.biortech.2007.12.012.

Noike T., Takabatake H., Mizuno O., Ohba M., 2002. Inhibition of hydrogen fermentation of organic wastes by lactic acid bacteria. *Int. J. Hydrogen Energy*, 27, 1367–1371. DOI: 10.1016/S0360-3199(02)00120-9.

Rafieenia R., Lavagnolo M.C., Pivato A., 2018a. Pre-treatment technologies for dark fermentative hydrogen production: current advances and future directions. *Waste Manage.*, 71, 734–748. DOI: 10.1016/j.wasman.2017.05.024.

Rafieenia R., Pivato A., Lavagnolo M.C., 2018b. Effect of inoculum pre-treatment on mesophilic hydrogen and methane production from food waste using two-stage anaerobic digestion. *Int. J. Hydrogen Energy*, 43, 12013–12022. DOI: 10.1016/j.ijhydene.2018.04.170.

Slezak R., Grzelak J., Krzystek L., Ledakowicz S., 2017. The effect of initial organic load of the kitchen waste on the production of VFA and $H_2$ in dark fermentation. *Waste Manage.*, 68, 610–617. DOI: 10.1016/j.wasman.2017.06.024.

Srivastava N., Srivastava M., Abd_Allah E.F., Singh R., Hashem A., Gupta V.K., 2021. Biohydrogen production using kitchen waste as the potential substrate: a sustainable approach. *Chemosphere*, 271, 129537. DOI: 10.1016/j.chemosphere.2021.129537.

Tamasiga P., Miri T., Onyeaka H., Hart A., 2022. Food waste and circular economy: Challenges and opportunities. *Sustainability*, 14, 9896. DOI: 10.3390/su14169896.

Tian Q.-Q., Liang L., Zhu M.-J., 2019. Enhanced biohydrogen production from sugarcane bagasse by *Clostridium thermocellum* supplemented with $CaCO_3$. *Bioresour. Technol.*, 197, 422–428. DOI: 10.1016/j.biortech.2015.08.111.

Tjørve K.M.C., Tjørve E., 2017. The use of Gompertz models in growth analyses, and new Gompertz-model approach: an addition to the Unified-Richards family. *PLoS ONE*, 12, e0178691. DOI: 10.1371/journal.pone.0178691.

Ubando A.T., Chen W.-H., Hurt D.A., Conversion A., Rajendran S., Lin S.-L., 2022. Biohydrogen in a circular bioeconomy: a critical review. *Bioresour. Technol.*, 366, 128168. DOI: 10.1016/j.biortech.2022.128168.

Zakład Wodociągów i Kanalizacji w Łodzi, 2022. *Dział Oczyszczalni Ścieków*. Available at: https://zwik.lodz.pl/pl/artykuly/326/dzial-oczyszczalni-sciekow.

Zhu H., Béland M., 2006. Evaluation of alternative methods of preparing hydrogen producing seeds from digested wastewater sludge. *Int. J. Hydrogen Energ*y, 31, 1980–1988. DOI: 10.1016/j.ijhydene.2006.01.019.