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EFFECT OF pH ON THE PRODUCTION OF VOLATILE FATTY ACIDS IN DARK FERMENTATION PROCESS OF ORGANIC WASTE

WPŁYW pH NA PRODUKCJĘ LOTNYCH KWASÓW TŁUSZCZOWYCH W PROCESIE FERMENTACJI CIEMNEJ ODPADÓW ORGANICZNYCH

Abstract: The aim of this study was to investigate the effect of pH on the dark fermentation process of kitchen waste by specifying the composition of the volatile fatty acids (VFA), H_2 and by drawing the carbon balance. Studies were carried out in 8 dm^3 batch bioreactor in mesophilic conditions. The kitchen waste from the city of Lodz were used as a substrate. Based on the study, it was observed that most of the VFA was produced during the first two days of the process, while in the following days the production was diminished. The highest production of VFA (19.5 g/dm^3) was obtained in the bioreactor, where the pH was 7 and 8. Analyzing the produced VFA it was observed that mostly the acetic and butyric acid had been produced. Most of acetic acid (over 70 %) was obtained in fermenter with pH 7 and 8. In contrast, most of the butyric acid (over 40 %) was in the bioreactor with a pH of 6. Production of H_2 was in the range from 4.29 to 26.5 dm^3, wherein the largest amount of H_2 was created in the bioreactor with a pH of 6.

Keywords: kitchen waste, volatile fatty acids, dark fermentation, hydrogen

Introduction

The activities we perform every day in the kitchen or home garden generate huge amounts of waste. A considerable percentage constitute biodegradable waste (up to 50 %), that most often are stored on landfills or are composted [1]. The amount of food waste generated by urban areas has increased significantly in recent years, which, among others, is inextricably linked to a rising standard of living. Considering the shrinking natural resources and the rapid progress of civilization, more often the reuse of food waste is taken into account as an alternative source of energy. Food waste, including household kitchen waste (KW) could be one of the cheapest and easiest sources of this substrate [2].

Kitchen waste are rich source of carbon, nitrogen and trace elements. It is used for example as feed for livestock (approx. 80% of KW) [3]. At the same time, high humidity and high salt content cause additional energy consumption in the combustion process of

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KW. This process is also accompanied by the production and release of toxic pollutants into the environment. These negative aspects does not favor the process of combustion, what restricts the use of KW [4]. There are many methods available for treatment of organic waste [5], but it seems that the process of anaerobic fermentation is the most promising method to deal with the problem of increasing amount of generated waste.

Methane fermentation involves series of steps such as hydrolysis, acidogenesis, acetogenesis and methanogenesis [6]. Under controlled conditions, this process provides a useful product, such as CH_4, which is considered to be relatively cleaner than fossil fuels. Dark fermentation is a modified version of classic fermentation process, where 3-stage anaerobic process can be distinguished: hydrolysis, acidogenesis and acetogenesis. During the hydrolysis step, decomposition of polymers contained in waste to monomers is taking place to make it more easily digestible for microorganisms. Then acetogenic and acidogenic bacteria metabolize collected monomers to volatile fatty acids (VFA), including acetic and butyric acid and H_2 [7]. In the process of dark fermentation, methanogenesis is eliminated to prevent the conversion of synthesized VFA into methane [8]. VFA serve not only as a substrate for the production of H_2 or CH_4. They are also used in microbial fuel cells, biological wastewater treatment plants or as a substrate for the production of lipids used in biodiesel production [2]. VFA may be also a substrate for the synthesis of biodegradable polymers (polyhydroxyalkanoate) by specially selected strains of bacteria, e.g. *Cupriavidus necator* [2].

The dark fermentation process is the most promising and at the same time, the least harmful to the environment among the available methods of H_2 synthesis. It solves the problem of obtaining energy from waste materials and eliminates the problem of collecting waste on landfills [9]. The bacteria involved in the dark fermentation reaction, such as *Clostridium*, convert VFA into acetate, CO_2 and H_2 during the acetogenic phase [10]. Nowadays, the H_2 generation in the dark fermentation process is gaining an increasing number of fans, mainly because this technology enables usage of a wide range of substrates, including renewable resources particularly rich in organic matter, such as sludge, leachate, pomace or waste of food origin [11].

A number of parameters affect the amount and the composition of the produced VFA, including pH, temperature, inoculum or the type and quantity of organic load [2]. Most studied parameter that can regulate those mentioned factors is the pH of the environment in which the process is carried out [9, 12-17]. Adjusting the pH affects, among others, intra- and extracellular transport of VFA, which is translated directly into an efficiency of hydrolysis and subsequent fermentation stages [18]. Temudo et al. [19] have shown that depending on the pH, different groups of microorganisms begin to dominate the fermentation process, which has a direct impact on the type of product generated by them, including VFA.

In the process of dark fermentation the compliance of carbon balance was hardly studied. The calculations were mainly limited to the total amount of generated carbon dioxide related to the amount of carbon indicated at the beginning of the process [12].

In the present study the effect of pH on the composition and amount of VFA obtained in the dark fermentation process of the KW was investigated. Experiments were performed at pH 6, 7 and 8, and without pH adjustment. In addition to the quantitative and qualitative VFA analysis, the changes in pH, the C/N ratio in the feedstock and the production of gases: CO_2 and H_2 were monitored during the process. On the basis of determined carbon

amount in liquid and solid phase and the amount of produced CO_2, the balance of the carbon fluxes during the fermentation process was calculated.

Materials and methods

The substrate for the production of VFA in the dark fermentation was selectively collected and then shredded KW from households, later stored at –20 °C. Inoculum for the process was the digested sludge from the anaerobic Wastewater Treatment Plant in Lodz. The digested sludge was heated at 70 °C for 30 minutes to eliminate the methanogenic bacteria growth [12]. The amount of volatile solids (VS) in sludge was 20.06 g/dm^3 and in organic matter it was 0.271 g/g. The characteristics of the sludge and KW properties are presented in Table 1.

Table 1

The amount of total solids (TS) and volatile solids (VS) in KW (substrate) and digested sludge (inoculum)

Digested sludge	TS [g/dm^3]	31.46 ±0.23
	VS [g/dm^3]	20.06 ±0.13
KW	TS [g/g]	0.286 ±0.004
	VS [g/g]	0.271 ±0.003

In presented study four different dark fermentation processes were conducted: in three of them the pH was kept constant at the level of 6, 7 and 8, and one process was performed with no pH adjustment. pH control was carried out with NaOH. The dark fermentation process for all pH series was conducted in batch bioreactor and it took 7 days. Reaction volume was 8 dm^3. The process run under fully anaerobic conditions at 37.0 ±0.2 °C. The volumetric ratio of organic matter to sludge was 1:1. The content of organic dry matter was in the range of 33.88 to 36.12 g/dm^3, and the C/N ratio between 10.02 to 12.04. To ensure completely anaerobic conditions each bioreactor at the start of the process and during each sampling was flushed with nitrogen for 5 minutes. Each experiment was performed in triplicate.

The samples for analysis were collected each day of the process. Three separate fractions were analyzed during the fermentation process: solid, liquid and gas. All fractions were analyzed in triplicates.

The separation of solid and liquid fraction was carried out in a centrifuge MPW 250 (MPW Med-Instruments) at 13,000 rpm for 15 min. For solid phase samples, there were carried out elemental analysis, calculated TS and VS. Elemental analysis, to determine the level of carbon, nitrogen and hydrogen in the samples, was carried out using an elemental analyzer 2500 (CE Instruments). The TS content and VS matter were determined by gravimetric method. All analysis were conducted in accordance with standard methodology [20].

In the liquid phase the concentration and composition of the VFA, total organic carbon (TOC), total nitrogen (TN) and pH were indicated. TOC and TN was measured using the IL550 TOC_TN apparatus from Lachat Instruments. The pH measurement was performed using an electrode Mettler Toledo InPro 3250/225/PT1000. The concentration of VFA was measured using Varian CP4800 chromatograph equipped with a capillary column type BP21 (25 m, 0.25 mm, 0.25 μm). Injector and FID detector temperature was 250 °C. Before the samples from bioreactor were loaded on chromatograph, they were filtered using filters

0.2 μm and acidified with formic acid. 1 mm^3 samples were injected into the dispenser with the 1:100 split. After starting the measurement the oven temperature was set 110 °C for 1 minute, then the temperature raised to 230 °C with 10 °C/min speed and finally maintained 230 °C for 2 min. Helium was the carrier gas set on 1.4 cm^3/min flow.

The gas fraction was examined to read the content of CO_2, CH_4 and H_2. The concentration of gases were measured using a gas chromatograph type 8610C (SRI Instruments) equipped with two packed columns (molecular sieve and silica gel) with a length of 1 m, 1/8" diameter and TCD detector. Oven and detector temperature was 60 °C and 150 °C, respectively. The carrier gas was helium, the flow through the column was set at 10 cm^3/min. The quantity of gas was measured with the water displacement method: bioreactor was attached to a 20 dm^3 cylinder with brine (30 % NaCl in water), which accumulated biogas produced during the process.

Results and discussion

Dark fermentation process was analyzed each day in terms of quantity and composition of the VFA in the liquid fraction. In all series the highest production of VFA was observed in the first two days of the experiment. In the subsequent days the process significantly slowed down (Fig. 1). Similar results were achieved in Liu et al. experiments [21], who studied the process of VFA synthesis using activated sludge at a pH range from 3 to 12 for 10 days. Although in total the highest amount of VFA were found on the last day of the process, the synthesis was the most intensive in the 2-3 day. Liu et al. [21] assumed that one of the reasons for this, was the high protein content of the fermentation broth (60 %). This also suggests an analogy for the process analyzed in this paper. Although the protein content was not measured directly, the low value of C/N can indicate this (data quoted later in the article).

Biosynthesis of VFA most extensively occurred at pH 7 and 8 reaching the concentration of 19.5 g/dm^3. For pH 6 the maximum value of generated VFA was 14.1 g/dm^3. Minimum value of VFA - 9.39 g/dm^3 was found in bioreactor without pH control. Comparing those data with the results of other authors, similar effects achieved He et al. [22], who obtained VFA concentration of 18.46 g/dm^3 from food wastes and Zhang et al. [23] reaching up to 36 g/dm^3 of VFA from kitchen wastes, both at pH 7. The results published by Hong et al. [24] also showed that pH 7 was optimal for VFA synthesis. In the semi-continuous process, with the hydraulic retention time of 9 days and the addition of food wastes on the level of 8.31 g/dm^3/d Hong et al. [24] obtained up to 29.1 g/dm^3. Feng et al. [25] and Yuan et al. [17] by fermenting sludge, reached a maximum of VFA production in 8-day fermentation process at pH 8 and 10, respectively, which was three times more than in the non-controlled pH. As confirmed above, maintaining an alkaline pH favors the production of VFA. One of the reason for this may be intensification of hydrolysis in alkaline medium, which affects the dissociation of the acid moieties of the polymers contained in the substrate and inoculum, which facilitates the distribution of the monomers [25]. It has been shown that maintaining a pH of 8-10 for the hydrolysis step results in increased availability of soluble organic compounds that are next consumed in the fermentation by microorganisms [26]. In addition, a significant increase of VFA production may be affected by the high protein content of the sludge, which are easily absorbed in the pH range of 7-10 [21].

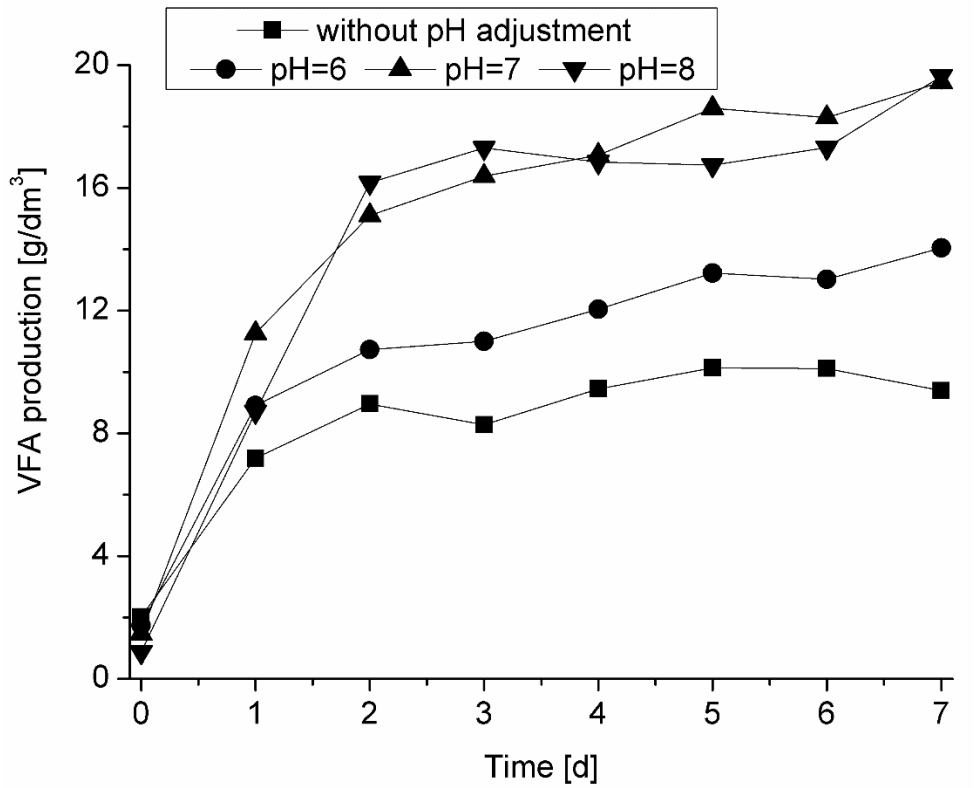

Fig. 1. The amount of VFA produced in the dark fermentation process carried out at pH 6, 7, 8 and without the pH adjustment

Wang et al. [27] conducted a similar process of anaerobic fermentation of food wastes at a pH range of 4-6 and without pH adjustment. In their studies, the highest concentration of VFA was observed at pH 6, and the lowest VFA amount was formed in fermenter without pH adjustment (more than 5-fold less than pH 6). This confirms the fact that the decrease in the pH value of the process has a negative impact on VFA production. This is probably because the simultaneous inhibition of acidogenic bacteria activity at low pH values occurs. In addition, lowering the pH increases the number of undissociated VFA, which inhibits the fermentation by enhancing the flow of undissociated acids across the cell membrane [14]. A high content of VFA in an acidic medium is also possible, as shown by Jankowska et al. [15], who performed the experiments using primary and waste activated sludge. In 5 days, the process achieved a high content of VFA at pH 4-5. In the following days, there was no significant increase of VFA, which could be due to increasing concentration of methanogens [15]. Longer retention time favored the synthesis of VFA while maintaining the pH above 6.

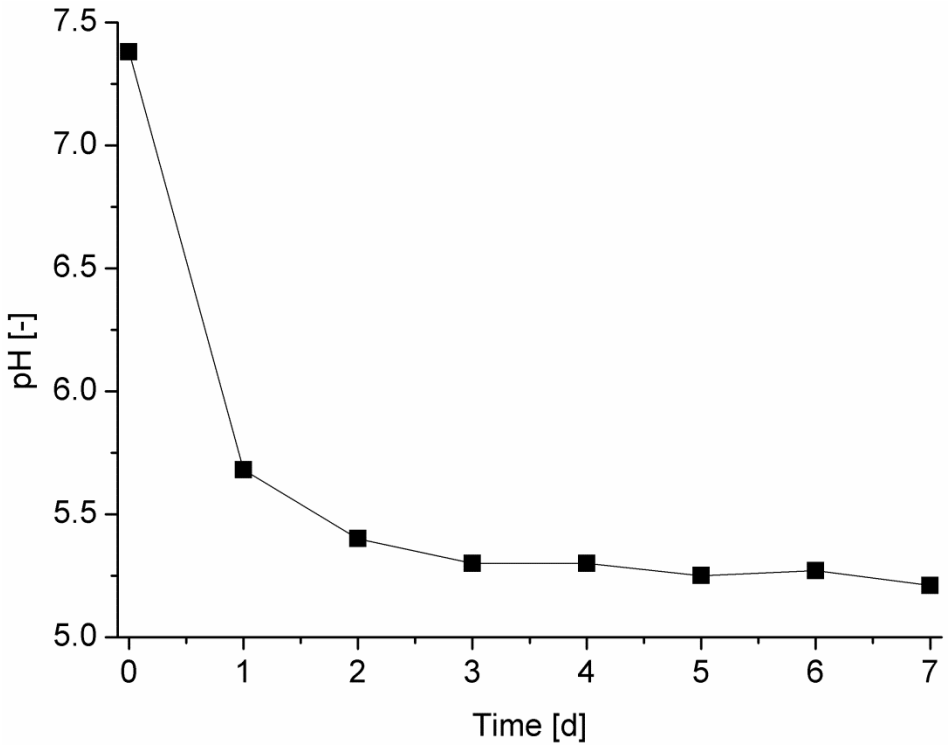

Fig. 2. pH fluxes during the fermentation processes conducted without adjusting the pH

In all bioreactors the pH fluxes were monitored during the process. Figure 2 shows that in fermenters with pH 6, 7 and 8 the pH value was constant (± 0.16), while in the fermenter without the pH control, the largest decrease in pH from 7.4 to 5.7 was observed on the first day of the process. It is associated with an intensive production of VFA during the first hours of dark fermentation. In the following days the pH was maintained at 5.3, although the VFA synthesis proceeded with much lower intensity (Fig. 1).

In the post-fermentation broth the composition of the synthesized VFA was also analyzed. It was observed that the largest part accounted for acetic and butyric acid. Acetic acid maintained at the highest level (approximately 80 % of total fatty acids) in a bioreactor with pH 7 and 8. In bioreactor without pH control the acetic acid constituted about 60 %. The lowest concentration of acetic acid was observed at pH 6 (approx. 40 %). In case of butyric acid, these values ranged from 13-15 % at pH 7 and 8, and 35-40 % for the unregulated pH to a value above 40 % at pH 6. The propionic acid maintained at the level of 2-6 % in all bioreactors, apart from pH 6 where it shared approximately 10 % of the total volatile fatty acids. This acid, in the acidogenic phase, is decomposed into CH_3COOH, CO_2 and H_2 [28]. Caproic acid in the samples with the pH unregulated, pH 7 and 8 were practically undetectable (< 1 %) but at pH 6 represented more than 7 %. Other recognized acids (isobutyric acid, valeric acid, isovaleric acid) were < 1 % of the total fatty acids. The detailed percentage composition of recognized VFA is presented in Figure 3.

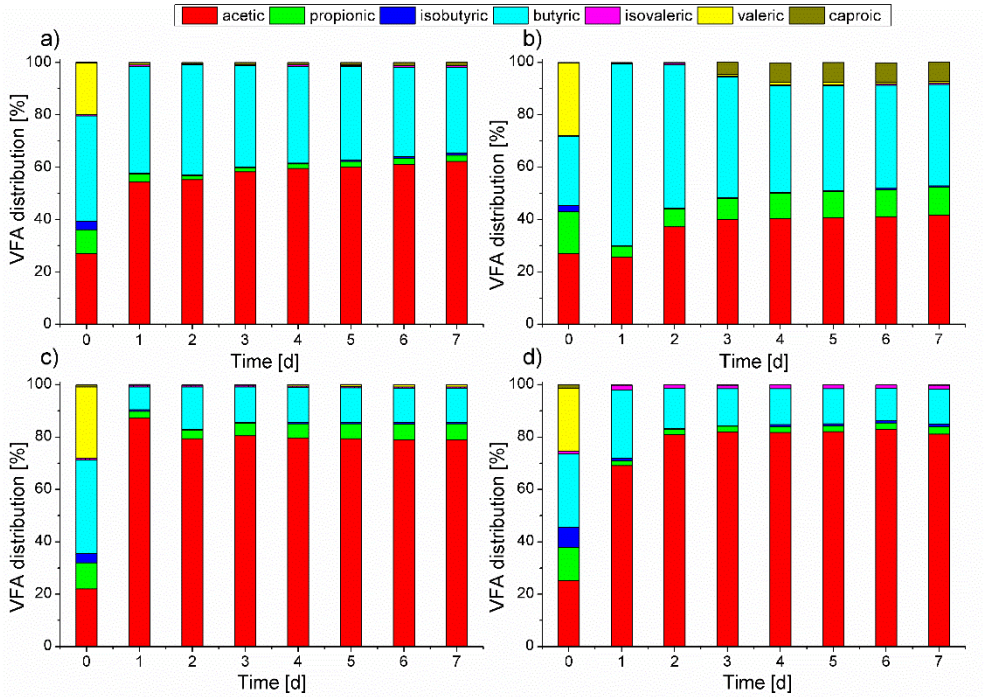

Fig. 3. Percentage share of acetic, butyric, isopropionic, propionic, isovaleric, valeric and caproic acids in the dark fermentation process: a) without pH control, b) with pH 6, c) pH 7, d) and pH 8

This is due to the microbial metabolism pathways dependent on redox potential in the cellular environment, which indicate that the reaction favors biosynthesis of butyric acid and H_2 at acidic pH. The inhibition of this process and redirection to alternative route of ethanol and acetic acid synthesis occurs at alkaline pH [29].

In Wang et al. [27] experiments, acetic and butyric acids constituted more than 90 % of all recognized acids. In the bioreactor without pH control they observed approx. 60 % of acetic acid and approx. 40 % of butyric acid. Due to the fact that in Wang et al. [27] process, they used waste with high carbohydrate content (over 40 %), it can be concluded that the use of substrates with increased carbohydrate level may direct the synthesis to acetic and butyric acid during dark fermentation process. Most of the propionic acid was recorded at pH 6, proving the observations referred in this article. The confirmation of the results presented in this study is also the work of Temudo et al. [16], who has studied the fermentation of glucose at a pH range from 4 to 8.5. Their results indicate that at lower pH values the most common product was butyric acid, but when the pH increased, the biosynthesis of butyric acid gradually decreased based on increasing acetic acid content.

From the data collected in presented experiments the changes of C/N ratio in the post-fermentation broth were calculated (Fig. 4). In the bioreactors with pH 6, 7 and 8, the ratio of carbon to nitrogen was approx. 12. Comparing it with initial C/N values for pH 6: 10.9; pH 7: 11.7 and pH 8: 12, very small change of C/N is seen. Only in fermenter without regulated pH the C/N ratio was at the level of 9.4, while the initial C/N value was

10.2. Analyzing the ratio between VFA concentration and TOC value, it was noted that in all bioreactors it reached approx. 0.8.

Referring the obtained results to available literature, the optimal C/N ratio for the growth of acetogenic bacteria and thereby VFA synthesis in dark fermentation process range between 20-30 [30]. Due to the fact that in described process the C/N was about half of this value, it may have a direct impact on the metabolism of microorganisms, and thus, on the amount of VFA produced. This hypothesis is confirmed by the results of [31], who showed that the optimal conditions for the production of VFA using identical substrate and inoculum as in the presented case was obtained at the C/N ratio of 22, pH 8 and 6-day long fermentation. As it is documented by Feng et al. [13], one way of increasing the efficiency of the dark fermentation process is to add carbohydrates to the activated sludge (in the cited experiment it was rice), what increased the C/N ratio of the reaction broth. It is particularly important since the C/N ratio in the sludge oscillates around 7 [32].

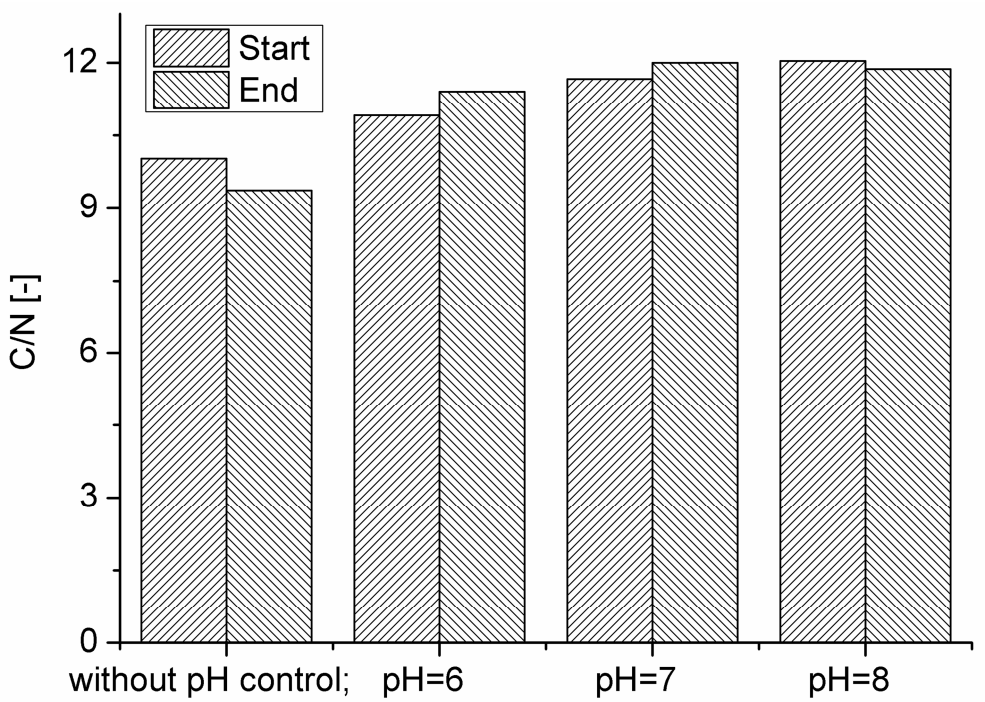

Fig. 4. C/N ratio measured at the beginning and at the end of a process run at pH 6, 7, 8 and at uncontrolled pH

In the conducted dark fermentation processes apart from VFA, there were also produced H_2 and CO_2 (Fig. 5). Most H_2 and CO_2 were detected during the process conducted at pH 6 (H_2 - 26.48 dm^3 and CO_2 - 13.55 dm^3). These amounts decreased with increasing the bioreactor's pH. The smallest amount of H_2 and CO_2 was formed in bioreactor with a non-regulated pH (H_2 - 4.29 dm^3 and CO_2 - 4.19 dm^3). For the rest of the processes the ratio between generated H_2 and CO_2 reached 1.03 in the fermentor without pH adjustment; 0.82 for pH 8 and 1.00 for pH 7.

These results confirm Temudo et al. [16] studies, who investigated the glucose fermentation at different pH values by mixed cultures of anaerobic bacteria. Monitoring the H_2 content in the various samples, they observed that it decreased with an increase of pH (from 4 to 8.5). They concluded also, that it is directly linked to the production of formic acid. In this study, formic acid was not identified, so direct correlation cannot be confirmed. Slightly different results were obtained in Lee et al. [9] work, where the dark fermentation process was carried out using KW at a pH range between 5.5-7. The H_2 production correlated with the synthesis of VFA in that case showed that at higher pH values dark fermentation process was aimed at H_2 synthesis (maximum at pH 7). In contrast, at pH 5.5 practically no H_2 was generated, however, the highest concentrations of VFA, reaching 6.0 g/dm^3, was reported. Those differences might appear due to the character of the fermentation processes, which ran under thermophilic conditions (55 °C). It probably has a direct impact on the activity of distinct microorganisms, different from those which lead the process in mesophilic conditions [9].

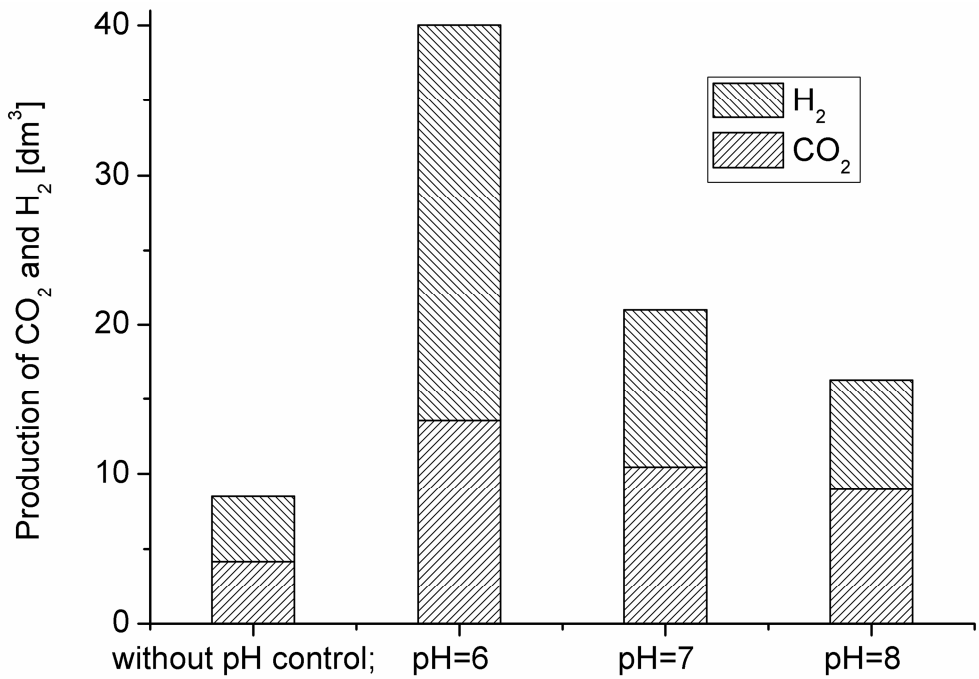

Fig. 5. The amount of H_2 and CO_2 produced at different pH values at the end of the process

Complementary to the analysis of data collected in this experiment there was performed a carbon balance of the dark fermentation process. In presented calculations it is assumed that the carbon included in KW was mainly converted into VFA and CO_2 as a result of biochemical processes. The correctness of the carbon balance was determined by comparing the amount of carbon at the beginning and at the end of the process (Table 2). During the procedure, the amount of CO_2 in the gas phase was measured. The concentration

of carbon inside each selected gas, liquid and solid fraction was related to the volume of the growth media in which the process occurred.

Table 2

Carbon balance of the dark fermentation process carried out at different pH values

pH	Start of the process		End of the process			Balance error [%]
	C-solid fraction [g C/dm^3]	C-liquid fraction [g C/dm^3]	C-solid fraction [g C/dm^3]	C-liquid fraction [g C/dm^3]	C-gas fraction [g C/dm^3]	
6	14.22	5.45	9.38	8.59	3.32	–8.18
7	14.55	5.60	9.33	9.90	2.56	–8.12
8	14.54	7.87	9.50	10.00	2.20	3.17
Without pH regulation	13.98	3.85	9.55	5.56	1.02	9.50

The highest error in the carbon balance has been found in the process conducted without pH control and it was 9.50 %. In the series carried out at a pH of 8, the mistake was 3.17 %, at pH 7: –8.12 % and at pH 6: –8.18 %. The error occurred due to inaccurate analytical apparatus, as well as non-homogenous samples of the solid fraction at the start of the process, which accounted for approx. 65 % of carbon at that point. In all cases the concentration of carbon in the liquid phase at the end of fermentation was higher than at the beginning of the process. The highest amount of carbon, 43.4 % related to the initial phase, increased in series with the pH 7, whereas the lowest, at pH 8 (approx. 21 % growth). As demonstrated in Table 2, together with an increase of pH, the carbon content of the liquid phase grew relatively to the amount of carbon marked at the beginning of the process. Detected carbon in the liquid phase at the end of the process was incorporated in synthesized VFA (80 %). Comparing the amount of carbon in the solid fraction at the beginning and at the end of the process, it is seen that about 65.6 % of organic carbon has not been transformed in conducted fermentation processes. This may indicate improper pH in the hydrolysis phase or too short duration of the fermentation process. Similar results were obtained by Cappai et al. [12], where 60-74 % of the carbon from the beginning of the process was found in post-fermentation broth, suggesting restricted bioavailability of carbon in the process [12]. Based on the amount of carbon detected in the gaseous fraction at the end and at the beginning of the process, the number of organic carbon converted to CO_2 was calculated. Most of the carbon from the beginning of the process (16.88 %) was transformed into gaseous phase at pH 6, the lowest value (5.72 %) at non-regulated pH.

Conclusions

Presented in following paper results confirm that environment, in which the dark fermentation process of KW was conducted, had a direct impact on the generated VFA quantity and composition. VFA synthesis proceeded most intensively in the first two days of fermentation. Maintaining pH in the range from 7 to 8 led to higher VFA production. Most intensive growth was observed at pH 7-8 (19.5 g/dm^3). No pH adjustment during the process caused a depletion in the production of VFA (9.39 g/dm^3). As shown in the presented article, adjusting the pH can significantly affect the profile and the composition of generated VFA. Lower pH (6) favored the production of H_2 (26.5 dm^3) and butyric acid (over 40% of total VFA), while higher pH values (7-8) directed the metabolic pathways into acetic acid biosynthesis (80 % of total VFA) and lower H_2 synthesis. The biggest error

in the carbon balance has been found for the process conducted without pH control, and it reached 9.5 %. In a series carried out at pH 8, the error was 3.17 %, pH 7: –8.12 % and at pH 6: –8.18 %. In all cases, the concentration of carbon in the liquid phase at the end of the fermentation process was higher than at the beginning. Most of carbon in the liquid phase - 43.4% relatively to initial phase, appeared in series with the pH 7. The smallest amount, approx. 21 %, was calculated at pH 8. At pH 6 the highest amount of carbon from the beginning of the process was transferred into CO_2 (16.88 %). The lowest carbon was transferred into CO_2 in the fermentor with unregulated pH (5.72 %).

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