Microbial Biogas Production From Pork Gelatine.

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Research Article

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Abstract

Dark fermentation of collagen (gelatine) results are shown in this research. The concentrations of applied gelatine were of VSS (volatile suspended solids) from 10 g VSS/L to 30 g VSS/L. The initial process pH was 5.5, depending on concentration reached pH values from 7.5 to 7.8 after 55 days. Although inoculum was heat-shocked in the case of 30 g VSS/L of collagen the process was hydrogenotrophic anaerobic digestion. In collagen concentration below 30 g VSS/L, hydrogen production was dominant only in the first 5 days of experiments. Then there also changed from dark fermentation into hydrogenotrophic methane production. In the case of optimal biogas production was due to accumulative production for a concentration of collagen 20 g VSS/L: 147.2 mL of hydrogen and 57.23 L of methane. In the case of optimal biogas production was due to accumulative production for a concentration of collagen 20 g VSS/L: 147.2 mL of hydrogen and 57.23 L of methane. The optimal hydrogen and methane yields were for concentration 10 g VSS/L (7.65 mL H_2 /g VSS, and 3.49 L CH_4/ g VSS). In 10 g VSS/L was also the lowest accumulated emission of hydrogen sulphide (10.3 mL of H_2S), while the lowest yield was for 30 g VSS/L (0.44 mL H_2S /g VSS). After a lag time, the hydrogen production and hydrogen sulphide grew with a specific ratio depending on concentration. Collagen, a protein with known amounts of sulphur allowed determining the origin of hydrogen sulphide in biogas. The hydrogen sulphide emission and sulphur added analysis proved that hydrogen sulphide origins in biogas from bacteria remains more than from substrate.

Highlights

- Dark fermentation of collagen after five days transferred into hydrogenotrophic anaerobic digestion;
- The heat shock was not enough to block methanogenesis if added enough collagen substrate (30 g VSS/L);
- Hydrogen sulphide originated in biogas of collagen from bacteria rests more than from substrate;
- Collagen is a promising source of hydrogen and methane;

1. Introduction

Dark fermentation (DF) is a special case anaerobic digestion (AD) of compounds into hydrogen and volatile acids that stops acidogenesis. Optimal conditions for the process are stressed inoculum and acidic pH (5.0 to 6.5). The temperature depends on bacteria mesophilic or thermophilic. Substrates are mostly glycerol and polysaccharides [1]. The stressing of inoculum stops methanogenesis influenced by heat, centrifuging, pH, and chemicals [1]. There is little research about the potential of proteins [2, 3] as a source of DF. Protein wastes (including collagen) utilization is an enormous problem in the tannery, fish, and butchery industries [4]. Therefore, collagen (one of the most common such waste) for its sustainability was tested as a substrate for DF.

Hydrogen sulphide emission is a vital problem of anaerobic digestion[5]. Thus, checking the appearance of this compound in dark fermentation is important before viable industrialization [6]. Another aim of the research was monitoring the relationship between hydrogen and hydrogen sulphide [7], observed in the fermentation of cotton wastes [8]. Among investigations aims, there were tested optimal load for the process also. Detection of the bottom products of the digestion of proteins allows for projecting potential pathways of biogas production. The bottom products of dark fermentation are usually volatile organic acids [9], but ammonia compounds also appear [10]. Those compounds cause pH changes, transforming the process from DF to AD (hydrogenotrophic [11] or methanotrophic) [12]. Eventual liquid products of dark fermentation can be bioresource for cosmetics and chemistry (low organic acids) [13] or fertilizers [14]. Some anaerobic digestion works applied protein-rich substrates like leathers or meat [2, 15, 16].

In the case of DF, many pieces of research are added to a substrate of a portion of agar plates, without published results of agar plates alone[17, 18]. Therefore, was chosen pork gelatine with a similar content substrate but more feasible. (In every grocery can be bought). In the experiment, it is worth checking process trends, their spontaneity, and the necessity of regulation of them.

Another aim of the research was checking stressed inoculum behaviour after switching pH after adding proteins. The digestion of proteins should increase ammonia concentration similarly to chicken manure [19]. Proteins composition of collagen is well-known and can serve as a model substrate for protein digestion like glucose (as the carbohydrate) in DF [20] and AD [21]. The addition of collagen with a known mass of methionine and cysteine allows identifying a source of hydrogen sulphide emission. Therefore it can be observed if hydrogen sulphide originates from added collagen, bacteria rests, or both. Hydrogen sulphide emission is a problem of odour in biogas plants and landfills [5]. In the paper were reported analysis and observations of dark fermentation of collagen with hydrogen and methane production.

2. Materials And Methods

The inoculum was collected from an agricultural biogas plant in Darżyno (near Gdańsk), working at a temperature of 38°C, mainly at maize silage. The inoculum was stored for about two weeks to minimize its biogas production and sieved before filling test reactors for removing large particles.

The experiments were carried out in the Laboratory of Biomass Energy Transformation in The Szewalski Institute of Fluid-Flow Machinery in Poland. Experiments were conducted according to the NREL procedure for biogas production [22]. Fermentation setups consisted of nine 2 L glass reactors with a working load of 1.2 L. The tested substrates were placed in the reactor with inoculum. The pH of the digestion of collagen was measured once per week but not regulated. A batch system is one of the most economical first experimental attempt for checking if a selected design/product is worth scaling up. One of the most commercially available is a one proposed by Dach et al. [23, 24] Therefore, such a model was used for checking gelatine hydrogen and methane availability.

Initially, the inoculum was treated by heat shock for 0.5 h at 105°C as recommended [25, 26]. Later, the initial pH of 7.84 was lowered by HCl to pH 5.5 and applied to the DF process. The bacterial layer was pretreated analogous to a Nasirian et al. [27] procedure for DF of wheat straw, but a cheaper 0.1 M solution of HCl replaced the 0.1 M solution of H_2SO_4. As a source of collagen were used pork gelatine from a grocery in powder form. Gelatine was added to the reactor with an inoculum of different concentrations. The collagen was at concentrations from 10 g VSS/L to 30 g VSS/L. The general characteristics are shown in Table 1.

Table 1. Physicochemical characteristics of the inoculum and substrates used in various tests

Material	pH	TS [%FM]	VSS [%TS]
Inoculum	7.6	1.09 ± 0.03	36.35 ± 1.02
Collagen (pork gelatine)	-	89± 0.03	96.5 ± 1.06

The process was established at the temperature of 38°C because it was used in the Darżyno plant and used in some glycerol fermentations [28, 29]. Biogas produced in every reactor was collected in a cylindrical vessel filled with barrier liquid to prevent biogas solubility. The system worked on the principle of connected vessels. All the experiments were carried out in triplicate. There were three reactors with the only inoculum, as prime samples, and 12 reactors with

gelatine. Results were the average values determined. Batch experiments were continued until daily biogas production was less than 1% of total biogas production according to NREL norms [22]. The setups were shown in Figure 1.

The volume of measured methane/biogas was normalized to standard conditions (0°C and 1.013 bar) using Equation 1.

$$V_s = \frac{V_m \cdot T_s \cdot P_m}{T_m \cdot P_s} \quad (1)$$

Where V_s - a volume of measured gas at standard temperature and pressure, V_m - a volume of measured gas at ambient condition, T_m - ambient temperature, T_s - standard temperature and P_s - standard pressure, P_m - ambient pressure.

Biogas measurements were carried out every day at the same time with an accuracy of ±0.0001 dm^3. The qualitative and quantitative assessments of the gases were performed and determined in two stages like it was written in [30]. During the first, the gas was measured using a portable biogas analyzer (GA5000, Geotech), with the volume of biogas in the cylinder being at least 0.45 dm^3. The analyser poses ATEX II 2G Ex ib IIA T1 Gb (Ta from -10°C to +50°C), IECEx and CSA quality certifications, and UKAS ISO 17025 calibration certificate. The equipment allowed measurements of CH_4, CO_2, O_2, H_2, and H_2S in the ranges 0-100%, 0-100%, 0-25%, 0-1000 ppm, and 0-5000 ppm, respectively. During the second stage, when hydrogen concentration was above 1000 ppm, the gas was assessed using a gas chromatograph (GC) GC SRI 8060 with a thermal conductivity detector (SRI) and argon as a carrier (gas flow rate was 0.6 mL/h). A Silco packed column Restek® with characteristics of 2m/2mm ID 1/8" OD Silica was used. The detector temperature was between 46°C and 196°C. The oven was working at a temperature from 23°C to 200°C. The injection temperature (splitless mode) method was 45°C.

Ammonium cuvette tests (Hach, UK) were used for the determination of mg $NH^{4-}N/L$. Samples of liquids were filtered before the analyses with a 0.45 µm membrane syringe-filter (Pureland). The error of measurement was ±0.01 mL. The volatile acid analysis is provided by Ekotechlab lab with characteristics in Table 2.

Table 2: Characteristics of volatile acid contamination and determination of compounds.

Technique and method:	Volatile acids contamination in sample using (GC-FID)
Equipment:	Gas chromatograph Thermo Scientific Trace 1300
Analysis conditions:	Column: Rxi 5MS 60m Gas carrier: helium Flow: 1.0 ml/min The temperature of injection: 250°C Stream separation: 1:10 Detector FID: 300°C Temperature program: from 40°C (3 min) - 20°C/min to 300°C -300°C (5 min)
Sample preparation:	To sample (6 mL) sulphuric acid (VI) (drop 0.25 mL) and sodium chloride (100 mg), then extracted with tert-butyl-methyl ether (2 mL)
Technique and method:	Determination of compound in gas chromatograph with a mass spectrometer (GC-MS)
Equipment:	Gas chromatograph of firm Shimadzu GC-2010Plus
Analysis conditions:	Column: Rxi 5MS 60m Gas carrier: helium Flow: 1.0 ml/min The temperature of injection: 250°C Stream separation: 1:20 Detector MS: 210°C Temperature program: from 50°C (4 min) - 20°C/min to 300°C - 300°C (5 min)
Sample preparation:	To sample (6ml) sulphuric acid (VI) (drop 0.25 mL) and sodium chloride (100 mg), then extracted with tert-butyl-methyl ether (2 mL)

3. Results And Discussion

At prime tests (without collagen), biogas production was not detected The biogas was observed in samples only with added collagen.

3.1 Biogas Pathways

Dark fermentation of collagen required writing some modifications of common reactions pathways for the process. Modifications were made according to the proper analysis mentioned earlier.

Hydrogen production was reported only from protein-rich substrates [31]. The proteins could be a source of hydrogen by DF only in some theories [32].

The volatile acids analysis determined the presence of ammonia, propionic, and butyric acids. The common pathway[33] should be modified to a current substrate, see Figure 2. Therefore the potential reactions of the process

after bottom analysis looked like this.

The processing time of methanogenesis occurrence depended on collagen concentration. In a concentration below 30 g VSS/L, methane production occurred after five days. In 30 g VSS/L, methane production lasted all the time. The heat shock and low pH were insufficient to stop methanogenesis in this case. The process can be classified as dark fermentation in all samples [34]. However, due to Angelidaki et al. [35], there is DF only in the first five days for collagen concentration limited from 10 g VSS/L to 20 g VSS/L and then was hydrogenotrophic anaerobic digestion. In the experiment, there were significant changes in measured parameters during all 55 days of the process: hydrogen production, hydrogen sulphide emission, methane production, ammonia, volatile acids, and pH changes. The results of earlier mentioned biogas components were given for the first 20 days and then all 55 days to stress the transition of the dark fermentation process into anaerobic digestion.

3.2 pH Change, Ammonia, and Volatile Organic Acids Concentrations

The change in pH was caused by the appearance of ammonia and volatile organic acids. Between the 3rd and 6th days, increased pH from 6.0 to 6.2 (10 g VSS/L), 6.3 (15 g VSS/L), 6.5 (20 g VSS/L), and 6.8 (30 g VSS/L). After 15 days pH was between 6.75 (10 g VSS/L), 6.8 (15 g VSS/L), 20 g 6.9 (20 g VSS/L), 7.1 (30 g VSS/L), 7.3 (20 g VSS/L and 10 g VSS/L). Looking at Figure 3 pH oscillations after earlier growth could be discerned between the 18th and 30th day. An increase in ammonia concentration resulted in changes in pH (see Figure 4). The pH growth depended on gelatine concentration. Between the 19th to 39th days of fermentation at 30 g VSS/L of collagen, the pH value reduced and then returned to the earlier point. In this period, pH was lowered by low volatile acids (see Figure 5). In the concentration of collagen from 15 g VSS/L to 20 g VSS/L, pH growth was slower. The sudden decrease in pH was on the 30th day of fermentation at those concentrations. In gelatine concentration, 10 g VSS/L, a drop in pH value started on the 38th day and decreased until the 43rd day (like in concentration 15 g VSS/L and 20 g VSS/L). Then increase of pH values for collagen 10 g VSS/L was higher than in 15 g VSS/L and 20 VSS/L. On the 51st day in concentrations of collagen from 15 g VSS to 30g VSS/L, pH increased beside 10 g VSS/L that reached the lowest value at the end of the process. The growth of pH in the case of 20 g VSS/L 'overtook' other concentrations. pH changes results were the background for liquid compound and biogas components analysis of reason of shifting the process from DF to AD.

Figure 4 showed an increase in ammonia concentration with the growth of collagen concentration. That was caused by the higher decomposition of collagen, resulting in ammonia growth. The highest pH growth coincided with the medium highest ammonia concentration.

Other bottom products were volatile organic acids: butyric and propionic acids. Production of volatile organic acids did not block the increase of pH at collagen concentration 20 g VSS/L. Figures. 6 and 7 showed low hydrogen production in those points caused by conversion with volatile organic acids to methane.. The liquid products influenced the transformation from DF to AD, like in [36] from wheat straw. The biogas relationship coincided with volatile acids and ammonia analysis.

The GC analysis determined the presence of propionic and butyric acid. The highest concentration of volatile acids coincided with the highest pH values see Figures. 4 and 5 – collagen concentration 20 g VSS/L. There was also a production of butyric and propionic acids that slowed down the increase of pH more in 30 g VSS/L than in 20 g VSS/L. Besides concentration 30 g VSS/L, propionic acids occurred in much lower volumes than butyric acids. In 30 g VSS/L, the ratio of butyric to propionic acid is the highest. But the propionic pathway is less spontaneous than butyric [37], and thus more special conditions are required [38]. In the concentration of collagen 30 g VSS/L, butyric acid was on the level of propionic acids. There both acids were in lower concentrations than in other cases. That was caused by immediate methane production that converted volatile organic acids [39]. Visible differences in volatile acid

concentration did not differ much 30 g VSS/L with 20 g VSS/L in pH value. The volatile acids absence in collagen concentration 30 g VSS/L resulted from pH slightly lower than in 20 g VSS/L. The propionic acid concentrations were similar to the digestion of collagen from 10 g VSS/L to 30 g VSS/L. Butyric acid concentration difference in gelatine concentration from 10 g VSS/L to 20 g VSS/L was shrinking. Butyric acids to propionic acids volumes were the lowest in collagen concentration 20 g VSS/L and the highest in 10 g VSS/L. In these concentrations, ranges lower the concentration of volatile acids, the higher pH. Propionic acid in the fermentation of collagen replaced usually occurring acetic acids [40, 41].

3.3 Hydrogen Production

Hydrogen production results were shown in the first 20 days (Figure 6) and 55 days (Figure 7). Division of two periods improved readability of hydrogen growth trends, responding to fluctuations in pH value in Figure. 3.

A significant pH growth from 5.5 to 7.3 (above one pH unit) lasted during the first ten days. Hydrogen production for a collagen concentration of 10 g VSS/L was the highest on the first day. Then until the fifth day, the produced biogas volumes in 10 g VSS/L were not measurable. Comparing Figures 6 and 7, significant progression of hydrogen production was occurring in the first 20 days. The highest collagen concentration 30 g VSS/L resulted in the lowest from all cases volume of hydrogen from the 5^{th} day. Before the 5^{th} day, the lowest volume of hydrogen was for collagen concentration 15 g VSS/L. That was caused by changes in pH that resulted in the growth of ammonia and volatile organic acids. When pH reached 6.8 in concentration 30 g VSS/L and 10 g VSS /L, hydrogen production slowed down, replaced by methane production. When pH reached 6.8 in concentration 30 g VSS/L and 10 g VSS /L, hydrogen production slowed down, replaced by methane production. The pH increased for concentrations 15 g VSS/L and 20 g VSS/L, at the same time like in other concentrations, but hydrogen production did not significantly mitigate methane production (see Figures 11 and 12). In figures, points were in places of measurable volumes of obtained biogas. In 15 g VSS/L, hydrogen production increased with stable and remarkable growth at the 6^{th}, 14^{th} day despite pH value higher than in other cases. pH grew on the 21^{st} day that coincided with the optimal growth of hydrogen production for concentration 15 g VSS/L. The pH changes did block hydrogen production. In collagen concentration, 15 g VSS/L, hydrogen production from the 28^{th} day overtook 10 g VSS/L. In back increase of pH in 30 g VSS/L, after 53^{rd} day met with hydrogen production growth. The constant hydrogen growth coincided with higher production volatile acids concentration and a significant amount of ammonia.

3.4 Hydrogen Sulphide Emission.

Figure 8 and Figure 9 presented cumulative hydrogen sulphide emission. Hydrogen sulphide emission trends (Figures. 8, to 9) were similar to accumulated hydrogen production, grew with collagen concentration from 10 g VSS/L to 20 g VSS/L. In 30 g VSS/L, hydrogen sulphide emission was lower than in collagen concentration 15 g VSS/L and 20 g VSS/L like in hydrogen production. Accumulated hydrogen sulphide emission volumes in those collagen concentrations were higher than in collagen concentration 10 g VSS/L. The emission of hydrogen sulphide increased for all samples, mostly in the first 5 to 7 days like hydrogen production. In the concentration of collagen 15 g VSS/L, hydrogen sulphide initially was lower than in collagen concentration 30 g VSS /L in initial five days as in hydrogen for this concentration. Ratios between hydrogen sulphide and hydrogen production stabilized during 5 or 6 days. After this period hydrogen sulphide and hydrogen production formed a permanent and stable ratio beside gelatine concentration 10 g VSS/L during all processes, see Figure. 111.

Since collagen has a known mass of sulphur, there was calculated by equation (2) using data from Figure 10 (shown in Table 3). Hydrogen sulphide accumulated emission volume multiplied by the density of hydrogen sulphide at room

temperature ρ_{H2S} (1.313 g/cm^3), percentage of sulphur in hydrogen sulphide (94.11%), and divided by the mass of sulphur added with collagen gave the ratio (2).

$$Ratio = \frac{94.11\,\% V_{H2S} \cdot \rho_{H2S}}{Mass\ of\ sulphur\ added\ with\ collagen} \quad (2)$$

The data of sulphur added and emitted in hydrogen sulphide were given in Table 3.

Table 3: Sulphur added with collagen in samples and the ratio sulphur ratio of sulphur converted in emitted hydrogen sulphide %.

Mass of collagen added [g VSS/L]	Mass of sulphur in added collagen [g VSS/L]	The ratio of sulphur converted in emitted H_2S %
10	0.02	46
15	0.03	108
20	0.04	96
30	0.06	26.1

The ratio of sulphur converted as hydrogen sulphide (Table 3) at a collagen concentration of 15 VSS/L proved its origin. It originated from bacterial rests, not only from substrates. This agrees with the conclusion of glycol ethylene fermentation [30]. Because the mass of sulphur in produced hydrogen sulphide was higher than sulphur added from collagen. Hydrogen sulphide emitted with biogas converted 108% of added sulphur added with collagen at gelatine concentration 20 g VSS/L then it could not originate only from substrates.

3.5. Methane Production and Overall Discussion.

Methane was the main biogas product after two days at a gelatine concentration of 30 g VSS/L (see Figures. 11 and 12). Figure. 11, illustrated that in collagen concentration from 10 g VSS/L to 20 g VSS/L until the fifth-day methane production did not occur with increasing pH. Therefore in this time range, there was classical dark fermentation [42]. Later at these three concentrations and collagen concentration of 30 g VSS/L [43] or by others as hydrogenotrophic anaerobic digestion [44]. In collagen concentration, 30 g VSS/L in a time range of the highest pH from the 10th day to the 19th day, the methane production was the highest from all collagen concentrations. Then decrease in pH caused that until the 25th-day increase of methane was lower than in 10 g VSS/L and 20 g VSS/L. On the 25th day, despite lowering pH, there was a relevant increase of methane production to the head before the 35th day (see Figure 12) when collagen concentration 30 g VSS/L reached its minimum pH value (after the 11th day of the process). Afterward increases in methane volumes were not as significant as at collagen concentrations 15 g VSS/L or 20 g VSS/L. The spring of methane production in collagen concentration 15 g VSS/L and 20 g VSS/L coincided with the highest hydrogen production growth, though, a usual increase of methane reduced hydrogen production like in [32]. The methane production from the fifth day for collagen concentrations from 10 g VSS/L to 20 g VSS/L changed rapidly during a time like pH (Figure 3). From the 18th day to the 24th day, the collagen concentration of 10 g VSS/L reached the highest methane accumulated volume from all concentrations (during its increase from pH 6.8 to 7.3). After pH 7.3, the increase in methane production was not as high as in other cases. In this range, methane production increase did not coincide with an increase in hydrogen production. The methane production from 15 g VSS/L was the most changeable of all collagen concentrations in time with rapid pH changes (Figures. 3, 11, and 12). At that concentration, until the 25th day was the lowest (in this time) hydrogen production and hydrogen sulphide emission. The increase of methane

production after the 25th day in collagen concentration was large enough to reach between 38th and 43rd day the highest accumulative methane volume, thus in this range pH was decreasing again to 7.2 value from 7.6. When pH increased the methane volume was lower than in the case of VSS 20 g VSS/L. These figures showed that heat shock stress for block methanogenesis depended on substrate load. If substrate contained proteins, depending on concentration, reduced effect of stress shifting (collagen from 10 g VSS/L to 20 g VSS/L) shifting sooner to hydrogenotrophic methane production (simultaneous dark fermentation and dark fermentation) after 6 days of dark fermentation or methane production completely like for 30 g VSS/L. The hydrogen yield from collagen was in the middle (see Table 4). Gelatine is a quite promising source of hydrogen by dark fermentation. Methane production was very high though inoculum was stressed. The hydrogen production from collagen with pH control was higher than in the case of uncontrolled cotton, potato, and wheat straw fermentation but still twice lower in the case of controlled pH [46]. Looking at Table 4 there can be observed that the highest accumulated biogas production concentrations were different from the most efficient concentrations. The yields of methane and hydrogen were the highest for collagen concentration 10 g VSS/L. The lowest hydrogen sulphide emission yield was in collagen concentration 30 g VSS/L though accumulated hydrogen sulphide emission was higher than at collagen concentration 10 g VSS/L. For the odourless process most suitable was a concentration of 30 g VSS/L. The worst hydrogen yield sulphide accumulated emission and yield is for collagen concentration 20 g VSS/L.

Table 4: Comparison of hydrogen and methane yield from collagen and substrates found in articles.

Substrate	Hydrogen Yield [mL/gVSS]	Accumulated Hydrogen Production [mL]	Methane Yield [L/gVSS]	Accumulated Methane production [L]d	Hydrogen Sulphide Yield mL/gVSS]	Accumulated Hydrogen Sulphide Emission [mL]	Reference
30 g	2.14	64.21	1.63	48.97	0.44	12.53	This study
20 g	7.36	147.2	2.86	57.23	1.53	30.6	This study
15g	6.12	91.77	3.47	52	1.2	18.5	This study
10g	7.65	76.47	3.49	34.88	1.03	10.3	This study
Rapeseed oil 15 g VSS/L			0.007	0.11			[47]
Glycerol 10 g VSS/L	0.08	0.8					[48]
Cow manure with food wastes (butter mixture, palm oil, meat, and margarine) of ratio 1:8			0.31	3.1			[49]
Lipid waste 1.67 g VSS/L (tuna 7.5 % butter 22.3%, apple 27%, banana 27 %, chicken breast 7.5%, bread 1.5 %, pasta 1.5%, minestrone soup 5.5%)	27.93		0.26	0.43			[50]

		46.64			
Protein waste 1.67 g VSS/L (tuna 31.1 % butter 5.5%, apple 7.85%, banana 7.85%, chicken breast 31.1%, bread 3.2 %, pasta 3.2%, minestrone soup 10.2%)	8.02		0.35	0.58	
		13.4			[50]
Switchgrass 5 g VSS/L			0.26	1.5	[51]
The organic fraction of municipal solid waste 5 g VSS/L			0.69	3.45	[52]
Cotton stalk hydrolysate 40 g VSS/L	179	7160			[3]

4. Conclusions

Dark fermentation of collagen from concentration 10 g VSS/L to 20 g VSS/L after five days transferred into hydrogenotrophic anaerobic digestion. At a concentration of gelatine 30 g VSS/L the process was from the beginning hydrogenotrophic anaerobic digestion. The heat shock was not enough to block methanogenesis if collagen concentration was too high (30 g VSS/L). Optimal biogas production was due to accumulative production for the collagen concentration of 20 g VSS/L: 147.2 mL of hydrogen and 57.23 L of methane. The highest yields of hydrogen and methane (7.65 mL H_2 /g VSS, 3.49 L CH_4/ g VSS) were at collagen concentration 10 g VSS/L. Thus collagen is a promising source of hydrogen and methane. In 10 g VSS/L also was the lowest accumulated emission of hydrogen sulphide (10.3 mL of H_2S) though, the lowest yield was for 30 g VSS/L (0.44 mL H_2S /g VSS). The hydrogen production and hydrogen sulphide after stabilization grew with a ratio depending on the concentration of collagen. Collagen can become a model protein substrate like glucose for polysaccharides and glycerol for fats. The hydrogen sulphide emission and sulphur added to the analysis proved that hydrogen sulphide originated in biogas of collagen from bacteria rests more than from substrate. Mass of sulphur emitted with hydrogen sulphide was 108% of added with collagen at concentration 15 g VSS/L- so it could not be the result of digestion gelatine only. The phenomena need further research.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data of the study can be shared, after sending a request to the corresponding author.

Competing Interests

The author declares that he has no competing interests

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Authors' contributions

Gaweł Sołowski performed all works of research beside done by Ekotechlab

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Figures

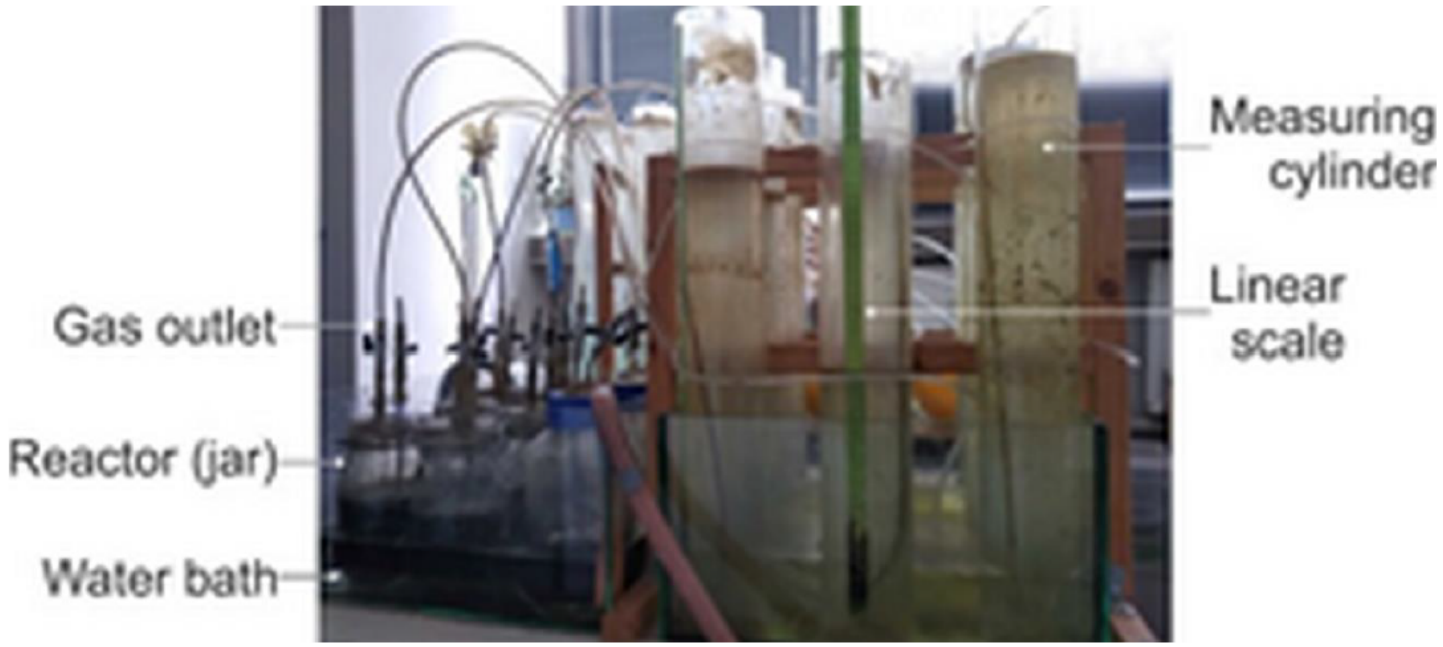

Figure 1

Fermentation setups used in the experiment: 1. glass reactors, 2. A cylindrical vessel for collecting biogas, 3. water bath chamber under mesophilic conditions (38±2°C).

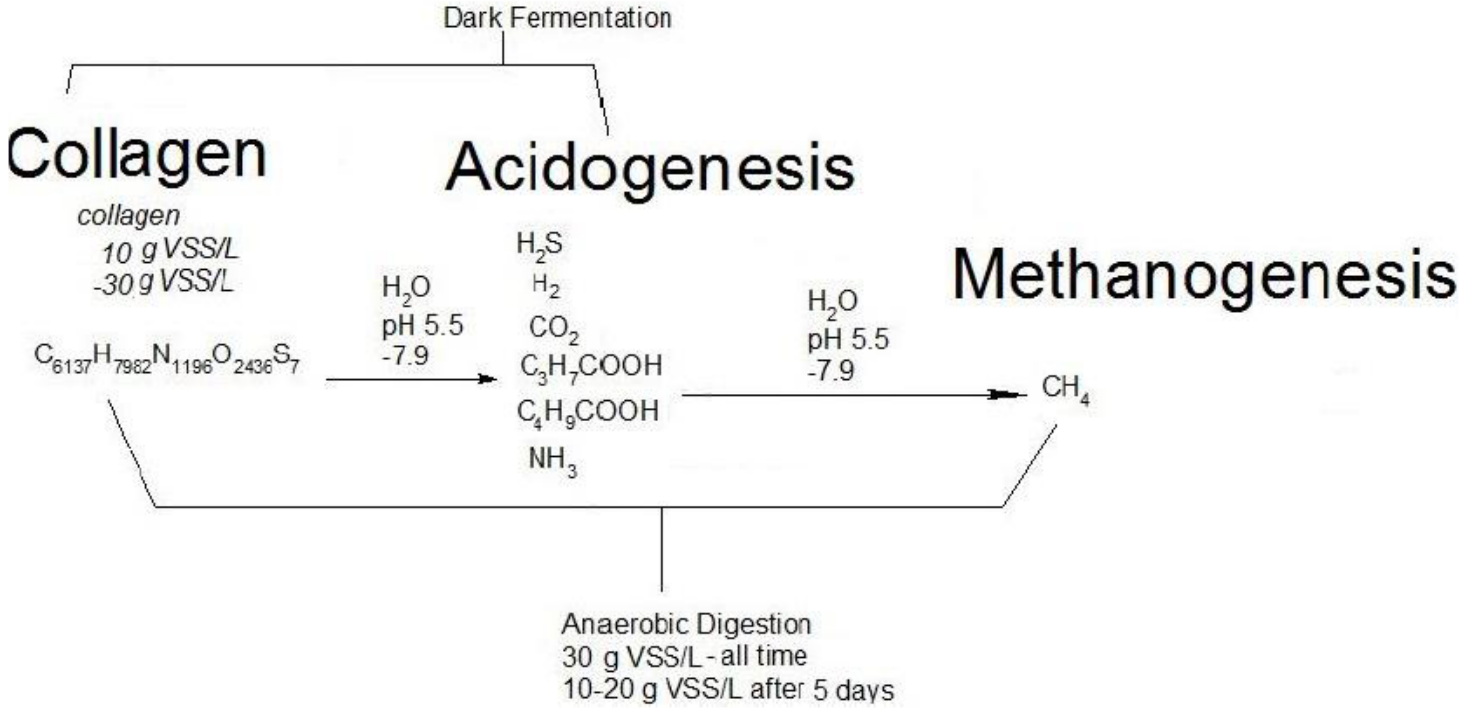

Figure 2

Potential pathways of collagen digestion by DF and AD.

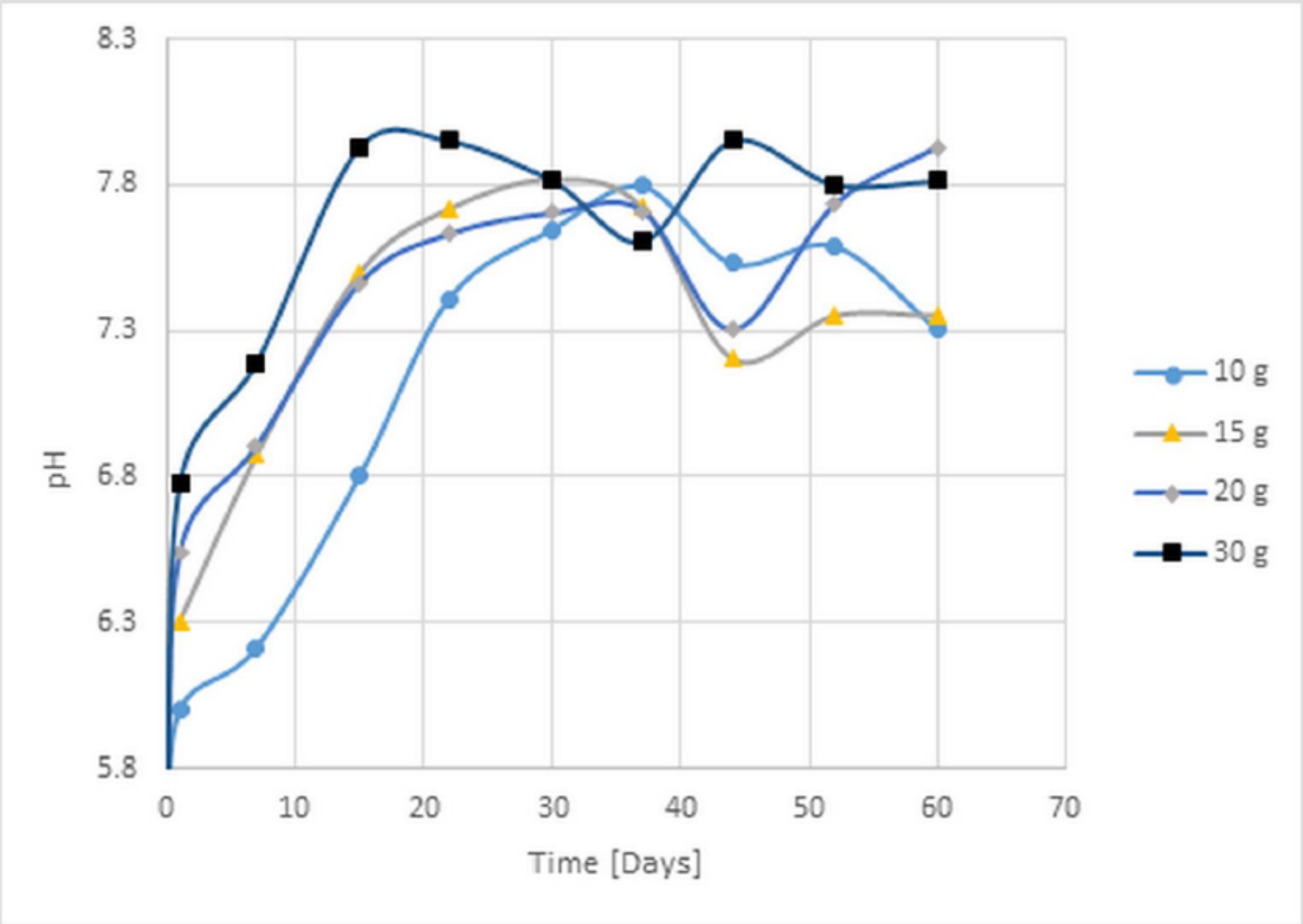

Figure 3

Change of pH value of collagen concentrations from 10 g VSS/L to 30 g VSS/L.

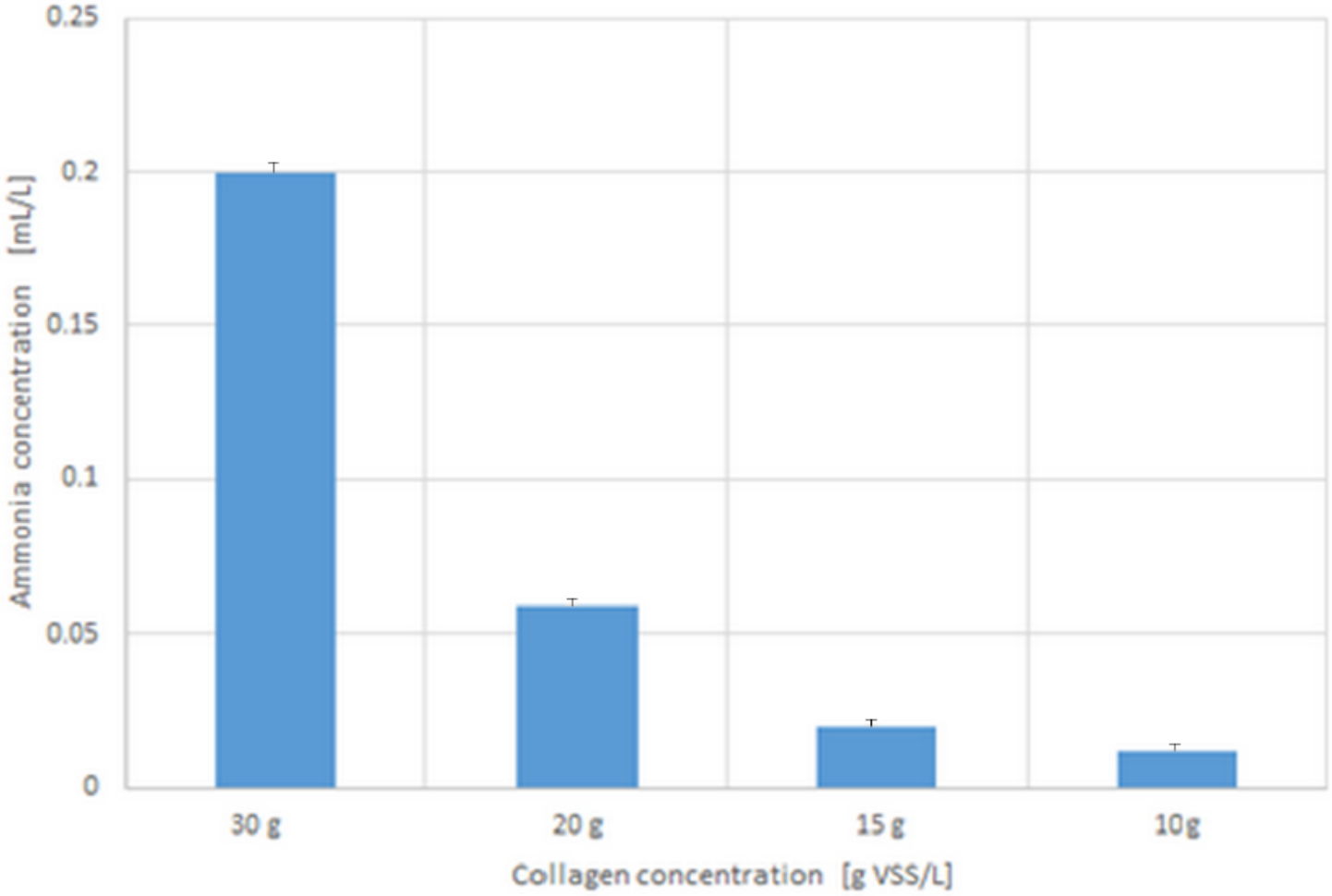

Figure 4

Ammonia concentrations change at dark fermentation of collagen concentrations from 10 g VSS/L to 30 g VSS/L after 55 days

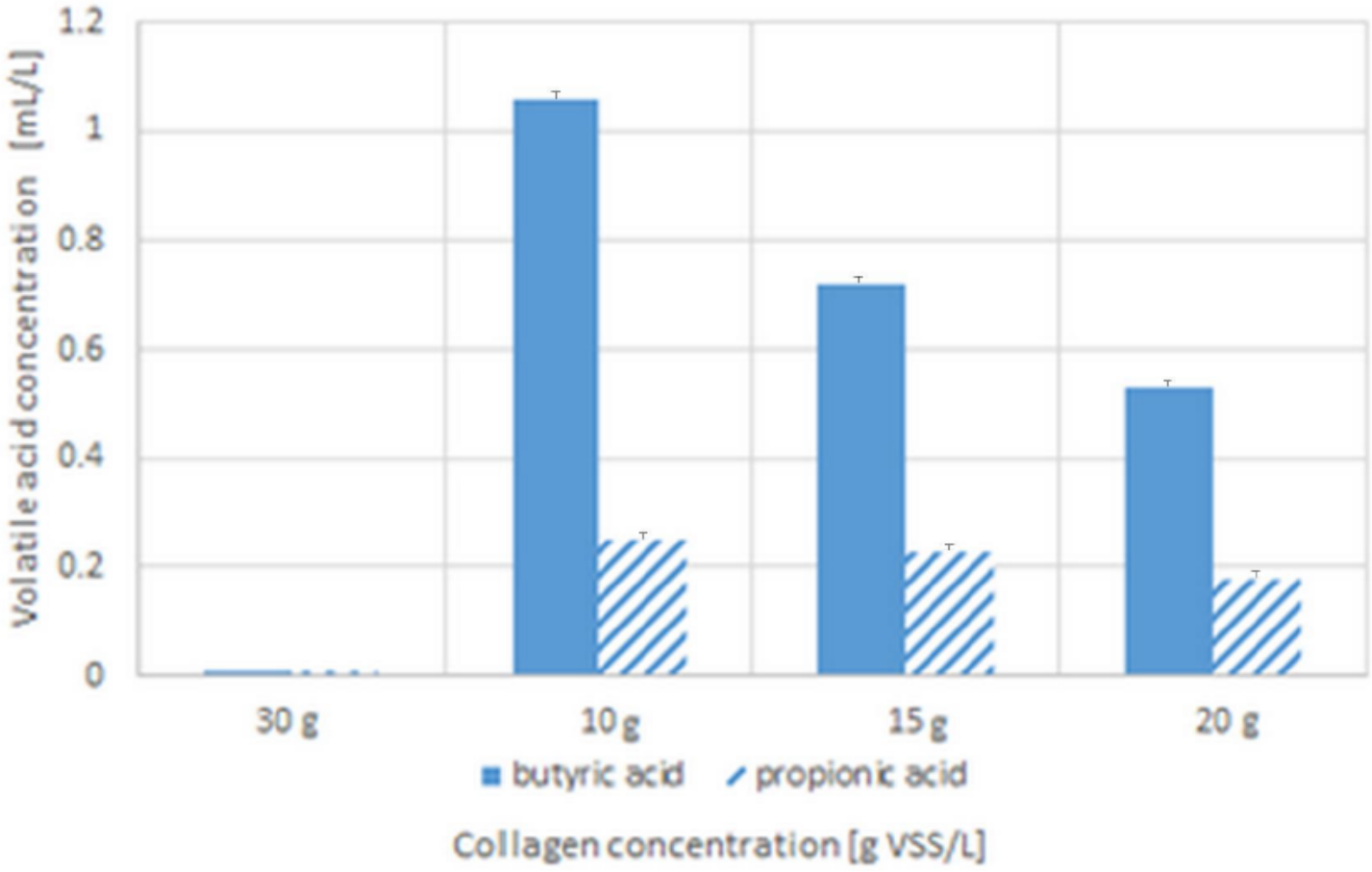

Figure 5

Volatile acid concentrations after 55 days of anaerobic fermentation of collagen concentrations from 10 g VSS/L to 30 g VSS/L.

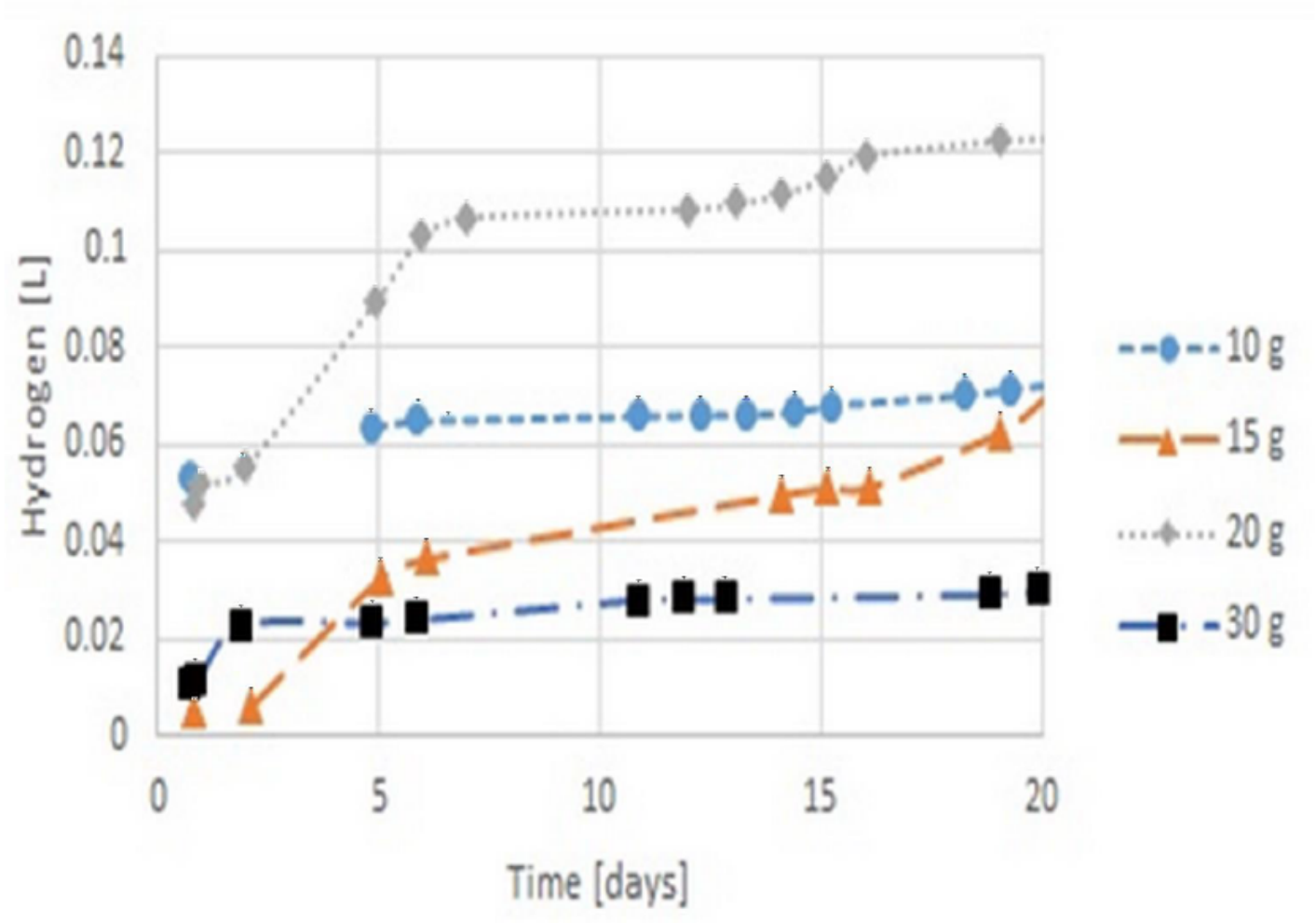

Figure 6

Accumulated hydrogen production from collagen in the first 20 days for collagen concentrations from 10 g VSS/L to 30 g VSS/L.

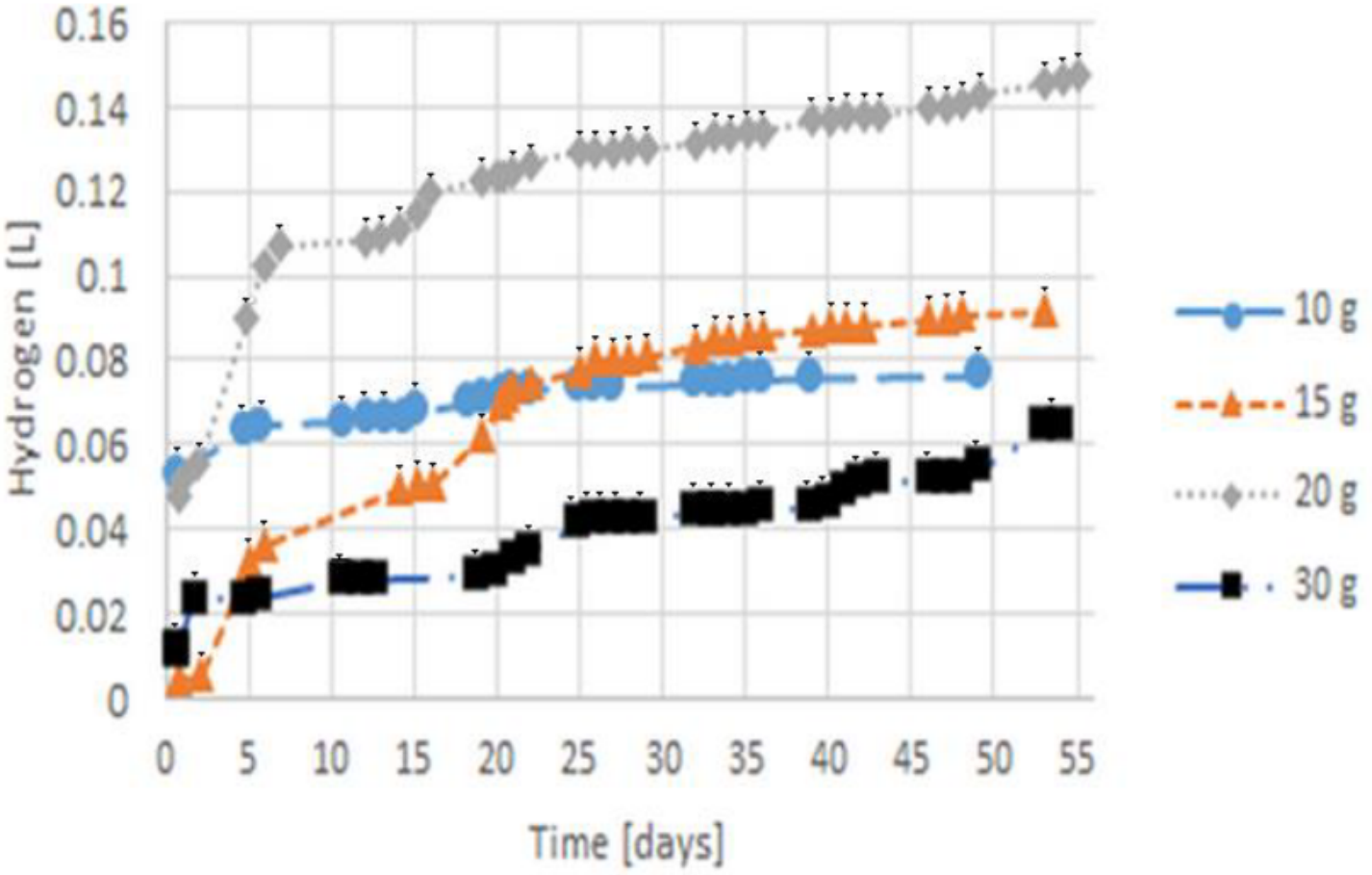

Figure 7

Accumulated hydrogen production from collagen during 55 days for concentrations from 10 g VSS/L to 30 g VSS/L.

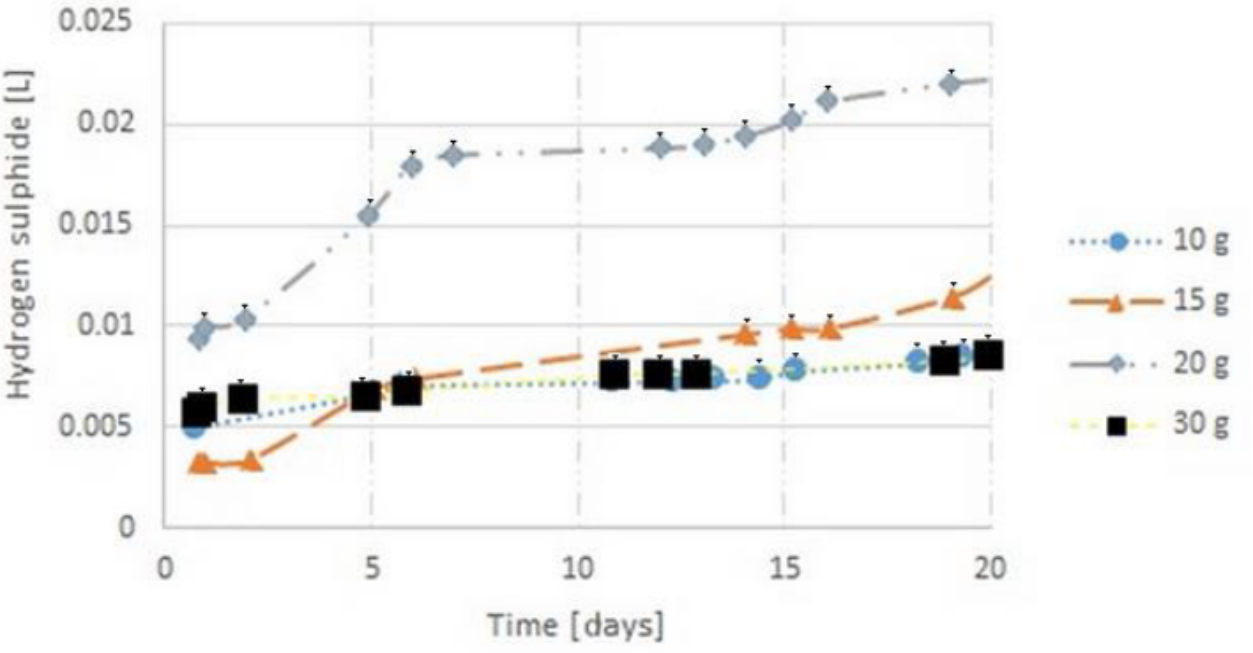

Figure 8

Accumulated hydrogen sulphide emission from collagen in the first 20 days for collagen concentration from 10 g VSS/L to 30 g VSS/L.

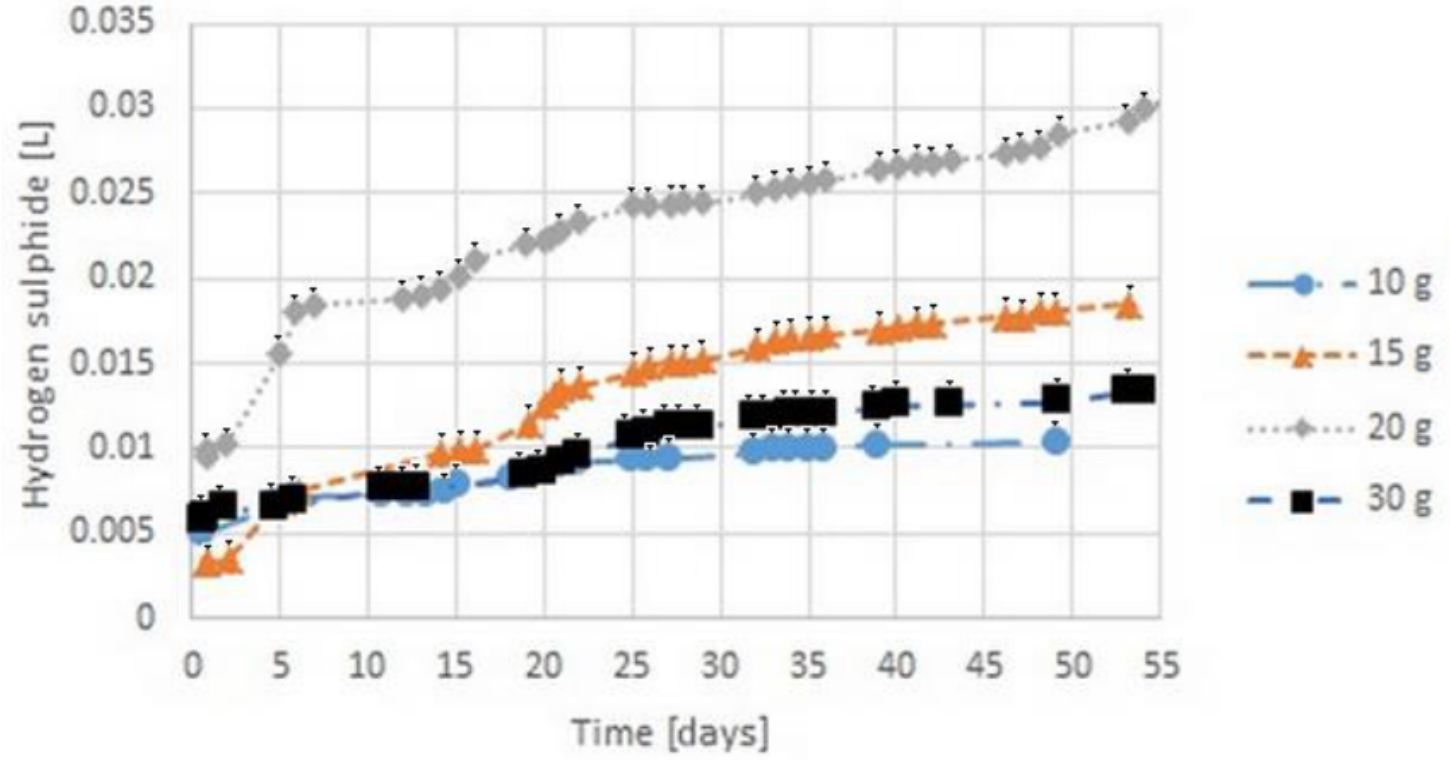

Figure 9

Accumulated hydrogen sulphide emission from collagen in all 55 days for collagen concentration from 10 g VSS/L to 30 g VSS/L

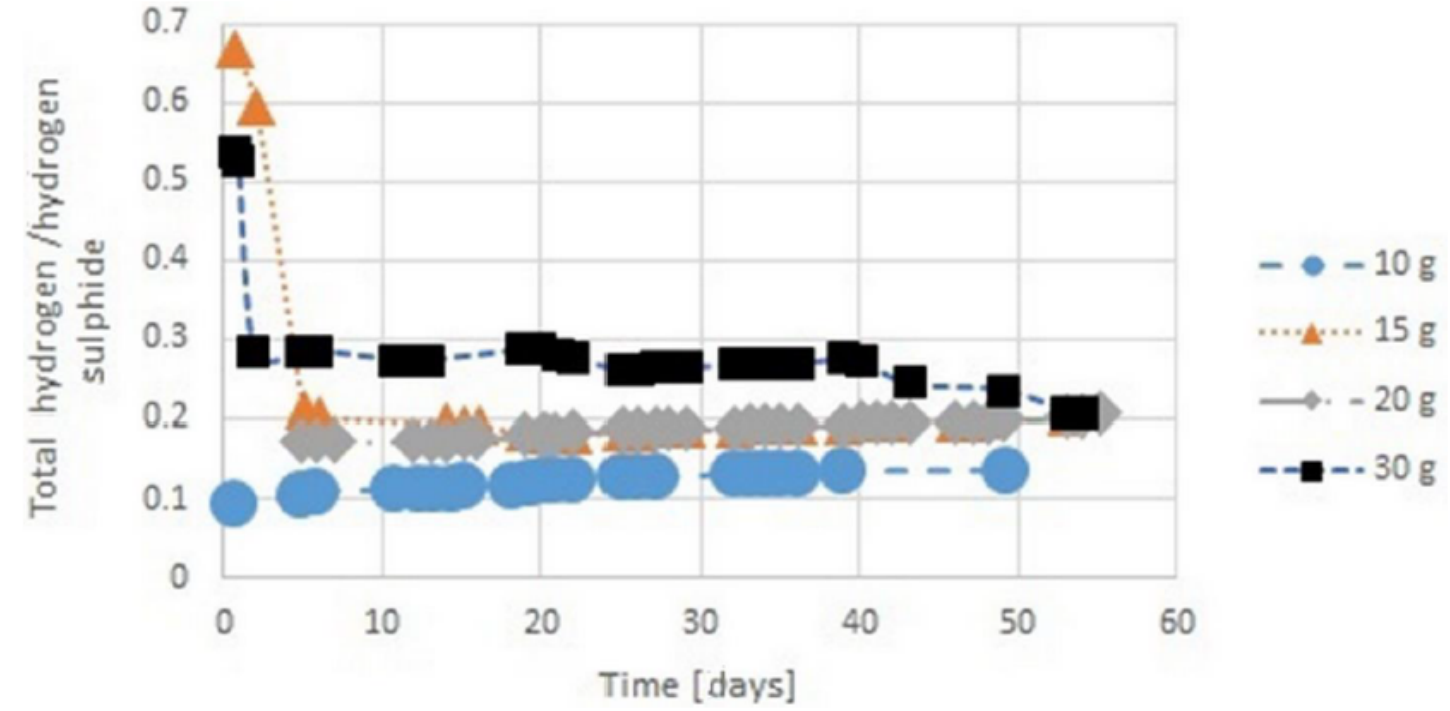

Figure 10

Ratio of total hydrogen to hydrogen sulphide ratio in all 55 days for collagen concentration from 10 g VSS/L to 30 g VSS/L.

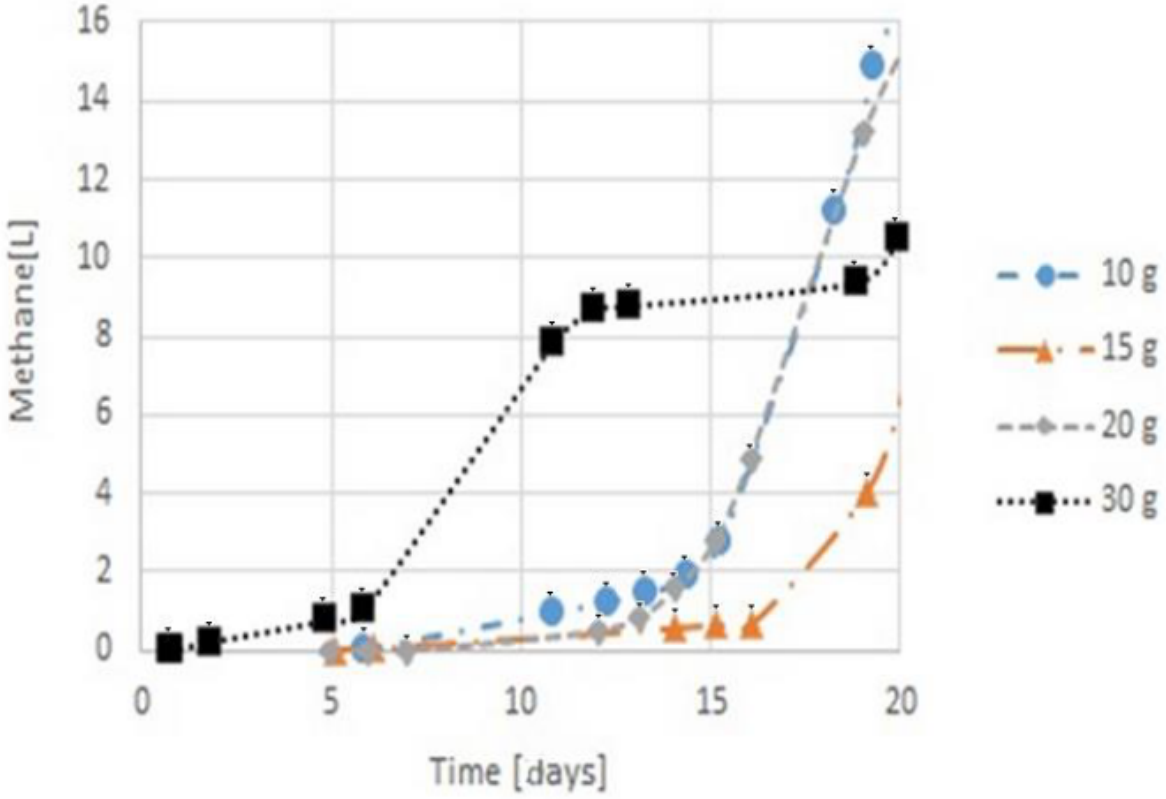

Figure 11

Accumulated methane production in the first 20 days for collagen concentration from 10 g VSS/L to 30 g VSS/L.

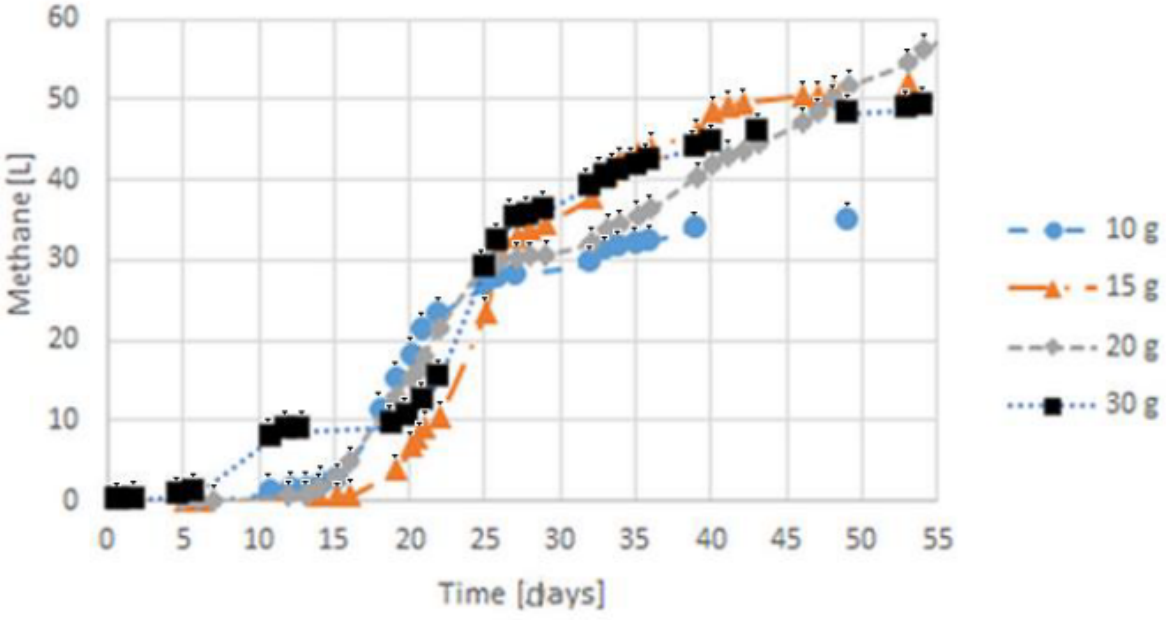

Figure 12

Accumulated methane production in the 55 days for collagen concentration from 10 g VSS/L to 30 g VSS/L.