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Comparison of GC and OxiTop Analysis of Biogas Composition Produced by Anaerobic Digestion of Glucose in Cyanide Inhibited Systems

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Abstract

The purpose of this study was to compare the composition of the biogas produced by the anaerobic degradation of glucose with or without the addition of cyanide (32.0 mg L^{-1}) during the digestion process using the qualitative OxiTop method and the quantitative gas chromatography (GC). For the non-inhibited systems the biogas composition obtained from OxiTop was in good correlation with the results obtained using gas chromatography analysis. On the other hand, a great difference was observed for the biogas composition of the inhibited system. We have shown that the necessary assumption, when using the OxiTop method, that the residual gas after carbon dioxide elimination was only methane, was not accurate for the inhibited reaction system.

Keywords: Anaerobic degradation, biogas, cyanide, gas chromatography (GC), inhibition

1. Introduction

Anaerobic digestion (AD) is a series of processes in which microorganisms break down biodegradable material in the absence of oxygen. AD has been widely used for the treatment of organic sludge and wastewater, biomethanation and recycling, and degradation of organic pollutants.[1–3] As part of an integrated waste management system, anaerobic digestion reduces the emission of landfill gas into the atmosphere. Anaerobic digestion is widely used as a renewable energy source because the process produces a methane- and carbon dioxide-rich biogas suitable for energy production, helping replace fossil fuels. Also, the nutrient-rich digestate can be used as fertiliser.

The biochemistry and microbiology of anaerobic digestion are complex biogenic processes involving a number of microbial populations (hydrolytic, acetogenic and methanogeneic bacteria), often linked by their individual substrate and product specificities.[4,5] There are several major issues to be addressed in methane production: protein content, the presence of salt, dry organic wastes containing less than 80% moisture, fats, oils, grease, inhibitors such as heavy metals ($Cr^{3+,}$ Pb^{2+}, Hg^{2+}, Ni^{2+}), organic pollutants and cyanides.[6]

The scarce literature available on the behaviour of cyanide in anaerobic wastewater treatment is concerned with two different aspects: (a) the toxicity of cyanide and acclimatization of the sludge; and (b) the anaerobic degradation of cyanide.[7,8] Yang et al. reported serious inhibition of methane production at CN^- concentrations less than 1 mg L^{-1}.[9,10] However, the cultures recovered after some time, and the recovery time was longer for higher initial cyanide concentrations. Fedorak and Hrudey using semi-continuous cultures demonstrated that methanogenic consortia can detoxify cyanide when exposed to total concentrations between 5 and 30 mg L^{-1}.[11] Gijzen et al. reported that UASB sludge is very sensitive to 5 mg cyanide per litre without previous acclimatization to cyanide.[12] When the sludge was acclimatized to cyanide, further step-wise increases of cyanide concentration up to 125 mg L^{-1} were possible, while maintaining high methane production and COD degradation efficiency. Acclimatisation and removal of cyanide have been tested for the treatment of cassava and starch processing wastewaters.[12,13] Zaher et al. proposed a pathway for the observed anaerobic aclimatisation and degardation of cyanide.[14] Anaerobic digestion acclimatisation to cyanide, which is an irreversible toxicant, was explained by modelling a population shift between

two acetoclastic methanogens that have different tolerances to cyanide toxicity.[14]

The biodegradation of cyanide under anaerobic conditions has also recently demonstrated the feasibility of concomitant biogas generation, a possible economic benefit of the process.[15] From this perspective the composition of the biogas produced is very important. The OxiTop (WTW, Germany) system offers a quick, simple and continuous method for investigation of the potential inhibition of the anaerobic digestion process over the whole time period.[16] In our previous study the main goal was to determine the impact threshold of cyanide concentration for anaerobic inhibition of a very simple system (glucose) using the OxiTop method. It was shown that substantial inhibition of the degradation of glucose occurred when the concentration of cyanide was above 2.6 mgL^{-1} (with no adaptation in 14 days at 40 °C).[17] It was also observed that the biogas composition depends on the concentration of cyanide. At a cyanide concentration of 2.6 mg/L the molar ratio between methane and carbon dioxide in the biogas produced was app. 1 : 1. At higher cyanide concentrations (up to 32.0 mg/L) the produced biogas contained only CO_2.[17] All these measurements of biogas composition using OxiTop system are based on the presumption that at the end of the degradation process carbon dioxide and methane were the only gaseous products of anaerobic digestion.

The purpose of this study was to re-evaluate the previously reported results of the biogas composition in the anaerobic degradation of glucose with or without the addition of cyanide (32.0 mgL^{-1}) during the digestion process using gas chromatography (GC) which represents a quantitative method.[17] GC allows for a more accurate determination of the biogas composition without any presumptions that are inherently necessary for the OxiTop qualitative method. The results were then compared to the data obtained with the results from the OxiTop method and significant differences were observed when the system was inhibited. Using the GC method also enabled a continuous measurement of the gas composition providing additional information about the biogas composition in particular time periods during the process as well as additional information about the inhibition. This informati on provides valuable insight in the interpretation of biogas composition data in wastewater treatment plants (WWTP) where the OxiTop method is frequently used, as it addresses an inherent flaw in the simple method.

2. Experimental

2. 1. Materials and Solutions

Gas production was continuously measured using the OC 110 OxiTop Control System (WTW Germany), with bottles of 1000 mL volume. The experiments were conducted according to SIST ISO 11734 (1999)[18] and are described in more detail by Tratar Pirc et al.[17]

Inoculum was anaerobic sludge collected from the WWTP. The anaerobic digesters operated at 40 °C and 40 days hydraulic retention time which is the same as sludge retention time. The inoculum was preconditioned for 7 days at 40 °C under argon (dry weight 31–33 g L^{-1}, it contained 53.6% of organic matter). The total content of metal ions in the sludge was: 19 mgL^{-1} Cu^{2+}, 52 mg L^{-1} Zn^{2+}, 416 mg L^{-1} Fe^{3+}, Al^{3+} 166 mg L^{-1} and other less than 1 mg L^{-1}. The buffer solution used in anaerobic digestion experiments consisted of: 4.30 g KH_2PO_4, 10.88 g K_2HPO_4, 16.70 g Na_2HPO_4 $7H_2O$ and 11.10 g, Na_2HPO_4 $2H_2O$, 0.90 g NH_4Cl in a 500 mL flask and dissolved in deionized water, pH value 7.5. This solution was also used for maintaining the appropriate pH value of the media and as a source of the macro elements. Glucose was used as a reference substance in all experiments. The mass concentration of glucose stock solution was 150.0 g L^{-1} (COD = 160 000 mg L^{-1}). Tap water was heated to 50 °C in order to remove dissolved gases, cooled to 40 °C and bubbled with argon for 30 minutes. 0.20 M KCN as a source of cyanide ions (Sigma-Aldrich) was prepared in a 50 mL flask to which 2–3 granules of NaOH (Fluka, Germany) were previously added and diluted the solution with deionized water to the mark. KCN, glucose and mineral salts were analytical-grade chemicals purchased from Sigma-Aldrich.

2. 2. Preparation of Suspensions

Each suspension was prepared in a 500 mL measuring cylinder as follows: 100 mL inoculum, 10 mL phosphate buffer solution and 2 mL glucose stock solution were diluted with previously prepared tap water to 500 mL. The suspension (3.6 g VSS/L) was stirred and 100 mL was poured off for other analysis (pH, determination of cyanide and ammonia concentrations), while the other 400 mL was transferred into the OxiTop bottles. The suspension in the OxiTop bottle was purged with argon and then sealed with plugs. Test sample solutions were prepared as described above, except that a 0.20 M cyanide solution was added in the OxiTop bottle just before sealing to prevent evaporation of HCN. The final concentration of the inhibitor in the suspension was 32.0 mgL^{-1}. Blank sample solutions were run in parallel without glucose or cyanide. The tests were run for 12 days in a thermostatic chamber at 40 °C. After app. 10 days (233 hours) of anaerobic degradation, 2.5 mL of 6 MNaOH was added in the rubber trap of each bottle. From the pressure drop (due to the absorption of carbon dioxide) the amount of carbon dioxide in the biogas was calculated.[17]

The reaction bottles used for the GC analysis were prepared in the same manner as described above, except that they were sealed with gas quick couplings which enabled a connection to the GC. All experiments were conducted in four series. In each set there were three bottles of blank samples (containing only inoculum and the buffer solution), three bottles of reference solutions (inoculum and buffer and

glucose solutions) and three bottles of samples containing inoculum, buffer solutions, glucose and cyanide (32.0 mgL^{-1}). These bottles served as controls and were not measured on the GC. Other bottles containing inoculum, buffer solutions, glucose and cyanide (32.0 mgL^{-1}) were equipped with quick couplings, which enabled the connection to the GC apparatus. Measurements on the GC instrument were performed at 40 °C when the pressure increase in the bottle was the steepest (for non-inhibited systems after 167 hours and for system containing cyanides after 20 hours).

2. 3. Analytical Methods

Cyanide content (SIST ISO 6703/1-1983)[19] and ammonium nitrogen (SIST ISO 5664:1996)[20] in the liquid phase (centrifugates)were measured before and after anaerobic degradation. The pH value of the suspensions was measured by a WTW (Weilheim, Germany) inoLab pH Level 2 pH meter equipped with a SenTixSp electrode. The pH value of the reaction mixture before and after anaerobic degradation did not change significantly (8.0 ± 0.5).

2. 4. GC Experimental Conditions

An Agilent 3000A Micro gas chromatograph was used at the following conditions: gas inlet 1/8" stainless steel capillary, 1 m in length and heated to 120 °C – 130 °C, reference gas Ar 5.0, inlet temperature 140 °C, injector temperature 100 °C, time of analysis 180 s, pre-run pumping time 45 s, post-run pumping time 10 s. Separation columns were Plot U (T = 100 °C, p = 2.068 bar), Plot Q (T = 70 °C, p = 2.068 bar), Molecular sieves (T = 80 °C, p = 2.206 bar), and a thermal conductivity detector.

The gas-tightness of the bottles was controlled over the whole time period of the measurements by measuring the concentrations of nitrogen and oxygen.

The pressure bottle was equipped with couplings and connectedto the GC (see Figure 1). During measurement on the GC the reaction vessel was thermostatted on 40 °C. Inert argon gas was purged trough the reaction bottle at a flow rate of 100 mL min^{-1} for 30 minutes. In that time the overpressure in the reaction vessel dropped almost to zero. A schematically diagram of the measuring system is presented on Figure 1.

For calibration and determination of the retention times of gases, commercial gas mixtures were purchased from Messer: Ar–CO_2 5.0 (96% – 4%), Ar–CH_4 5.0 (96% – 4%), Ar–CO_2–CH_4 5.0 (92% – 4% – 4%), Ar–CH_4–CO–CO_2–H_2 5.0 (84 % –4 –4% –4% –4%), Ar–H_2 5.0 (95% – 5%), Ar–H_2S 5.0 (99.9% – 0.1%). The area of each gas peak was normalized to 1 vol%, except in the case of H_2S where it was normalized to 0.1 vol%.

For determination of the retention time of gaseous HCN, a water solution of sodium cyanide was prepared in the pressure bottles (OxiTop) at different pH values. The boiling point of hydrogen cyanide at a pressure of 101.3 kPa is 26 °C. Since pK_a of HCN is 9.31, the equilibrium between gaseous HCN and CN^- in the solution is strongly pH dependent.[21] 400 mL of distilled water was added to the OxiTop bottle, then purged with argon and 2.40 mL of 0.20 M KCN was added trough the septum. The pH value of the obtained solution was 8.5. The GC apparatus did not detect any HCN gas. In the next experiment the pH value of the sodium cyanide solution was lowered by 0.10 M HCl to 5.0. The GC analyzer detected HCN on Plot U at a retention time of 2.69 min and on Plot Q at a retention time of 1.97 min.

3. Results and Discusion

3. 1. Biogas Composition Using OxiTop Method

As reported previously, the impact threshold of cyanide concentration for anaerobic inhibition is at 2.6

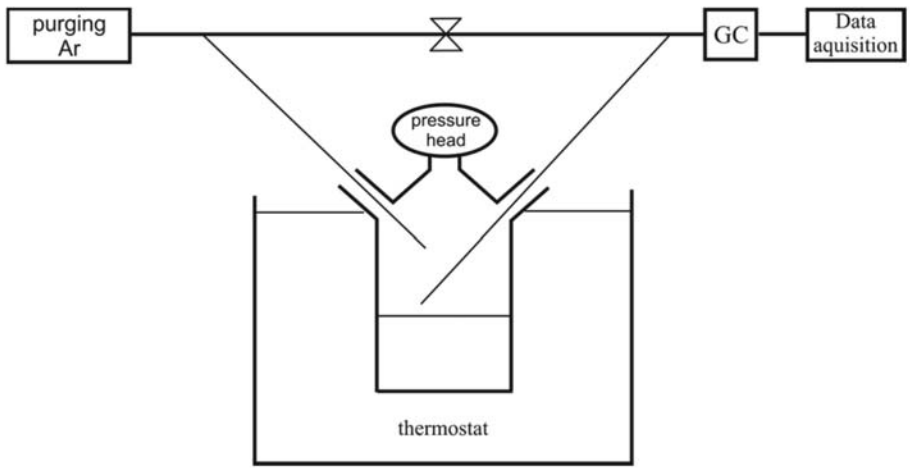

Figure 1. Schematical diagram of the measuring system.

mgL^{-1}.[17] Figure 2 shows the average pressure curves of anaerobic degradation of glucose and glucose with cyanide (32.0 mg L^{-1}) over 12.2 days

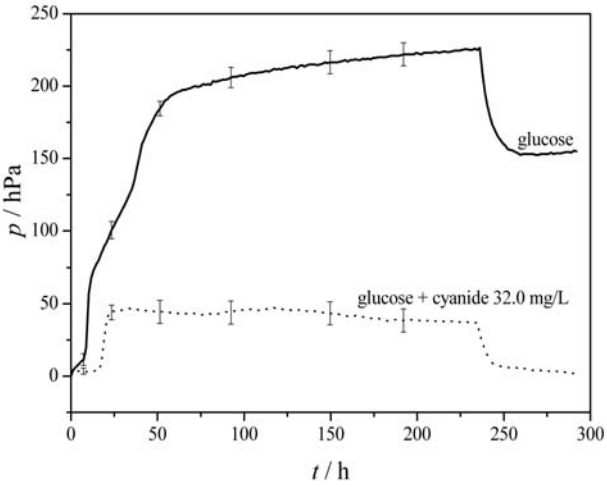

Figure 2. Average pressure values in the OxiTop bottles for the system containing only glucose and for the system containing glucose and cyanide (32.0 mg L^{-1}). The blank curve was subtracted. The drop of the pressure in the reaction bottles is due to the addition of 6 M NaOH in the rubber trap. The bars on the curves represent mean standard deviations of four series of experiments (each concentration in three replicates).

The increase of pressure was the highest in the bottles containing only glucose. The average maximal pressure was 227 hPa (before the addition of NaOH), while the maximal pressure in the bottles containing cyanide was app. 50 hPa. It is shown that inhibition due to cyanide is substantial.

The concentration of cyanide before the anaerobic process was found to be in good agreement with the nominal concentration (32.0±0.5 mgL^{-1}). In the blank and reference samples the measured concentration of cyanide was under the LOD. The content of cyanide after degradation was also under the LOD. On the basis of these results it could be concluded that CN^- was not present in the liquid phase after the degradation process. According to the literature data[14], anaerobic decomposition pathways of cyanide end with ammonia formation, but the ammonia concentration was almost the same before and after degradation (215±25 mg L^{-1}) and the pH value of the reaction mixture after anaerobic digestion was 7.3 (±0.3). It is possible that cyanide was present in the gaseous phase (as HCN) or that it was degraded and incorporated into the active biomass.

After app. 10 days (233 hours) of anaerobic degradation, 2.5 mL of 6 M NaOH was added to the rubber trap of each bottle. The pressure of the biogas dropped due to the absorption of carbon dioxide (see Figure 2). The pressure dropped to almost zero in the bottles containing cyanide ions. From the drop of pressure the molar concentration of carbon dioxide could be calculated.[17] The residual pressure in the bottle after the addition of NaOH is assumed to be mainly due to methane. Assuming that in the gas phase in the pressure bottle no other gases were present, the fraction of methane could be estimated.[16] The biogas produced in the non-inhibited systems was composed of 67 vol% of methane (the rest was carbon dioxide), whereas the concentration of methane in the biogas produced in the inhibited system was below 1 vol%.

To verify this data, the next step of our study was the determination of the biogas composition produced in anaerobic degradation of glucose with and without cyanide (32.0 mgL^{-1}) using the GC.

3. 2. Results of GC Analysis Coupled to Oxitop Bottles for Glucose Digestion

The increase of pressure in the reference bottle containing glucose was the highest in the first 85 hours of anaerobic digestion (up to the beginning of the stationary phase, see Figure 2). In that time period measurements of the gaseous phase in the pressure bottle were carried out. The last measurement was performed at the end of anaerobic degradation (after 167 hours) when the system was in the stationary phase. The Oxitop bottle equipped with a quick coupling was purged with argon, so that all the gases produced were transported to the GC. This protocol ensured a sufficient quantity of gas in the GC apparatus as well as good reproducibility. Consequently a steep drop in the pressure occurred and conditions in the Oxitop bottle changed. The results are collected in the Figure 3.

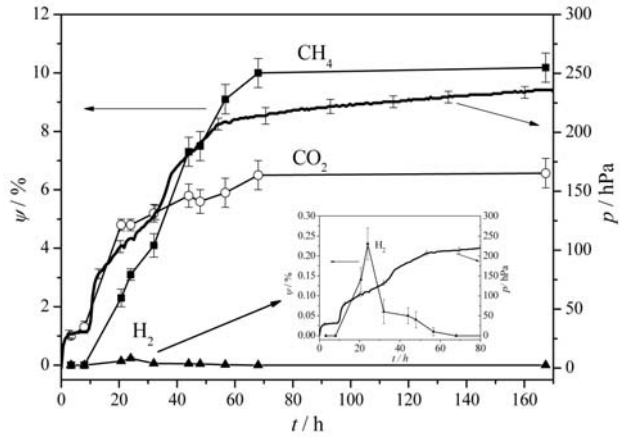

Figure 3. The molar concentrations of CH_4, CO_2 and H_2 in the biogas dependent upon the time of anaerobic degradation of glucose. The bold curve represents the pressure curve of the reference bottle of glucose (not connected to GC). Inserted figure is the enlargement of the increasing concentration of hydrogen in the biogas.

Figure 3 shows the composition of the biogas during the first 167 hours (app. 7 days) of anaerobic digestion of glucose. Carbon dioxide was present at the beginning of

the anaerobic degradation. During the first 25 hours (first step of degradation) the concentration of methane and carbon dioxide increased linearly and the molar ratio between them was app. 1 : 1.5 (Figure 3). In that time period the concentration of hydrogen reached its maximum and after the inflection point on the pressure curve, the concentration of hydrogen decreased (see inserted figure in the Figure 3). Hydrogen was used in the methanogenic phase and after 32 hours of degradation the molar ratio in the gas phase between methane and carbon dioxide was app. 1 : 1.3. Then the molar ratio increased in favour of methane and the pressure in the reaction vessel increased until the stationary phase was reached (67 hours). In the stationary phase the molar ratio of methane to carbon dioxide in the biogas was 1 : 0.67, while the apparatus did not detect any hydrogen. This is in good correlation with the data obtained from the OxiTop system.

The GC detected the presence of other gases in the biogas produced. Nitrogen and oxygen were present in insignificant and constant amounts due to insufficient purging with argon in the preparation of the pressure bottles. Oxygen in higher concentrations could inhibit the anaerobic process since methanogens are very sensitive to oxygen.[22] Although our samples contained trace amounts of oxygen, the experimental results for the reference system (only glucose) showed no inhibition. The concentration of water vapour was as high as expected from the water vapour pressure at 40 °C (temperature of the thermostatic chamber). The GC detector did not register the presence of ammonia or hydrogen sulphide.

3. 3. Results of GC Coupled to Oxitop Bottles for Glucose Digestion with a Cyanide Concentration of 32.0 mgL^{-1}

The increase in pressure in the bottle containing glucose and cyanide (32.0 mgL^{-1}) was the highest in the first 27 hours of anaerobic digestion (Figure 2). In that time period measurements of the gaseous phase in the pressure bottle were carried out and the last measurement was performed at the end of the experiment (after 167 hours).

Anaerobic degradation of glucose with a cyanide concentration of 32.0 mgL^{-1} is shown in Figure 4 (bold curve). After 27 hours of anaerobic digestion the pressure in the reaction vessel increased to app. 50 hPa and the system reached the stationary phase.

In the lag phase the main component of the biogas produced in the AD of glucose with a cyanide concentration of 32.0 mgL^{-1} was carbon dioxide. After app. 17 hours of anaerobic digestion, production of carbon dioxide was intensive and hydrogen formation began. This also caused a steep rise in pressure gain. The molar ratio between carbon dioxide and hydrogen in the log phase was almost constant at 1.5 : 1 in favour of carbon dioxide. After 27 hours, when the system reached the stationary

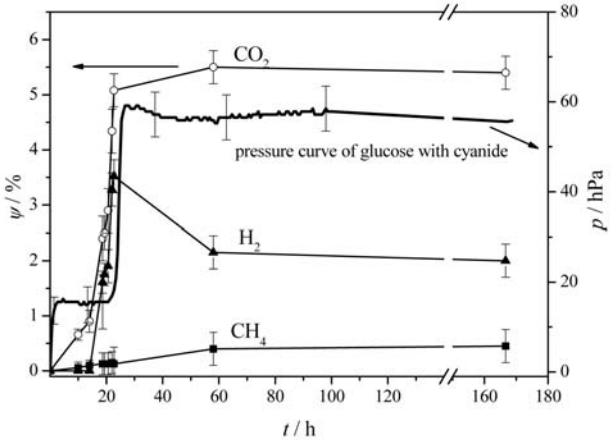

Figure 4. Composition of the biogas produced in anaerobic decomposition of glucose with a cyanide concentration of 32.0 mg L^{-1}. The bold curve represents the pressure curve of the reference bottle of glucose with a cyanide concentration of 32.0 mg L^{-1} (not connected to the GC). Standard deviation bars are shown on the curves.

phase, the concentration of hydrogen decreased slightly due to the formation of methane (see Figure 4). This indicates that hydrogenotrophic archaea were not completely inhibited in the process of methanogenesis, and after adaptation (a longer time period), started to produce methane from hydrogen and carbon dioxide.

The concentration of carbon dioxide in the biogas produced in the AD of the inhibited system was practically the same as in the biogas produced in the reference bottle in the first 27 hours of digestion. In the same time period the concentration of hydrogen in the biogas of the inhibited system was almost 15-times higher than in the biogas produced in the bottle containing only glucose (see Figure 3). This indicated that acetogenesis was not inhibited by cyanides; whereas methanogenesis was substantially inhibited since the concentration of methane was very low (relative to the concentration in the reference biogas).

The GC also detected other gases in the biogas produced, namely nitrogen, oxygen and H_2S in insignificant amounts, and water. The GC did not detect any ammonia or gaseous HCN.

The biogas produced after 167 hours in the AD of glucose at a cyanide concentration of 32.0 mg L^{-1} was composed of 5 vol% of methane, 70 vol% of carbon dioxide and 25 vol% of hydrogen according to the GC analysis. The results for the gas mixture composition calculated from the Oxitop data showed that the biogas is composed of 99 vol% of carbon dioxide and less than 1 vol% of methane. The assumption that the residual gas after carbon dioxide elimination was only methane was thus not accurate for the inhibited reaction system, since the hydrogen concentration was five times higher than the methane concentration and cannot be neglected.

4. Conclusions

The purpose of this study was to determine the qualitative and quantitative biogas composition in particular time periods of the anaerobic digestion process of glucose with or without the addition of cyanide.

The OxiTop respirometric system enabled continuous, quick and simple measurement of the biogas pressure increase during the anaerobic process in the reaction bottle. With the addition of NaOH in the rubber sleeve of the bottle, the gaseous carbon dioxide is absorbed. From the drop of the pressure in the reaction bottle the amount of the carbon dioxide and methane could be calculated on the assumption, that these are the only gases in the biogas produced. The final biogas composition from the anaerobic degradation of glucose, determined using GC, was in good agreement with the results obtained from the Oxitop bottles (30 vol% of carbon dioxide and 70 vol% of methane). The biogas produced from the anaerobic digestion in the cyanide inhibited system contained less than 1 vol% of methane, the rest being carbon dioxide according to the OxiTop method. However, the GC analyses showed that the biogas produced in this process was composed of 70 vol% of carbon dioxide, 25 vol% of hydrogen and 5 vol% of methane. It is shown then that the necessary assumption,when using the OxiTop method, that the residual gas after carbon dioxide elimination was only methane was not accurate for the inhibited reaction system.

Continuous measuring of the biogas composition using the GC method also showed that production of carbon dioxide in the first 27 hours of anaerobic degradation of glucose was the same as for the biogas produced in the systems containing glucose and cyanide (32.0 mg L^{-1}). The molar concentration of hydrogen produced in the first 27 hours in the glucose bottle was low, whereas the molar concentration of hydrogen produced in the glucose bottle with cyanide was app. 15x higher in the same time period. The concentration of methane in the biogas of the inhibited system was much lower than in the non-inhibited one. This indicates that acetogenesis was not substantially inhibited by cyanide, whereas methanogens were. Aceticlastic methanogens were inhibited to a greater extent by cyanide than hydrogenotrophic methanogens since the hydrogen concentration dropped as it was used up in methane production by hydrogenotrophic methanogens.

It is also important to mention that the OxiTop method, while relatively quick and simple, has inherent flaws and should be re-evaluated when dealing with inhibited systems as the assumptions necessary to evaluate the resulting data can vastly differ from the actual system. We have shown in the above example that using the OxiTop method we could determine only methane and carbon dioxide in the biogas from the inhibited system, when in fact the presence of hydrogen could not be ignored as its concentration was actually five times higher than that of methane.

5. References

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Povzetek

Namen raziskave je bil s kvalitativno OxiTop metodo in kvantitativno plinsko kromatografijo (GC) primerjati sestavo bioplina, ki nastane pri anaerobni razgradnji glukoze, če je sistem neihibiran oziroma v prisotnosti cinaida (inhibiran sistem). Sestava bioplina, ki smo jo določili z OxiTop metodo za neihibiran sistem, je primerljiva sestavi bioplina, določeni z GC metodo.Sestava bioplina, določena z OxiTop in GC metodo, pa se je za inhibiran sistem razlikovala. Predpostavka, da je preostali plin, po odstranitvi CO_2, le metan, ki je pri določevanju sestave bioplina z OxiTop metodo nujna, je za inhibiran sistem napačna.