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Anaerobic Digestion of Brewery Spent Grain in a Semi-Continuous Bioreactor: Inhibition by Phenolic Degradation Products

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Abstract

In this study anaerobic digestion of selected lignocellulosic substrate, namely brewery spent grain (BSG), was studied. In order to facilitate anaerobic digestion several types of pretreatment methods were tested such as: mechanical, chemical (alkali and acid) and thermo-chemical. The anaerobic digestion experiments were carried out in a semi-continuous stirred bioreactors with the organic loading rates between 2.9 and 3.9 $kg_{COD}\ m^{-3}\ d^{-1}$ (1.9 and 2.5 $kg_{VSS}\ m^{-3}\ d^{-1}$ respectively) and corresponding hydraulic retention times of 33–39 days. Biogas production and composition, pH, COD, TSS and VSS, short chain fatty acids and phenolic compounds were measured. A significant inhibition of biogas production occurred, depending on the type of substrate pretreatment. There are indications that *p*-cresol is responsible for process inhibition when its concentration in the reaction mixture exceeds critical value between 115 and 240 mg L^{-1}. Anaerobic digestion of chemically pretreated BSG (acid and alkali) and untreated-raw BSG was inhibited between the days 56 and 63 of the experiment, followed by thermo-chemically pretreated BSG on day 112 and mechanically pretreated BSG on day 126. Analyses of the substrates showed no phenolic compounds either in raw-untreated BSG or pretreated substrates, therefore the recorded *p*-cresol is an intermediate degradation product, responsible for process inhibition.

Keywords: Anaerobic digestion, brewery spent grain, inhibition

1. Introduction

The brewing industry generates relatively large amounts of by-products and wastes, such as brewery spent grain (BSG), spent hops and yeast. Utilization of these by-products in a form of animal fodder or compost is well known. However with increasing energy costs the brewing industry, which consumes approx. 4 m^3 of natural gas per hectolitre of beer, strives to convert most of its wastes to alternative energy sources.[1] In such perspective, anaerobic digestion has become an alternative to produce renewable energy through biogas from these waste substrates. Such a process however has not yet been developed due to low biodegradability of the waste. Brewery spent grain contains about 16.8–25.4% cellulose, 21.8–28.4% hemicellulose (mostly arabinoxylans) and 11.9–27.8% of lignin.[2] The major part of BSG constitute the kernel husk, pericarp and seed coat, which are rich in cellulose, non-cellulosic polysaccharides, lignin and also some proteins and lipids. The husks also contain considerable amounts of minerals (silicates).[3] In general, BSG is considered as a lignocellulosic material rich in fibres and proteins, which account for about 70% and 20% of its composition, respectively. However, the structural complexity of lignin, its high molecular weight, chemical stability and insolubility make the biodegradation of this lignocellulosic substrate quite difficult.[4] Lignin is a cross-linked polymer of phenolic compounds, having a very complex molecular structure. It is present in the primary cell wall, imparting structural support, imper-

meability and resistance against microbial attack.[4] Lignin is biodegradable in an aerobic process,[5] although it can get slowly degraded under strictly anaerobic conditions as well. Very little is known about the mechanism, kinetics and products of anaerobic digestion of lignin.[6]

Barley is the world´s most important cereal besides wheat, maize and rice. It is mainly used as an animal feed and as a raw material in beer production.[7] Ezeonu et al.,[8] reported about proposed use of barley wastes like BSG in energy production, either by direct combustion or by fermentation to biogas (a mixture of 60–70% CH_4, 40–30% CO_2 and small fractions of H_2, N_2, H_2S and CO). Production of biogas from BSG by anaerobic fermentation can be efficient only if it is divided into a hydrolytic and a methanogenic step. The hydrolysis of the fibre material in BSG is hindered by the presence of lignin and it is at the same time the limiting step for the complete degradation of the substrate.[9] Microbial hydrolysis of the lignocellulose generates weak acids, furan derivatives and phenolic substances which might inhibit the subsequent microbial degradation steps – acidogenesis, acetogenesis and methanogenesis. Especially the last step is very sensitive, that makes biogas production from lignocellulosic monosubstrates very difficult and practically unsolved.

One possible solution that is yet to be investigated may be pretreatment of the lignocellulose material in order to make it more susceptible to biodegradation. Pretretment can be – in principle – achieved by physical, thermal, chemical or biochemical means. It is known that higher temperature and acidic conditions stimulate cellulose and hemicellulose hydrolysis, followed by formation and release of a range of low molecular weight compounds; similar effect was reported using alkali and certain enzymes.[10,11] Some of the products, however, may have an adverse effect to the consecutive biodegradation process.[12] Phenolic compounds, originating from lignocellulose degradation, have been suggested to exert a considerable inhibitory effect to the biogas production process. The most toxic are low molecular weight aromatic/phenolic compounds: benzene, phenol, cresols, ferulic acid, vanillin, gallic acid, caffeic acid etc...[13–16] Batch anaerobic toxicity assays performed on ten phenolic compounds showed that acetoclastic methanogenesis was progressively inhibited with increasing concentration of these compounds.[14] Different studies showed that aromatic compounds mainly affect final methanogenic step and only partially hydrolytic and acidogenic steps,[16,17] whereas phenol apparently affected all trophic groups of microorganisms.[18]

The aim of this work is to assess the effect of selected pretreatment methods on anaerobic biodegradability of BSG as a potential lignocellulosic substrate for industrial biogas production. In the course of the experiments, due attention was also devoted to the possible causes of the process inhibition.

2. Materials and Methods

2. 1. Raw BSG Composition

Raw BSG from local brewery with annual production of about 100 million litres of brew was used. BSG is a solid material with granular structure of 2–4 mm in size. In order to provide appropriate rheological properties of the inlet mixture, raw BSG was mixed with brewery wastewater in mass ratio 1:2. In these substrates COD and VSS were determined (in order to calculate initial organic load to the reactor) as well as the low molecular aromatic/phenolic compounds and lignin content. Properties and composition of BSG and brewery wastewater are shown in Table 1. Applied analytical methods are presented in section 2.5.

2. 2. Substrate Pretreatment

In order to enhance the anaerobic digestion of BSG, several pre-treatment methods were tested:

- Mechanical pretreatment of BSG was performed by high shear IKA Ultraturrax 65 homogenizer for 10 minutes at 7200 rpm, which fragmented the substrate particles during recirculation through high shear field of the stirrer to the mean particle size below 0.5 mm.
- Chemical pretreatment was performed by maceration of alkalized or acidified substrate by addition of 20% NaOH or 37% HCl solution in order to obtain pH values 10.0 and 2.0, respectively, for 5 days at ambient temperature. In either case, approx. 20 ml of alkali/acid solution per kg of BSG was necessary.
- Thermo-chemical pretreatment was made on the acidified substrate (pH = 2.0), which was heated to 140 °C for 2 hours in a pressurised thermochemical stirred vessel.

Table 1. Composition of the raw substrate

Substrate	COD $g\ kg^{-1}_{sample}$	TSS $g\ kg^{-1}_{sample}$	VSS $g\ kg^{-1}_{sample}$	NDF $g\ kg^{-1}_{TSS}$	ADF $g\ kg^{-1}_{TSS}$	lignin $g\ kg^{-1}_{TSS}$	LMP ($mg\ kg^{-1}_{sample}$)
BSG	320–340	229.32	210.68	539.36	253.29	68.23	n.d.
brewery wastewater	2.8–4.1	1.4–2.8	1.1–2.1	–	–	–	n.d.

COD-chemical oxygen demand, TSS-total suspended solids, VSS-volatile suspended solids, NDF-neutral detergent fibre, ADF-acid detergent fibre, LMP-low molecular phenolics; n.d. – not detected (below limit of detection), – not determined

The pretreated substrates were kept in storage at 4 °C and neutralized with the noted alkali/acid solutions to pH 7.0 just before being fed to the reactor.

Fresh non-treated raw substrate suspension of particle size 2–4 mm was used as a control sample.

2. 3. Bioreactor Inoculum

The anaerobic digestion process started with inoculation of substrate with active anaerobic methanogenic sludge, obtained from a local full-scale CSTR mesophilic digester, fed by pig slurry. The inoculum contained in average 13.0–14.0 $g_{TSS}\,L^{-1}$ and 12.0–13.0 $g_{VSS}\,L^{-1}$. Its specific methane activity (as recorded at the treatment plant) was 0.048 $g_{CH4–COD}\,g_{VSS}^{-1}\,d^{-1}$. The activity was determined according to the procedure of Angelidaki et al.[19]

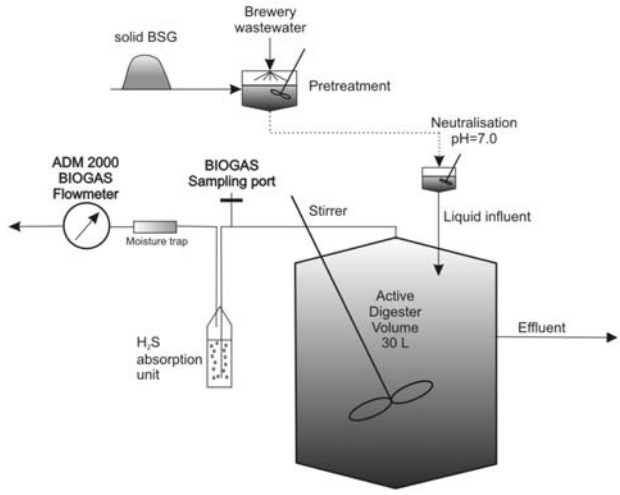

Figure 1. Experimental setup

2. 4. Experimental Setup

Anaerobic digestion process of BSG was studied in a series of parallel pilot-scale semi-continuous bioreactors of 30 L working volume, schematically shown in Figure 1. They were made of stainless steel, equipped with a paddle agitator operating at 50 rpm in order to provide complete dispersion. Temperature in the bioreactor was kept constant at 37 ± 1 °C by means of warm water recirculation through the double jacket.

The volume of biogas produced was recorded continuously by Agilent ADM 2000 biogas flow meters, which were equipped with a H_2S and moisture trap. Anaerobic digestion tests, using the freshly pretreated substrates and raw BSG of pH value 7.0, were conducted at pH between 6.9 and 7.8. Progressive organic loading rate (OLR) was applied to provide adaptation of the microbial community and to avoid overloading the reactor. The initial OLR was gradually increased to maximum OLR in 21 days of operation. The influent substrate was introduced to the reactor once daily. The range of operating conditions in the experiments using various treated and untreated substrates are given in Table 2.

The performance of the reactors was monitored:

- continuously: temperature, pH and biogas production;
- weekly: chemical oxygen demand (COD), short chain fatty acids (SCFAs), phenolic compounds and ammonia-N.

The experiments were terminated shortly after the process began to cease. The inhibition starting point was defined as the time when the average daily biogas production irreversibly decreased for at least 20% in comparison to the previous period of stable biogas production.

2. 5. Analytical Methods

TSS and VSS were analyzed according to APHA Standard Methods.[20] COD was measured by the procedure according to APHA Standard Methods (2005).[20] Ammonia-N was determined by distillation and titration method according to APHA Standard Methods (2005). Short chain fatty acids (SCFAs) were extracted from the samples with diethyl ether according to the procedure of Holdeman et al.[21] and analysed by gas chromatograph Hewlett Packard 5890 with split/splitless injector and flame ionization detector (FID), equipped with fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25 μm stationary phase thickness (Supelco 20988–03A). Quantification was performed by an internal standard method. The injector temperature was 185 °C, the detector temperature 290 °C, the column temperature 75 °C. Analyses of NDF, ADF and ADL were performed following the protocols of Van Soest.[22] Biogas composition was determined by gas chromatograph

Table 2. Bioreactor operating conditions

Pretreatment	HRT	OLR ($kg_{VSS}\,m^{-3}\,d^{-1}$)		OLR ($kg_{COD}\,m^{-3}\,d^{-1}$)		Duration	pH (/)		operating temperature
	(days)	initial	max.	initial	max.	(days)	max.	min.	(°C)
mechanical	33.7±0.1	0.93	3.94	0.59	2.51	126	7.73	6.96	37±1
thermal-chemical	35.1±0.1	1.15	2.91	0.73	1.85	119	7.73	7.02	37±1
chemical-alkali	39.5±0.2	1.50	3.26	0.95	2.07	77	7.67	7.24	37±1
chemical-acid	39.5±0.8	1.40	3.26	0.89	2.07	77	7.65	6.89	37±1
raw BSG	38.7±0.1	1.56	3.22	0.99	2.05	84	7.79	7.32	37±1

Agilent 6890N, equipped with MS detector and capillary column Agilent 113–4362 GS-GASPRO (diameter 0.32 mm, length 60 m), the carrier gas was helium and the temperature of the column was 60 °C . The samples for the detection of aromatic/phenolic compounds were extracted by methyl isobutyl ketone in mass ratio 1:1. Extraction was partially adapted from Zhou et al.[23] They were determined in MIBK phase by gas chromatograph Agilent 6890 N, equipped with MS detector and capillary column HP-5MS (Agilent 19091S-433), length 30 m, diameter 0.25 mm and thickness of stationary phase 0.25 μm. Phenol and p-cresol that appeared during the experiments in detectable amounts were quantified according to the calibration curves. Calibrations were determined by linear regression (R^2 = 0.993, R^2 = 0.996). For the calibration procedures the following concentrations of chromatography grade chemicals were used: phenol in the range from 69 mg L^{-1} to 2451 mg L^{-1} and p-cresol in the range from 58 mg L^{-1} to 2665 mg L^{-1}.

3. Results and Discussion

3. 1. Lignin Content in the Pretreated Substrates

Figure 2 shows the lignin content in the pretreated as well as the raw substrate and the degradation effect of each pretreatment procedure. Lignin content in pretreated samples was analyzed to elucidate lignin fate during the pretreatment. In raw substrate the lignin content was 64.45 g $kg^{-1}_{TSS;}$ the maximum pretreatment effect was achieved in alkali pretreatment (lignin content 5.64 g kg^{-1}_{TSS}) and acidic pretreatment (lignin content 5.94 g kg^{-1}_{TSS}), following the thermo-chemical pretreatment with lignin content 19.40 g kg^{-1}_{TSS}. With the mechanical pretreatment very little degradation was achieved (lignin content 57.17 g kg^{-1}_{TSS}), which was expected since the mechanical force cannot change chemical structure of lignin. According to other authors,[24] using newsprint paper and acetic acid – nitric acid mixture (in concentration of 35% and 2% respectively), about 80% of lignin was degraded. Optimal conditions for 60% delignification and dissolution of hemicellulosic polysaccharides of wheat straw was found using pretreatment with 1.5% sodium hydroxide for 144 h at 20 °C.[25] He et al.[26] reported about 23% lignin degradation of rice straw achieved by 6% sodium hydroxide pretreatment at room temperature for 3 weeks. Cheng[27] stated that about 60–70% of wheat straw lignin was degraded at temperature about 100 °C and 90% at 160 °C. At temperature 200–230 °C for 15 minutes approx. 35–60% of the lignin was degraded.[28] One can conclude that our lignin degradation results using noted types of pretreatment (Figure 2) are comparable to other research done in this field.

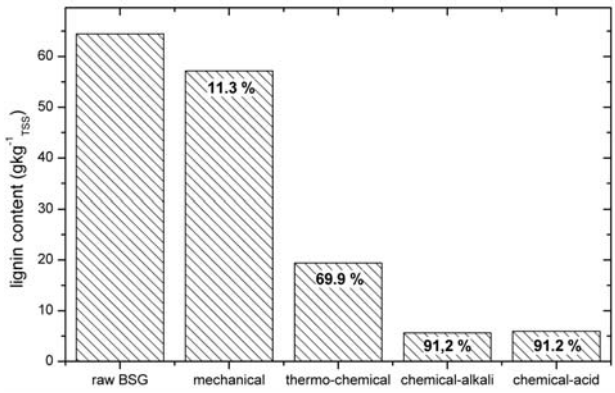

Figure 2. Lignin content and its percentage of degradation in raw and pretreated substrates

3. 2. Biogas Production

All anaerobic digestion experiments were terminated in the time period between 2–3 HRT due to irreversible reduction of biogas production (Figure 3). The phenomenon was first detected on both types of chemically pretreated samples and the raw BSG, and later on thermal-chemically and mechanically pretreated samples of BSG. On the basis of literature data, inhibition of biogas production from the selected lignocellulosic waste was to some degree expected; it was only the question when it would happen for different types of pretreated BSG. The inhibition of biogas production from chemically (acid and alkali) pretreated BSG and raw BSG occurred between the days 56 and 63 of semicontinuous anaerobic digestion process and it appeared evidently much earlier than in biogas production from thermochemically and mechanically pretreated BSG where it started on the day 112 and 126 respectively.

The inhibition time shows no correlation with the extent of lignin degradation, given in Figure 2. Presuming that lignin is likely to be responsible for low biodegradability of such a substrate in anaerobic conditions, the reverse results would be expected. Obviously it is not lignin itself, but its degradation products (polyphenolic and low molecular phenolic substances) which play important role in digestion process. Pretreatment of BSG, regardless of type, makes little help in the respect of the inhibition time; however it does a lot in the respect of physical properties of the substrate particles.

Steady state biogas production in all experiments, prior to inhibition, produced in average 550 L of biogas per kg of BSG VSS with average methane content of 71 ± 1%. Figure 4 shows the cumulative biogas production in the experiments. The gradient of biogas volume produced shows that the biogas production rate in all experiments was in a similar range, between 0.98 and 1.64 $m^3\,m^{-3}\,day^{-1}$ (average values are also shown in Table 3). In the steady state COD removal was between 65 and 77% in all expe-

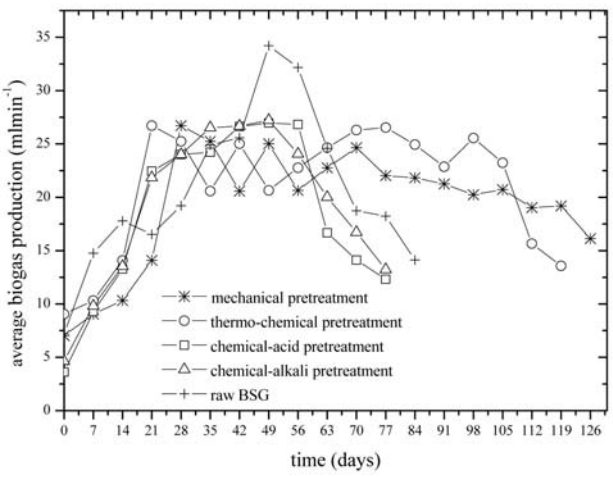

Figure 3. Average biogas production during the experiments

riments. COD removal decreased drastically when inhibition occurred.

Considering possible factors responsible for the process inhibition under given conditions it can not be attributed to the most trivial variables such as organic overload, ammonia load or acidification with SCFAs, as shown later. Attention was focused on low molecular weight phenolic compounds, which had been detected in increased concentrations just before marked inhibition of the biogas production process was observed.

With the onset of inhibition the feeding of the reactors was stopped. During next two days gradual decrease towards 50% of the last value of biogas production was observed. In seven days biogas production nearly stopped, whereas the pH values remained unchanged. Since no recovery of the system biogas production had occurred, the experiments were terminated.

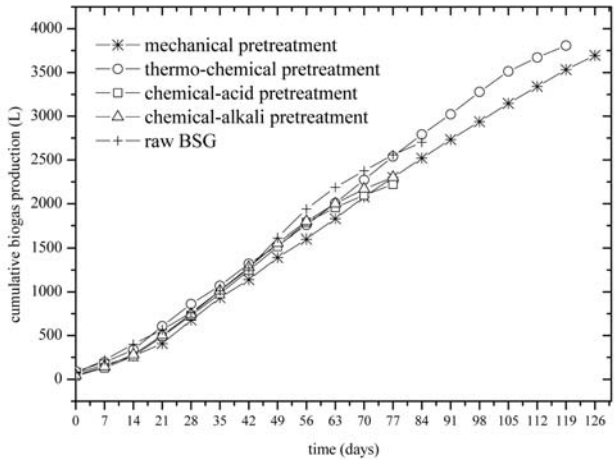

Figure 4. Cumulative biogas production with respect to the waste pretreatment

3. 3. Concentration of Aromatic/phenolic Compounds

In raw BSG as well as in all pretreated substrates before digestion no low molecular aromatic or phenolic compounds were detected. During the experiments using four types of pretreated BSG substrates and untreated raw BSG, progressively increasing concentration of *p*-cresol were mainly detected in the last period of experiments, just before inhibition. Anaerobic digestion of chemically (both alkali and acid) pretreated BSG resulted in p-cresol detection in increasing concentration after the day 56, in thermo-chemically pretreated BSG after the day 63, in raw substrate (control) after the day 77 and in mechanically pretreated BSG after the day 105 (Figure 6 and Figure 7). From these days onward concentrations of *p*-cresol were increasing exponentially until the process collapse in all experiments. Phenol was detected only during anaerobic digestion of thermo-chemically pretreated BSG in concentrations up to 100 mg L^{-1}.

Biogas production decrease in the control experiment using raw BSG occurred on day 60, simultaneously with increasing concentration of acids (Figure 6 and Figure 7), whereas the presence of *p*-cresol was observed with delay – on day 70. This may be attributed to the effect of substrate accumulation in the reactor due to coarse suspension (particles size of raw BSG was 1–4 mm). It was observed that largest particles tended to accumulate, so that the substrate concentration was gradually increasing, which resulted also in larger biogas production before inhibition, compared to other treated substrates. In this experiment inhibition occurred due to combination of physical barrier effect and the consequent organic overload and not due to mechanisms of chemical inhibition by phenolic compounds.

Table 3. The COD inlet concentration, the COD removal rate and the biogas average production rate

Pretreatment	Average COD influent (g L^{-1})	Average COD removal(%)	COD removal at inhibition(%)	Average biogas production rate ($m^3m^{-3}day^{-1}$)
mechanical	107.2	69.6	60.4	1.10
thermal-chemical	101.0	70.4	56.0	1.16
chemical-alkali	101.4	73.1	62.3	1.20
chemical-acid	103.3	74.3	68.2	1.21
raw BSG	104.1	68.6	58.5	1.10

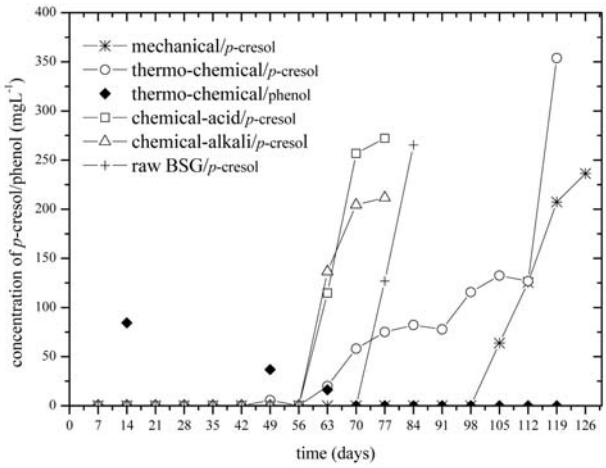

Figure 5. Concentrations of *p*-cresol and phenol during the experiments

Examination of Figure 3 and 5 reveals a correlation between the decreasing biogas production and the increasing of *p*-cresol concentration in anaerobic degradation of all types of pretreated substrates. The inhibitory effect of *p*-cresol on anaerobic digestion of mechanically pretreated BSG became significantly evident when the *p*-cresol concentration reached 236 mg L^{-1}, for thermo-chemically pretreated BSG the detected inhibitory *p*-cresol concentration was 232 mg L^{-1}, for chemically (alkali) pretreated BSG it was 204 mg L^{-1}, for raw-untreated BSG it was 127 mg L^{-1} and for chemically (acid) pretreated BSG 115 mg L^{-1}. In our study, no other aromatic or phenolic compounds were detected in concentration higher than 5 mg L^{-1} (LOD of the method) in any experiment. It may be tentatively assumed that *p*-cresol and to lesser extent phenol are the intermediate degradation products of BSG, which are responsible for the process inhibition. They are formed by slow biodegradation of lignin or its polyphenolic fragments, produced during thermo-chemical pretreatment. Consortia of microorganisms is to some extent able to conduct their degradation, however when production prevails, accumulation can reach the critical concentration 100–240 mg L^{-1} of *p*-cresol that eventually leads to inhibition of rate limiting step – methanogenesis and irreversible decay of the process.

The inhibition effects of pure p-cresol, pure phenol and their mixtures on biogas production in various arrangements have been demonstrated on laboratory scale by Hwang & Cheng,[29] Veeresh et al.,[30] Watson-Craik et al.[16] and Ho et al.[31] The inhibitory concentrations ranged from 100 to 400 mg L^{-1} for *p*-cresol and up to 1000 mg L^{-1} for phenol, depending on the experimental system and source of methanogenic biomass. Suspended microbial cells were more susceptible to *p*-cresol inhibition than immobilized e.g. granulated biomass. Razo-Flores et al.[32] demonstrated for example that *p*-cresol concentrations higher than 700 mg L^{-1} caused severe inhibition to the granulated methanogenic consortium in an UASB reactor. Phenol was demonstrated to be less toxic than *p*-cresol and it was degraded to propionic acid under anaerobic conditions.[16]

3. 4. Concentrations of SCFAs (Mainly the Acetic Acid and Propionic Acid)

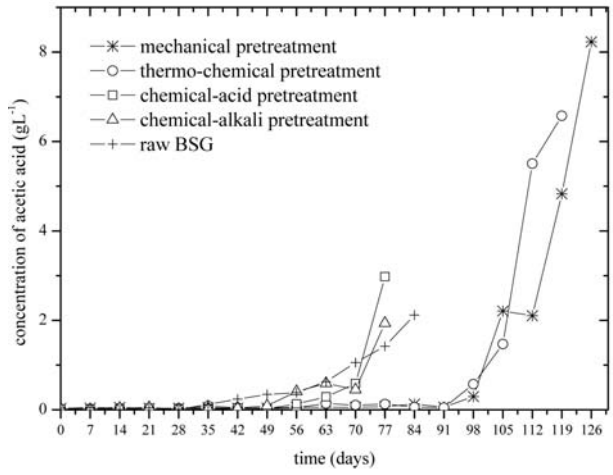

Figure 6. Concentration profile of acetic acid in the experiments

During the process of biogas production from pretreated BSG substrates and raw-untreated BSG mostly acetic and propionic acid were detected among all different SCFAs. Extent of propionic acid occurrence was noted to a much lesser extent than the acetic acid (Figures 6 and 7). Acetic acid began to accumulate constantly from day 56 on (both chemically pretreated BSG and raw BSG) and reached the inhibitory concentration for methane production of 1.0 g L^{-1} at pH of 7.0,[33] between days 70 and 77. The inhibitory concentration of acetic acid in anaerobic digestion of mechanically and thermo-chemically pretreated BSG was reached much later, between days 98 and

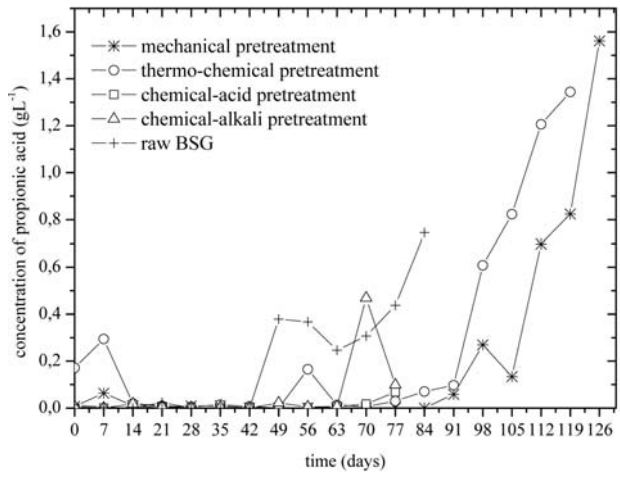

Figure 7. Concentrations of propionic acid during the experiments

105. Observing the data on Figures 3 to 7 conclusion can be made that accumulation of acetic acid is matched with premature increase of *p*-cresol (in all types of pretreated substrates), with the consequence of reduced microbiotic activity and biogas production.

The concentrations of propionic acid in anaerobic digestion of all pretreated BSG substrates and raw-untreated BSG generally followed the same trend (Figures 6 and 7) although in some cases (both types of chemically pretreated BSG experiments) the increasing of concentrations was not strictly regular. The inhibiting concentration of propionic acid[32] at pH = 7.0 is approx. 0.7 g L^{-1}, although it may vary substantially in different methanogenic systems and may be much lower.[34] Comparing the concentration profiles of acetic and propionic acid (Figures 6 and 7) during biogas production from pretreated BSG it could be concluded that stronger inhibition of acetoclastic than hydrogenotrophic methanogenesis occurred by lignin degradation products. In opposite case propionic acid would accumulate to a higher degree. The coinciding increasing concentrations of SCFAs, accumulation of *p*-cresol and decreasing biogas and methane production confirm the inhibition of methanogens in anaerobic microbial consortium by *p*-cresol.[16] SCFAs are important intermediary degradation products in the metabolic pathway of anaerobic fermentation. In case of inhibited methanogenesis, acetate and H_2 accumulate and inhibit acetogenic bacteria, resulting in SCFAs accumulation and a decrease of pH, what ultimately leads to failure of the bioreactor performance.[35,36] The acids concentration in this study do rise to the critical concentrations for inhibition however as a consequence of factors, characteristic for lignocellulosic substrates.

3. 5. Concentration of Ammonia-N

The concentrations of ammonia-N in anaerobic digestion of chemically-alkali pretreated BSG increased from initial 1.08 to 2.29 g L^{-1}, in chemically-acid pretreated BSG from 1.24 to 2.20 g L^{-1}, in thermo-chemically pretreated BSG from 2.03 to 2.32 g L^{-1}, in mechanically pretreated BSG from initial 1.99 to 2.95 g L^{-1} and in control experiment with raw-untreated BSG from 1.23 to 2.25 g L^{-1} (Figure 8).

Ammonia-N is produced as a by-product of anaerobic digestion, mainly from proteins and amino acids. Considering the pH range (Table 2) and the concentrations of ammonia-N (and consequently free ammonium) during our study, the concentration could not be considered inhibitory for the methanogenesis. Mata-Alvarez et al.[37] proved the sufficient adaptation of the anaerobic microflora to the ammonia-N concentration of 3.1 g L^{-1} in the bioreactor. Significant inhibition of the methanogenesis may be expected at concentration between 5.4 and 5.8 g L^{-1} ammonia-N.[38, 39]

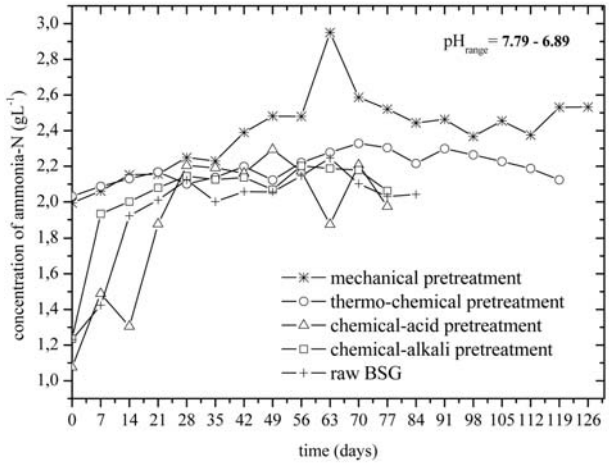

Figure 8. Concentration of ammonia-N during the experiments

4. Conclusions

As has been shown from the experimental results, BSG as a mono substrate cannot be successfully digested in a semi continuous anaerobic bioreactor, regardless of its pretreatment. None of the four types of tested pretreatment methods produced the material that would be readily converted into biogas by a steady state process. The conventional criteria for this type of operation, i.e. stable operation of the process for at least three HRTs, has not been met. The inhibition of biogas production from chemically (acid and alkali) pretreated BSG and raw BSG occurred between the days 56 and 63 of anaerobic digestion and from thermo-chemically and mechanically pretreated BSG inhibition was noted on the day 112 and 126 respectively. The HRT in the experiments was 33–39 days. In the best case the experiments were carried out 3.1–3.7 HRTs, however this figures must be reduced for one HRT needed for initial biomass adaptation.

It can also be concluded from the results that biogas production from BSG as a potential lignocellulosic substrate is inhibited by intermediate lignocellulosic biodegradation products, p-cresol being the most responsible. Different pretreatment procedures of the selected substrate, tested in the study (mechanical, thermo-chemical, chemical) did not prevent the inhibition. Mechanical and thermo-chemical pretreatment only delayed the inhibition for about 60 days in comparison with chemically (acid or alkali) pretreated BSG. Different type of pretreatments of BSG evidently influenced microbial degradation of lignocellulose and/or further metabolism of lignin degradation products; they could have also influenced the structure of microbial consortium by selection pressure.

Different pretreatment methods resulted in very different levels of lignin degradation in BSG. Chemical

(acidic or alkali) achieved over 90% of lignin degradation, whereas the mechanical and thermo-chemical treatments showed very little effect. Degree of lignin degradation during pretreatment adversely affects the digestion efficiency. It seems that lignin degradation products (e.g. polyphenolic compounds), released during thermo-chemical pretreatment, are stronger precursors to anaerobic process inhibition than lignin itself.

Further work is needed in order to develop a stable biogas production process from BSG as a mono substrate without inhibition. Here are some suggestions:

- biological pretreatment of raw BSG with lignolytic fungi or bacteria that might produce non-inhibitory or less inhibitory substrate for anaerobic digestion;
- selective adaptation of anaerobic microbial biomass to higher concentrations of p-cresol;
- selection of alternative inoculum that would contain microbial biomass, more resistant to p-cresol intoxication;
- co-digestion of BSG and complementary (non-cellulitic) easily degradable substrate that would prevent phenolic intermediates to reach critical (inhibition) concentration.

5. References

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Povzetek

Pivovarniške tropine so zaradi visokega bioplinskega potenciala izredno atraktivne za proces anaerobne razgradnje. Vendar pa so zaradi vsebnosti lignina težko biorazgradljive, ker nekateri produkti delujejo zaviralno na metanogene bakterije in arheje. Poskusi anaerobne razgradnje so se izvajali v polkontinuirnih pretočnih reaktorjih s popolnim premešanjem (CSTR) v mezofilnem območju (37 °C); delovni volumen reaktorjev je bil 30 litrov. Za inokulum smo uporabili sveže anaerobno metanogeno blato iz predelave prašičje gnojevke. Kot substrat smo uporabili pivovarniške tropine s 6,5 % s.s. lignina in velikostjo delcev 1–4 mm v kombinaciji s pivovarniško odpadno vodo (masno razmerje 1:2). Substrat je bil predhodno obdelan z naslednjimi tehnikami: mehanska, kemična (kisla in alkalna) in termo-kemična (kisla). V vzorcih, odvzetih iz biokemičnih reaktorjev enkrat tedensko, smo analizirali amonij, kratkoverižne maščobne kisline, kemijsko potrebo po kisiku in vsebnost aromatskih spojin. Dnevno se je izvajala meritev pH vrednosti in produkcija bioplina. Meritve produkcije bioplina so se izvajale *online* z merilcem pretoka bioplina ADM 2000. Opisana tehnika se je obnesle kot zelo učinkovita (povprečna proizvodnja bioplina 550 L/kg TSS), vendar pa so se po določenem času anaerobne razgradnje substrata pojavile težave z inhibicijo. Le-to pripisujemo sproščanju in kopičenju *p*-krezola in deloma fenola, vzporedno pa tudi ocetne in propionske kisline. Pri anaerobni razgradnji mehansko predobdelanega substrata smo zasledili pojav inhibicije pri koncentraciji *p*-krezola 236 mg/L, pri termo-kemičnem v mezofilnem območju pri koncentraciji 232 mg/L, pri termo-kemični v termofilnem območju pri koncentraciji 184 mg/L, pri kemični-alkalni pri koncentraciji 204 mg/L in pri kemični-kisli pri koncentraciji 115 mg/L. Ostali za inhibicijo potencialni parametri niso bili v kritičnem območju. Pri neobdelanem substratu je bil najverjetneje vzrok za inhibicijo preobremenjenost reaktorja, zaradi kopičenja nerazgrajenih velikih delcev. Na podlagi rezultatov lahko zaključimo, da je čas nastopa inhibicije produkcije bioplina iz pivovarniških tropin v CSTR reaktorju odvisen od vrste predobdelave substrata, ki vpliva na razgradnjo lignina. Noben tip predobdelave ni zagotovil stacionarnega obratovanja reaktorja v trajanju najmanj treh hidravličnih zadrževalnih časov, kar je običajni kriterij za te procese. Podani so predlogi za dodatne raziskave za doseganje tega cilja.