The influence of pre-incubation, storage and homogenization of inoculum for batch tests on biogas production

M. Gerber*, N. Schneider*, A. Kowalczyk*, S. Schwede*, Z. Rehman* and R. Span*

* Institute of Thermodynamics, Ruhr-Universität Bochum, Universitätsstr. 150, Bochum, Germany
(E-mail: *m.gerber@thermo.rub.de*)

Abstract
Batch tests are used to determine biogas production potential and degradability of substrates and their mixtures. There are guidelines for the experimental setup, but nevertheless the variation of biogas production rates of the same substrate is very high, what can be seen from results of ring tests. Therefore, different experimental setups were investigated to evaluate influencing parameters. This paper addresses the influence of pre-incubation, storage and homogenization of digestate as inoculum. After one week the biogas production of pre-incubated digestate was reduced by 50%. A pre-incubation of digestate for batch tests longer than one week is not reasonable, due to the very slow decrease of the biogas production rate after that time. Biogas production of cooled stored samples decreases as well. For this reason samples should be used immediately. A comparison of filtered and homogenized digestate compared to untreated one shows a high gas production for homogenized samples caused by disintegration. Filtered inoculum can be recommended for usage as inoculum, due to the lowest biogas production rate and a higher homogeneity.

Keywords
Batch test; inoculum; pre-incubation; storage; homogenization; filtration

INTRODUCTION

Batch or biochemical methane potential (BMP) tests are used to determine the potential biogas production rate and degradability of substrates and substrate mixtures as well as to qualitatively evaluate the speed of anaerobic degradation and possible inhibitions at certain concentrations. In spite of existing guidelines, such as VDI 4630 or ISO 11734, there is an ample scope for the experimental setup of batch tests. Depending on test equipment and test procedure the determined biogas production rate and quality can vary significantly as shown in ring tests. Despite using cellulose as a very homogeneous test material the variation coefficient at a ring test of KTBL for 30 laboratories was 8%, for silage 10% (Wulf & Döhler, 2009). Rozzi & Remigi (2004), Guwy (2004) and Müller et al. (2004) give an overview to a variety of standardized methods for anaerobic biodegradability tests and define influencing parameters such as equipment, operating conditions, methods of analysis, sample properties and inoculum. Even if the batch test procedure can be harmonized, there will be variability in the results due to biological nature of inoculum, due to the origin, concentration, activity, pre-incubation, acclimation/adaption and storage source (Raposo et al., 2011). The presented work shows the influence of pre-incubation, storage and homogenization of digestate used as inoculum.

MATERIALS AND METHODS

For the batch tests a digestate of an industrial biogas plant fed with cow manure, corn silage, chicken manure, ground ear maize, an iron supplement and trace elements was used as inoculum. All tests were performed without any substrate, only with the described digestate. Filtered digestate was used to reduce inhomogeneity of the samples to a minimum. In every batch test inoculum with 7 g of volatile solids (VS) was mixed with tap water up to a sample volume of 400 ml. The tests were operated at a temperature of about 40°C.

Produced biogas volume was usually measured daily by a 400 ml eudiometer using a barrier liquid according to DIN 38414-8 and converted to standard conditions (0°C, 101.325 kPa). At the beginning and at the end of the tests the content of total solids (TS) and the content of VS were analyzed (triplicate, standard deviations were stated at the results). Methane content (CH_4) was analyzed with an uncertainty of 1.52 mol-% by a gas chromatograph equipped with a micropacked column and a thermal conductivity detector.

EXPERIMENTS AND DISCUSSION

Pre-incubation of inoculum

Especially to investigate the degradation of low concentrated micro-pollutants with a BMP test, it can be difficult to distinguish between biogas produced by inoculum and by the investigated substrate (Raposo et al., 2011). Therefore, the gas production rate of inoculum should be very low, which results in an improved precision of a BMP test. Regarding VDI 4630 and DIN 38414-8 a pre-incubation of sewage sludge is recommended (not only for micro-pollutants) for the usage as inoculum at anaerobic conditions and 35°C in a closed system for at least one week and up to four weeks respectively to reduce the gas production rate; 2 to 7 days are suggested by ISO 11734. Studies of Battersby & Wilson (1988) or Elbeshbishya et al. (2012) could not find a significant effect of pre-incupation up to 3 weeks to the methane yield and biodegradability of sewage sludge used as inoculum. To investigate the biogas production of energy crops, digestate of a biogas plant fed by energy crops is more suitable, because the microorganisms are already adapted to these substrates. In this study the influence of pre-incubation to digestate used as inoculum was investigated. For this purpose, digestate was stored in a closed system in a heated room at about 40°C as a typical operating temperature of biogas plants. Every 3 days 3 batch tests were started for 9 days.

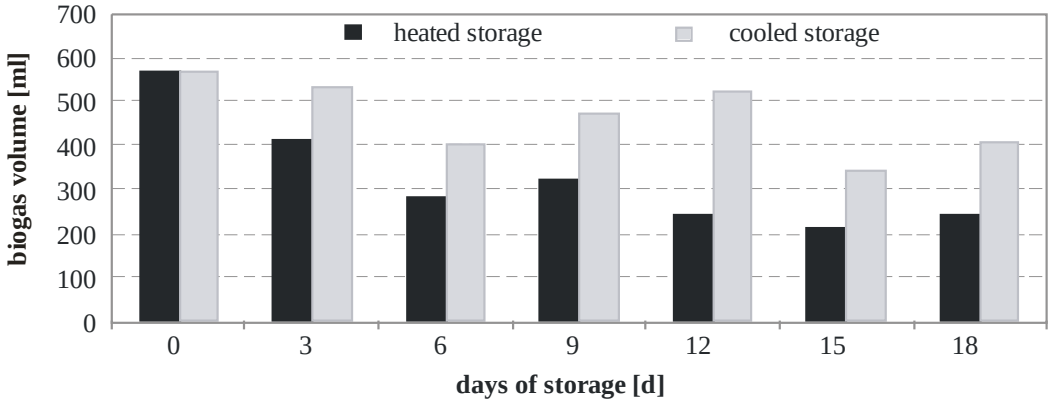

Figure 1. Biogas volume after 9 days batch tests using heated and cooled stored digestate as inoculum after different pre-incubation durations.

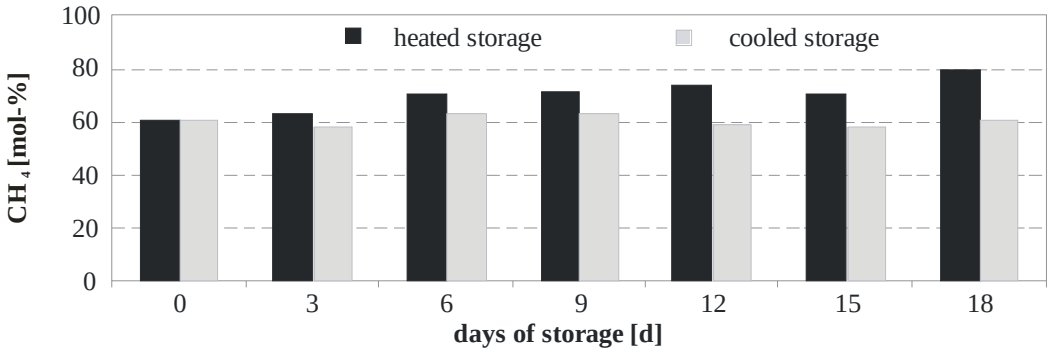

Figure 2. Content of CH_4 after 9 days batch tests using heated and cooled stored digestate as inoculum after different pre-incubation durations.

Results. At the batch tests 17.7 ± 2.2% of TS of the fresh digestate was degraded. With two weeks pre-incubation the degradation ratio at the tests was only 3.5 ± 3.1%, because the pre-incubation leads to a higher degradation of the digestate before the batch tests. Left VS is less degradable and results in a lower degradation ratio after pre-incubation. This can also be seen by a decrease of the gas production caused by pre-incubation (figure 1, heated stored digestate). Even if the constitution of digestate is different to sewage sludge, it takes only one week to reduce the biogas volume of around 50%. A pre-incubation of digestate for batch tests longer than one week is not reasonable, due to the very slow decrease of the biogas production rate after one week of "degassing". In figure 2 the increase of CH_4 for the biogas produced by digestate can be seen, which probably is caused by an intensified degradation of fats and proteins.

Storage of inoculum

If degradation tests or analysis cannot start immediately, substrate and digestate samples were often stored in a fridge at low temperature, because of the lower activity of microorganisms. Therefore, constitution, physico-chemical properties and biogas volume of a sample should be similar at any time after the storage. To investigate the influence of storage on digestate, samples were stored in a closed system in a fridge at about 4°C. Batch tests were performed as described for the investigation of pre-incubation. Both tests (cooled and heated stored digestate) run at the same time, so a straight comparability is given.

Results. No impact of storing digestate at low temperature can be seen in the CH_4 content (figure 2) and the pH value. A small variability is within the uncertainty of measurements. Compared to the heated stored digestate (TS degradation ratio of fresh material at the batch tests: 17.7 ± 2.2%, with 2 weeks of pre-incubation: 3.5 ± 3.1%) the TS degradation ratio of cooled stored digestate was 12.9 ± 1.0% at the batch tests after two weeks of storage. That means the degradation is less for cooled stored digestate, but there is still an obvious degradation of TS and VS. This can be seen in the decrease of gas volume (figure 1) with increasing storage periods as well. Therefore, organic samples should be used as fast as possible.

Homogenization

Pre-treatment influences the availability of biodegradable compounds, which are incorporated into complex, hardly biodegradable structures such as lignocelluloses or microbial cell walls and the actual surface area is increased by particle-size reduction, solubilisation, biodegradability enhancement, formation of refractory compounds or loss of organic material (Carlsson et al., 2012). In this study homogenization and filtration of digestate was investigated as pre-treatment to reduce the standard deviation of BMP tests. Mixing can homogenize materials, but also disrupt microbial cells or fibres, which influences the degradability. Filtered materials can reduce the formation of sedimentation or floating layers as well as the gas production of inoculum and the addition of water. Water addition to BMP tests changes the physico-chemical properties of a sample, which complicates the comparability with biogas production of industrial plants. For the batch tests digestate was filtered with a sieve (mesh size 2 mm, fluid phase was used) and homogenized with a Moulinette mixer (about 30 s), respectively.

Results. After one week a different gas production can be seen (figure 3). The homogenized samples reached a higher gas production of 200.9 ± 13.5 ml_N/g_{VS} compared to the untreated sample (161.3 ± 10.1 ml_N/g_{VS}), because the disintegration increases the degradability. The lowest biogas volume (91.5 ± 7.2 ml_N/g_{VS}) was produced with filtered digestate, although VS was the same for all batch tests. By the filtration of digestate fibers and crude parts were separated. Probably the low gas production is caused by a separation of immobilized microorganisms. Degradation ratios of TS confirm a worse degradation for filtered digestate (untreated: 23.6 ± 2.4%, homogenized: 23.2 ± 2.4%, filtered: 9.0 ± 0.8%) and a lower standard deviation. The concentration of volatile fatty acids (VFA) and carbonate was determined titrimetrical (table 1). The concentration of carbonate is very

similar for all samples. A higher VFA concentration leads to a higher gas production rate.

Because of the very low gas production potential and the highly homogenous material the filtration of inoculum can be recommended for batch tests. But further tests are required whether the concentration of (certain) microorganisms decreases and how this influences the degradation of substrates.

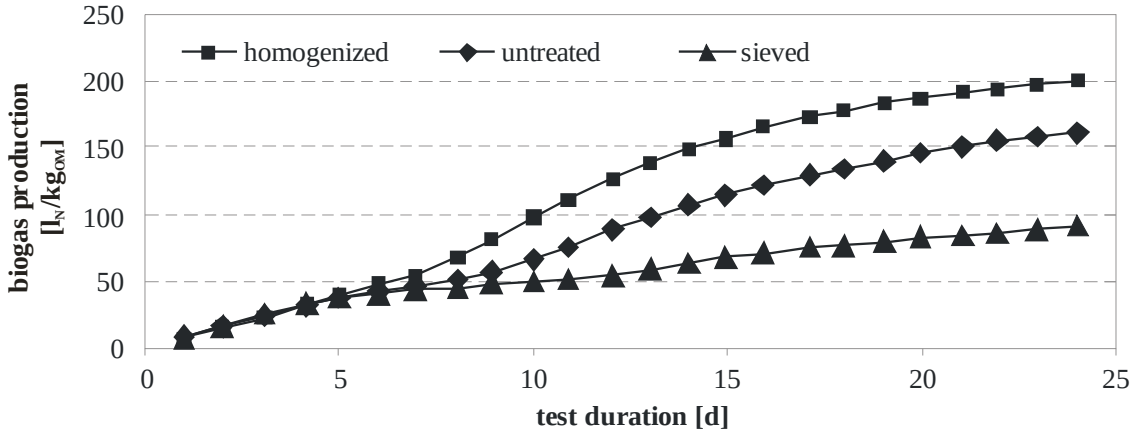

Figure 3. Biogas production of homogenized, filtered and untreated digestate.

Table 1. Concentration of VFA and carbonate of untreated, filtered and homogenized digestate.

	Untreated	Filtered - fluid phase	Filtered - solid phase	Homogenized
VFA (mg/L)	2,458	2,308	3,130	2,677
Carbonate (mg/L)	11,258	11,198	9,890	11,176

CONCLUSION

This study showed that guidelines (VDI 4630, DIN 38414-8, ISO 11734) for pre-incubation and storage of sewage sludge can be transferred to digestate as inoculum as well, and that a pre-treatment such as storage and homogenization obviously influences the biogas production. Storage in a fridge cannot avoid the activity of microorganisms and should be reduced to a minimum. Filtered inoculum can be recommended for use as very effectively inoculum due to the low biogas production rate with low standard deviation.

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