

Application of hydrolytic enzymes for improving biogas feedstock fluidity

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ABSTRACT

The composition of feedstock for biogas plants has changed during recent years. There has been an increase in the share of energy crops and residue from agriculture. As a consequence the contents of digesters are less fluid and hence, the effort for mixing has increased. Applying enzymes seems to be a promising way of improving fluidity. In this study the effects of enzyme application were investigated for a set of materials – grass silage, feed residue, maize silage, and rough-ground rye as feedstock for anaerobic digestion. After enzyme application these materials were mixed with cattle slurry and the probable effect was assessed with three different apparatus – fluidmeter, rotation rheometer, and torquemeter. The instruments proved applicable, in general, whereas grass silage could only be measured with the torquemeter. Fluidity of untreated material increased in the following row: grass silage<feed residue<maize silage<rough-ground rye. The pretreatment with enzymes, in general, enhanced fluidity. This effect was best measurable for feed residue and maize silage. Grass silage was so coarse that other effects superposed the enzyme effect. Rye had already a high fluidity and enzyme effect could only be small.

Keywords: anaerobic digestion, fluidity, feedstock, hydrolytic enzymes

1 INTRODUCTION

The increase in biogas installations and the demand for higher methane output led to an increasing demand for effective feedstock and hence, for a shift from liquid manures to solid energy crops and residue from agricultural production (Heiermann et al., 2009). Some kinds of this feedstock have considerable coarse structure and therefore, increase the demand of energy for mixing the digester content (Hopfner-Sixt et al., 2007). Insufficient mixing may result in less intensive digestion of the material and to the formation of a swimming layer. The latter leads on the one hand to also less digestion but to serious problems like a blocked passageway for the biogas and to an increasing risk for further thickening of the swimming layer. The increase of the swimming layer would further decrease the efficiency of the process and at worst it would become necessary to open the digester in order to remove the swimming layer. Both would increase the costs of the biogas plant and hence to a lower profit (Reinhold et al., 2009).

Hydrolytic enzymes enhance the decomposition of lingo-cellulose and hemi-cellulose to smaller molecules (Hendriks and Zeeman, 2009). It can be supposed that this enhanced decomposition leads to increased fluidity of material for anaerobic digestion treated with enzymes.

Although hydrolytic enzymes are supplied to biogas plants in considerable numbers there are only few systematic investigations of the effects of enzymes either on fluidity, the inverse of viscosity, or on increased biogas yields (Gerhardt et al., 2007). Hitherto the effects of enzymes on fluidity have been investigated in the context of animal feed, i.e. how the pumpability of liquid pig feed can be enhanced by applying enzymes (Türk and Jakob, 2006).

Liquid manure as well as digested residue is regarded as non-Newtonian thixotropic fluids, i.e. that viscosity cannot be described by a single value and that it decreases at constant shear stress over time (El-Hashad et al., 2005). These properties are the result of the mixture of pure liquids like urine and particles of different shapes and sizes. The liquid properties of liquid manure may change with the addition of further solid matter dependent on its size and shape. So we expect that spherical and smooth material like grains or ground grains will enhance liquidity while coarse structures like the squares of maize silage or the longish fibrous grass silage will decrease liquidity. The application of enzymes to these materials is supposed to enhance the degradation of the material and may modify their structural component in order to decrease viscosity.

In this study the effect of hydrolytic enzymes on fluidity of typical feedstock for biogas plants – grass silage, feed residue, maize silage, and rough-ground rye – was investigated. In order to determine these effects a set of different measuring devices has been used. This allows to administer the problems which may arise from the diverse thixotropic properties of the mixtures and to investigate in parallel their applicability for measurements in practice. These measuring devices were a self-constructed fluidmeter, a typical laboratory rotation rheometer, and a torquemeter for laboratory use.

2 MATERIAL AND METHODS

2.1 Feedstock

All experiments were conducted with liquid cattle manure as basic material (Table 1). The liquid cattle manure was collected at Rhinmilch Agricultural Company (Fehrbellin, Germany) several times – liquid cattle manure I: 22-04-08; liquid cattle manure II: 08-07-08; liquid cattle manure III: 10-23-08. The additional input material was provided also mainly from Rhinmilch – grass silage, feed residue, maize silage – and in case of rough-ground rye from Agricultural Company Trebbin (Trebbin, Germany). These materials, except grass silage, were already collected in 2007, 2 or 3 days in advance to chemical analyses. The materials were then kept at 4° C. Basic properties and additional information of the materials are summarized. Cattle manure was analyzed several times in order to consider changes in composition during storage. The range of heterogeneity of the material which could not be reduced by vigorous stirring was also reflected in the chemical properties. As liquid cattle manure III showed substantial high organic dry matter (ODM) values it was diluted with water to the content of organic dry matter comparable to liquid cattle manure II.

Table 1: Basic properties and description of the analyzed material

<i>Material</i>	<i>Date of analyses</i>	<i>pH value</i>	<i>ODM</i> ($\text{g}\cdot\text{kg}_{\text{FM}}^{-1}$)	N_{NH_4} ($\text{g}\cdot\text{kg}_{\text{FM}}^{-1}$)	N_{tot} ($\text{g}\cdot\text{kg}_{\text{FM}}^{-1}$)	<i>Description</i>
Liquid cattle manure I	04-22-08	6.9	82.1	1.3	4.0	Tixotropic liquid composed of urine, excrements, straw, and traces of feed residue
Liquid cattle manure II	08-12-08	7.0	45.7	2.1	4.8	
	09-17-08	6.9	41.4	1.4	2.7	
	10-13-08	7.1	52.9	1.5	3.7	
	10-14-08	7.0	44.1	1.4	2.8	
	10-16-08	7.0	41.0	1.4	2.8	
Liquid cattle manure III	10-23-08	6.9	63.7	1.5	3.5	
Liquid cattle manure III, diluted	10-30-08	7.0	42.7	1.1	2.4	
	11-04-08	7.0	39.5	1.0	2.3	
	11-10-08	6.8	42.5	1.1	2.4	
Grass silage	04-24-08	5.3	331.6	0.9	9.5	Longish, fibrous material with tendency to interlock, hardly degradable
Feed residue	10-09-07	4.7	385.2	0.5	9.7	Mixture of mainly silages and grain
Maize silage	10-09-07	3.8	285.1	0.3	4.2	Grains and squarish, easy degradable material
Rye, rough-ground	10-22-07	6.2	766.2	0.1	15.4	Small-sized grains, smooth and easy degradable

ODM: organic dry matter; FM: fresh matter; N_{NH_4} : ammonium nitrogen; N_{tot} : total nitrogen

2.2 Enzyme preparation

The fungal hydrolytic enzyme mixture, which was used to improve fluidity of feedstock for anaerobic digestion, is a mixture of fermentation product developed and produced by Bioreact GmbH¹. The enzyme rich fermentation product is particulate solid brown powder. The density of the product is approx. $304 \text{ g}\cdot\text{cm}^{-3}$, moisture content is approx. $50 \text{ g}\cdot\text{g}_{\text{FM}}^{-1}$, and the product can completely suspend in water. The major components are cellulase, hemi-cellulase, xylanase, pectinase, xylan esterase, pectin esterase, lipase, amylase, glucosidase, and protease. In addition there are non-identified enzymes in traces. Enzyme concentrations in the product vary depending on the fungi and the substrate used for production. The product contains also residues of the substrate.

¹ www.bioreact.de

2.3 Preparation of feedstock mixtures

The mixtures were adjusted to an organic dry matter ratio of 2:1 (liquid cattle manure to additional material). The mixing occurred at room temperature, i.e. approx. 20° C. At this temperature some measurements were conducted directly. In the case of experimental temperatures of 40° C the mixtures were pre-heated in a joggling bath and final temperature was then adjusted in the measuring apparatus.

In order to assess the impact of enzymes on the fluidity of the materials these were mixed with the enzyme in a ratio of 1:25 mass of enzyme with respect to mass of ODM in the material three hours before measurements. The enzyme was buffered with acetate buffer. The volume of acetate buffer added was set to $\frac{1}{10}$ of the volume of the material. After the enzyme treatment the materials were handled in the same way as the untreated materials.

2.4 Analytical methods

Feedstock material was analyzed according to standard analysis methods of Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB) and Association of the German Agricultural Investigation and Research Institutions (VDLUFA), summarized in Table 2. The analyses include pH value, organic dry matter, ammonia nitrogen, and total nitrogen.

- pH value was measured with the measuring electrode Sen Tix 41 (WTW) after homogenizing 10 g of sample FM with 100 ml distilled water for a period of 20 min.
- ODM was measured by determination of the ash content of dry samples, drying at 105° C until weight constant, in a muffle furnace at 550° C.
- The ammonium nitrogen content (N_{NH_4}) was converted to ammonia by addition of magnesium oxide, further distillation of the ammonia into a boric acid solution using a Vapodest 20 (Fa. Gerhardt). The content of ammonium nitrogen was finally quantified by back titration with sulphuric acid according to the method.
- The content of total nitrogen (N_{tot}) was determined using an elements analyzer (vario EL, Analysensysteme GmbH) operating at the principle of catalytical combustion under supply of oxygen and high temperatures. Elements analyzing was conducted according to the DUMAS method.

Table 2 Analytical methods

Parameter	Method	Reference	Literature
pH	VDLUFA	VDLUFA MB. BD. 3, ch. 18.1	VDLUFA, 1997
ODM	VDLUFA	VDLUFA MB. BD. 3, ch. 8.1	VDLUFA, 1997
N_{NH_4}	VDLUFA	VDLUFA MB. BD. 3, ch. 4.8.1	VDLUFA, 1997
N_{tot}	ATB	DIN EN ISO 16634	DIN, 2006
		VDLUFA MB. BD. 3, ch. 4.1.1	VDLUFA, 1997

ODM: organic dry matter; N_{NH_4} : ammonium nitrogen; N_{tot} : total nitrogen

2.5 Measurements

2.5.1 Overview

In Table 3 is given an overview on the combination of feedstock mixtures and measuring devices as well as an indication of the number of replicates for each experiment.

Table 3 Overview of conducted experiments, values indicate the number of replicates for each experiment

Material	Fluidmeter			Rheometer			Torquemeter		
	20° C		20° C 40° C	20° C			40° C		
	50 rpm	100 rpm	200 rpm	50 rpm	100 rpm	200 rpm	50 rpm	100 rpm	200 rpm
Cattle manure	9	6	6	6	6	6	6	6	6
w. enzyme	-	6	6	-	-	-	-	-	-
Grass silage	9	-	-	6	-	-	6	6	6
w. enzyme	9	-	-	6	-	-	6	6	6
Feed residue	9	6	-	6	-	-	6	6	6
w. enzyme	9	6	-	6	-	-	6	6	6
Maize silage	9	6	-	6	-	-	6	6	6
w. enzyme	9	6	-	6	-	-	6	6	6
Rye, r.-ground	9	6	-	6	-	-	6	6	6
w. enzyme	9	6	-	6	-	-	6	6	6

2.5.2 Fluidmeter

The principle of the measurements with the fluidmeter relies on the assumption that the time a specified volume of a liquid flows through an outlet in a cone is proportional to its fluidity.

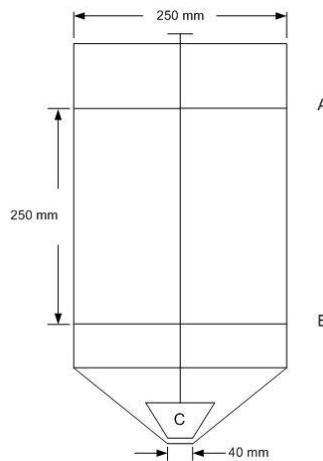


Figure 1 Schematic of fluidmeter; A = upper level, B = lower level, C = removable plug

The fluidmeter construction (Figure 1) was first developed at the former German Federal Research Institute for Agriculture². It was constructed from a perspex pipe of 250 mm diameter and 600 mm length attached above a cone with the tip pointing to the bottom. The cone had an angle of 60° and an outlet in the tip of 40 mm diameter. The outlet could be shut with a plug on a stick. The stick had guidance in the lid of the apparatus. The perspex pipe had two marks in 250 mm distance.

Conducting the experiments the apparatus was filled above the upper mark while the outlet was closed. After opening the outflow the time span was stopped, by hand, the liquidity between the two marks needed to flow out.

The experiments were conducted with three shares (20 l) of each mixture and each share had three replicates. In the case of liquid cattle manure one series was conducted at 40° C in addition, whereas all other experiments were conducted at approx. 20° C (cf. Table 3).

2.5.3 Rotation Rheometer

The rheometer (Paar Physica MC1 RM300³) measures viscosity as shear stress dependent on shear rate of a liquid placed in the annulus of two cylinders (Figure 2). While the outer cylinder is static the inner one rotates at a set of speeds. The cylinders have walls with a saw tooth tread design of 1x2 mm. The radius of the inner cylinder was 30 mm and of the outer one 38 mm, hence, the annulus was 8 mm wide. In order to heat the apparatus and hence, the liquid to given temperatures the installation is equipped with a water bath. The software tool RHEO 2000 v7⁴ was used to convert the raw data into measurement values.

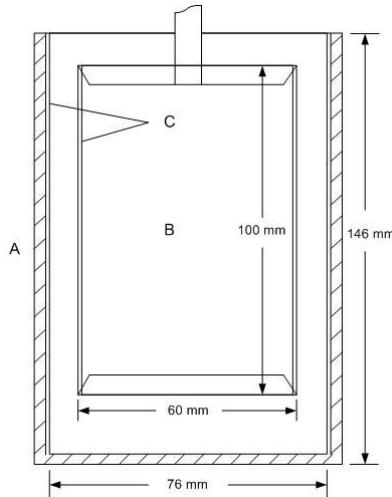


Figure 2 Schematic of rotation rheometer; A = outer cylinder with inner wall (C), B = inner cylinder with outer wall of saw tooth tread design of 1x2 mm (C), the annulus between these cylinder is 8 mm wide.

² www.vti.bund.de

³ www.anton.paar.com

⁴ www.rheotec.de

The experiments were conducted at 20° C and in the case of liquid manure at 40° C as well (*cf.* Table 3). The samples were kept at room temperature and adjusted to the temperature in the measuring apparatus. Each sample was approx. 350 ml. The experiments were conducted with each mixture in six replicates. Each replicate actually underwent two measurements: one during the shear rate accelerated to 180 s⁻¹ and the second one during the deceleration. The first phase was used as pre-shearing while the second phase then was used for measuring the shear stress.

2.5.4 Torquemeter with anchor shaped mixer

The torquemeter is based on an IKA⁵ Eurostar stirrer equipped with an anchor shaped mixer impeller (Figure 3). The mixer stirs the liquid, which is placed in a one-liter vessel which is attached to a water bath, at several preset revs per minute. The torque is recorded and analyzed with Labworldsoft 2.62⁵.

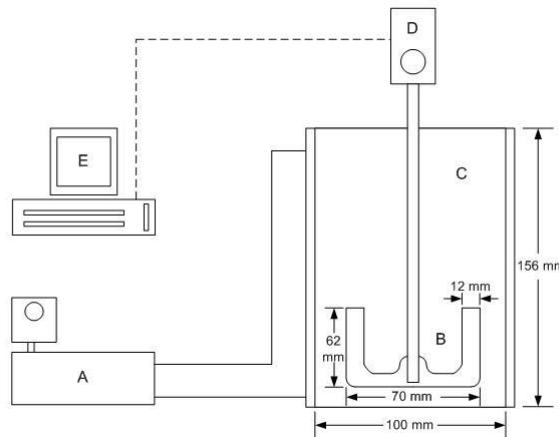


Figure 3 Schematic of torquemeter equipped with anchor shaped mixer; A = water bath, B = anchor shaped mixer impeller, C = temperature-controlled vessel, D = torque-controlled stirrer, E = computer running measure programme

The experiments were usually conducted at 40° C and three preset revs per minute (units in rpm): 50, 100 and 200 (*cf.* Table 3). An additional experiment was conducted at 20° C and 50 rpm for all materials, liquid cattle manure also was investigated at 20° C and 100 rpm and 200 rpm. Each experiment was conducted in six replicates. Thousand (1000) ml samples were pre-heated in a joggling bath and temperature than was adjusted in the apparatus. Pre-shearing with 50 rpm for 300 s was sufficient to reach homogenized conditions of the tixotropic material. The measuring interval was 300 s as well for each sample.

2.6 Statistical analysis

All measured values were analyzed with Instat+ v3.36⁶ as well as with Microsoft Excel 2003⁷. Significance was tested using a two-side T-test assuming an error probability of $\alpha = 5\%$.

⁵ www.ika.net

⁶ www.reading.ac.uk/ssc/software/download.html

3 RESULTS

3.1 Initial tests with liquid cattle manure

The torquemeter was used for the determination of some principles of measurement with the basic material. First the influence of pre-shearing was tested. Measurements were taken at 200 rpm and 20° C, pre-shearing was achieved in 5 minutes at 50 rpm.

While the standard deviation was small for six measurements (V1 to V6) in one sample without pre-shearing the standard deviation across six samples is considerably high (Figure 4). Pre-shearing reduced slightly the overall values of torque. The standard deviation within the measurements of one sample increased compared to the samples without pre-shearing (approx. ±0.07 Ncm to approx. ±0.80 Ncm). In the same course repeatability was enhanced and hence, the standard deviation of the medium value across six samples decreased (±0.65 Ncm to ±0.35Ncm).

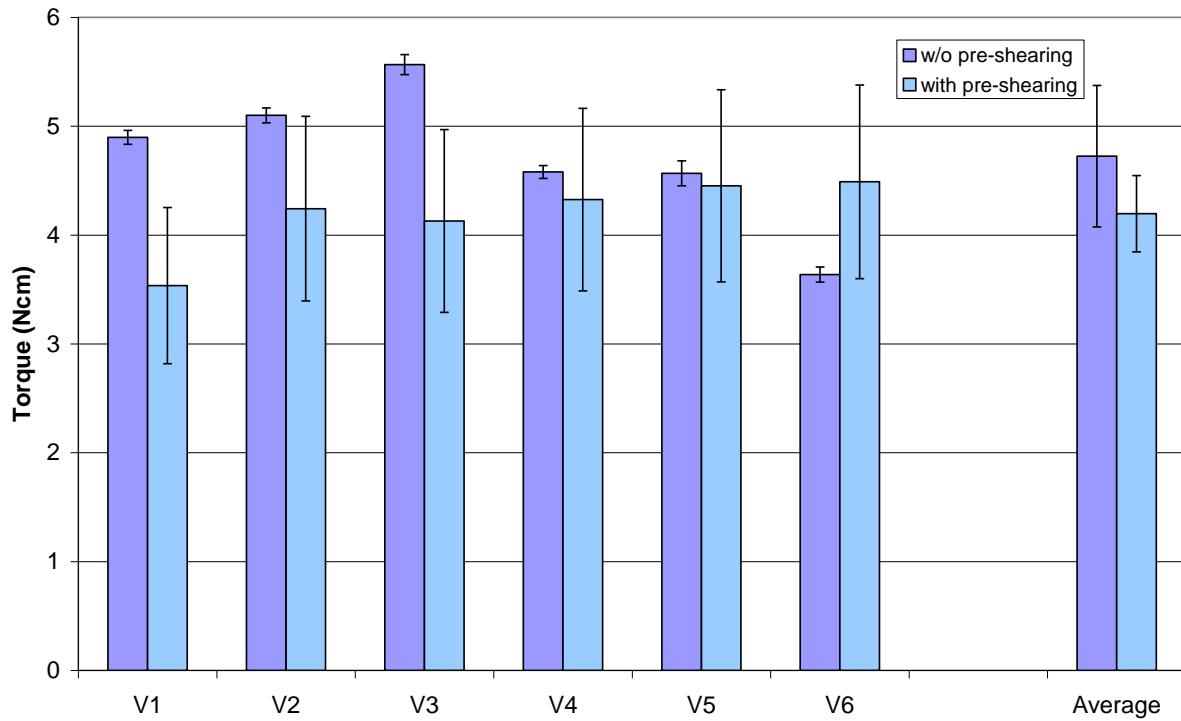


Figure 4 Torque of liquid cattle manure at 200 rpm without pre-shearing and with 5 minutes pre-shearing at 50 rpm, error bars represent standard deviation

A further test was done on the influence of temperature and shear rate (revs per minute). From the results presented in Figure 5 we can conclude that there are almost linear conditions for increasing temperature and revs per minute. We consider that measuring the torque at 50 rpm at 20° C and 40° C as well as at 100 rpm and 200 rpm at 40° C allow us to conclude on further temperatures and revs per minute.

⁷ www.microsoft.com

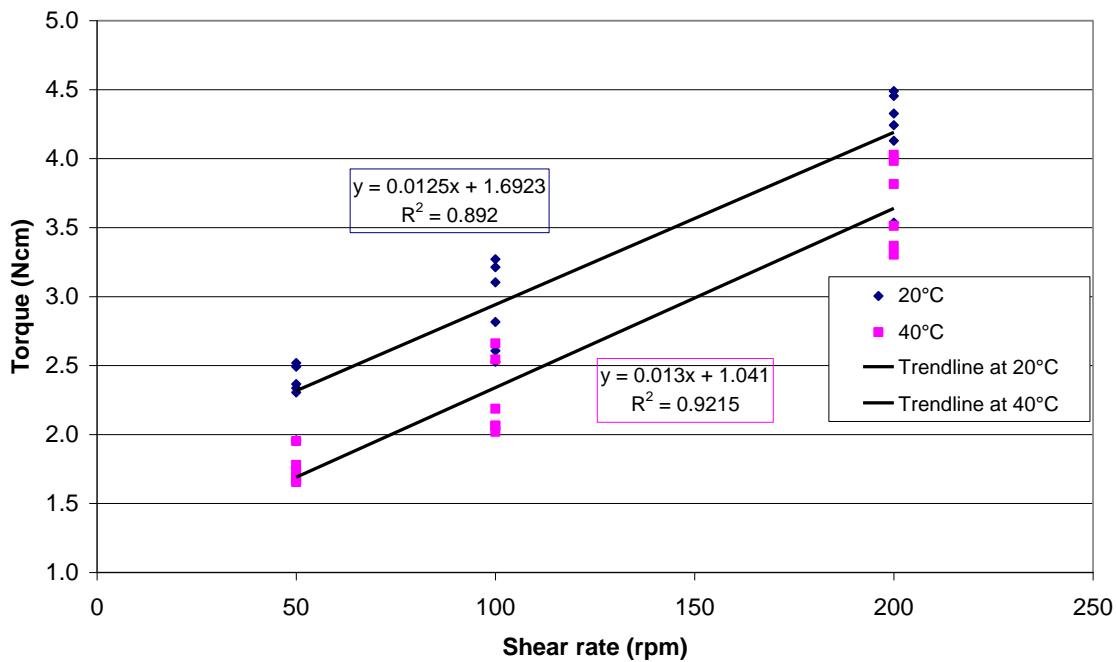


Figure 5 Torque of liquid cattle manure at 50, 100 and 200 rpm at 20° C and 40° C, respectively

3.2 Fluidmeter

The measurements with the fluidmeter delivered a clear picture of the influence of coarseness of the material and the effect of enzyme treatment (Figure 6). Liquid cattle manure had an average flow rate of 5.2 s with a rather high standard deviation of 0.63. Feed residue, which can be considered a very coarse material, had an increased flow rate of 7.0 s and an even larger standard deviation of ± 0.94 . The flow rate of maize silage was 4.6 s and that of rough-ground rye was 4.1, both with small standard deviations of ± 0.17 and ± 0.08 respectively. The effect of enzyme treatment was very clear with feed residue (flow rate = 5.1 ± 0.21) and maize silage (flow rate = 4.2 ± 0.07). Rough-ground rye as a smooth material did not show a large effect and had almost the same value as the untreated variant (flow rate = 4.0 ± 0.08). Grass silage proved to be a very coarse material and did not work in this apparatus at all.

3.3 Rheometer

The rheometer, a standard device for rheological investigations, worked quite well with liquid cattle manure. There is a clear difference in shear stress if determined at 20° C (approx. 145 Pa at 150 s^{-1}) and 40° C (approx. 105 Pa at 150 s^{-1}). The treatment with enzymes insignificantly decreased these values (Figure 7). Hence, we assume that effects of enzyme treatment on the other material will not be covered by effects on liquid cattle manure.

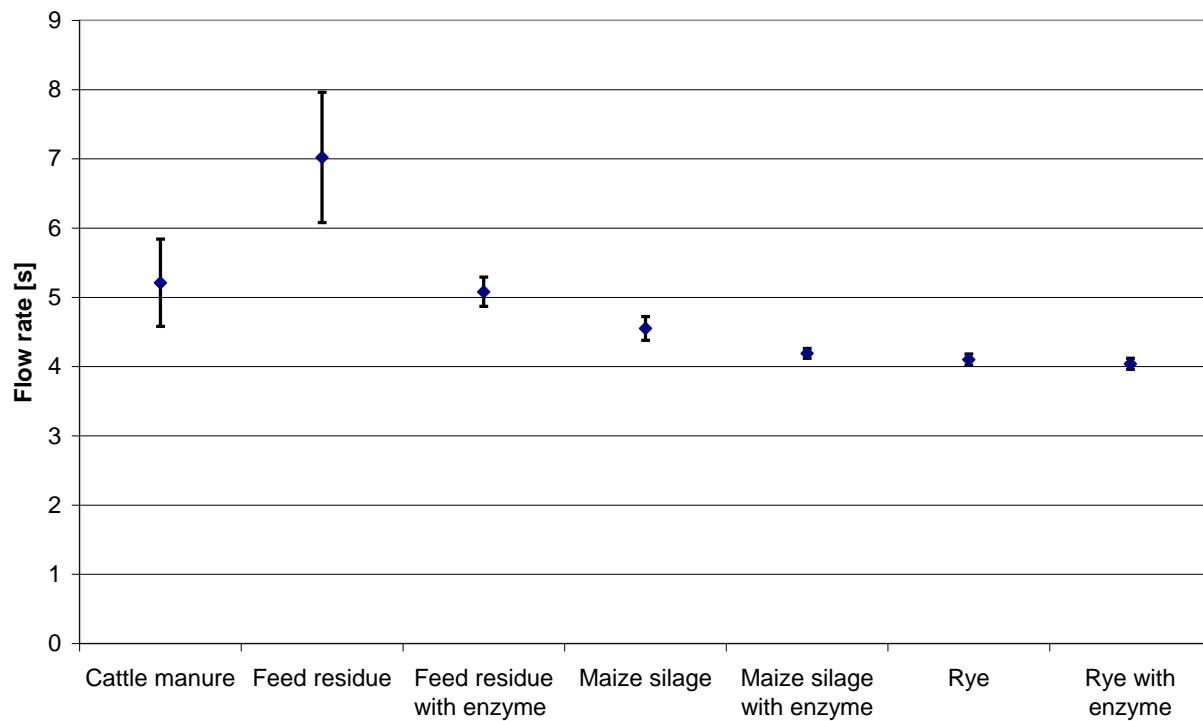


Figure 6 Flow rate of different material determined in fluidmeter, error bars represent standard deviation

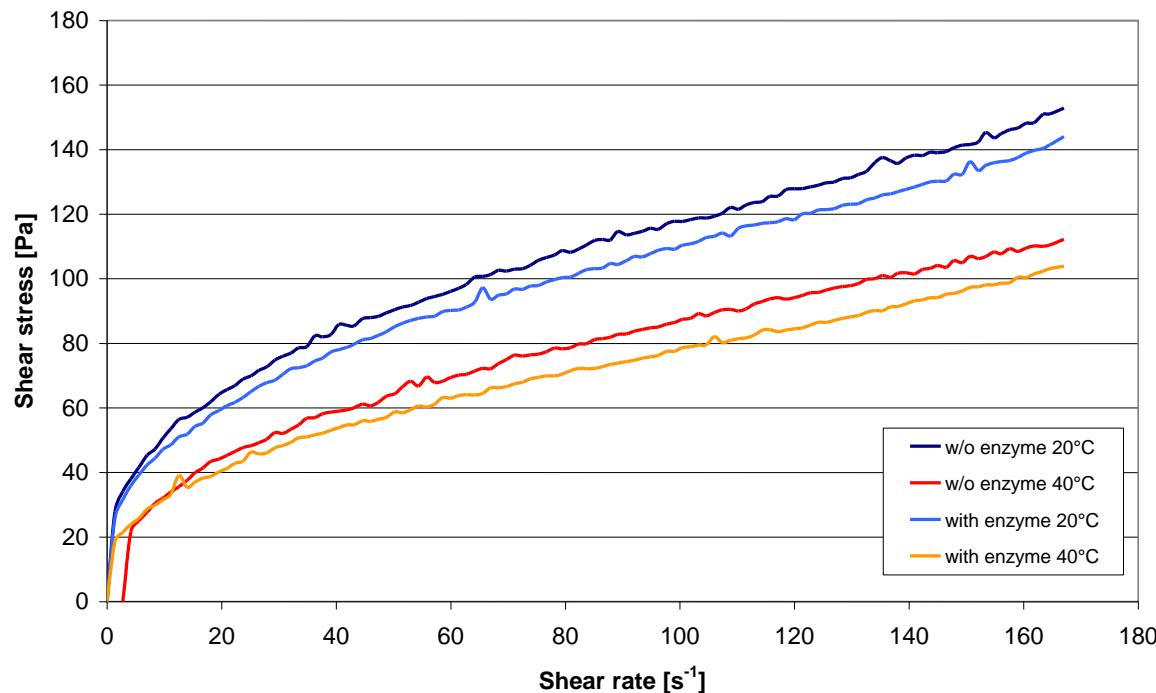


Figure 7 Shear stress of liquid cattle manure untreated and treated with enzymes at 20° and 40° C, respectively

Feed residue and maize silage delivered much less smooth curves of shear stress, therefore central moving average method with 30 periods was applied in order to analyze the data (Figure 8, Figure 9). In the case of maize silage a significant effect of enzyme treatment could be detected. The difference between untreated and treated feed residue was insignificant although visible. In both cases the curve of the treated variant showed much smoother behavior than the ones originating from the untreated variants. This also led to the assumption that there is an effect of enzyme treatment on the structural properties of these materials.

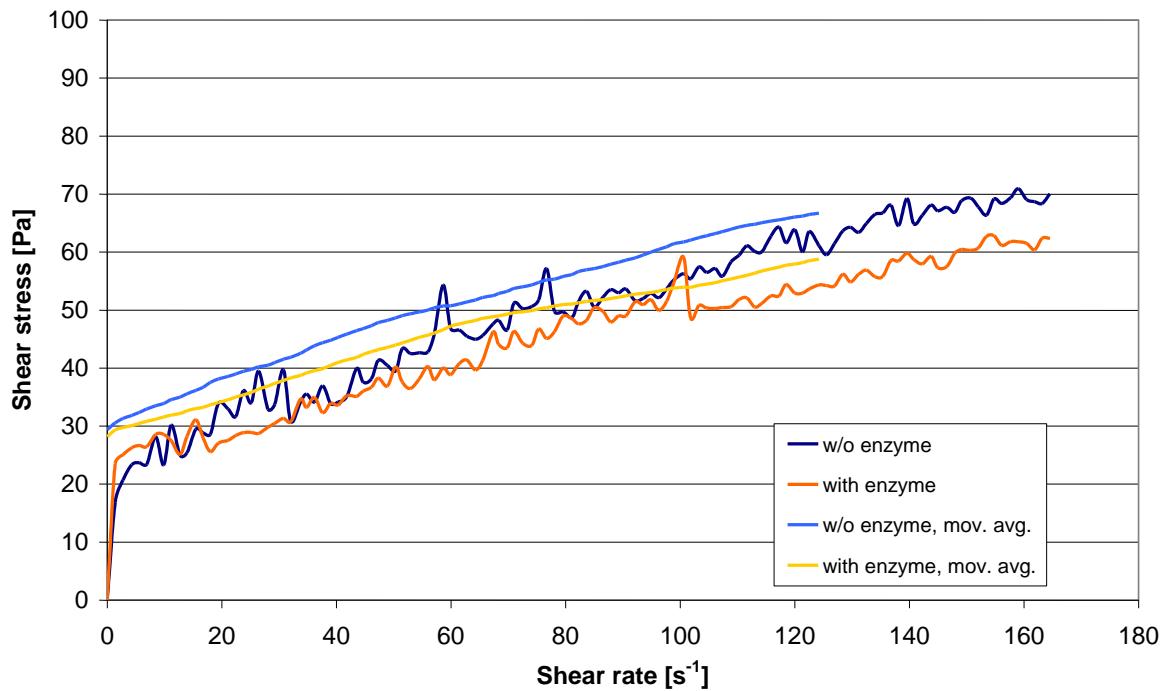


Figure 8 Shear stress of feed residue untreated and treated with enzymes, mixed with liquid cattle manure at 20° C, mov. avg. is moving average of 30 periods

There was only little increase of shear stress of the rough-ground rye mixture, both for the treated and the untreated variant (Figure 10). This reflected clearly the smooth structure of the ground rye and showed the decreasing effect of it on the viscosity of liquid manure. The effect of enzyme treatment on viscosity was insignificantly low although visible.

3.4 Torquemeter

The torquemeter proved to be a robust measuring device which could cope with all investigated materials (Figure 11). Grass silage and feed residue increased the torque of plain liquid cattle manure (e.g. at 40° C and 200 rpm from 3.7 Ncm to 5.9 and 4.6, respectively) and in the case of grass silage the standard deviation increased as well (from ±0.32 Ncm to ±0.75 Ncm). Maize silage and rough-ground rye decreased, as expected, the torque of the basic material and showed smaller standard deviations.

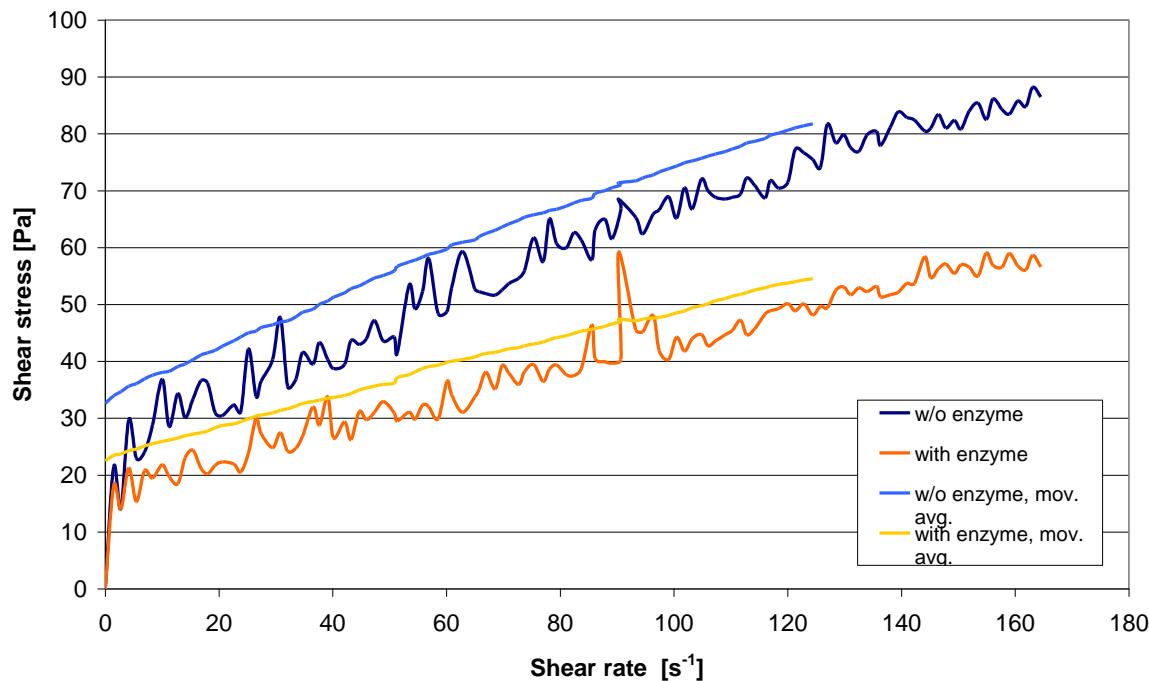


Figure 9 Shear stress of maize silage untreated and treated with enzymes, mixed with liquid cattle manure at 20° C, mov. avg. is moving average of 30 periods

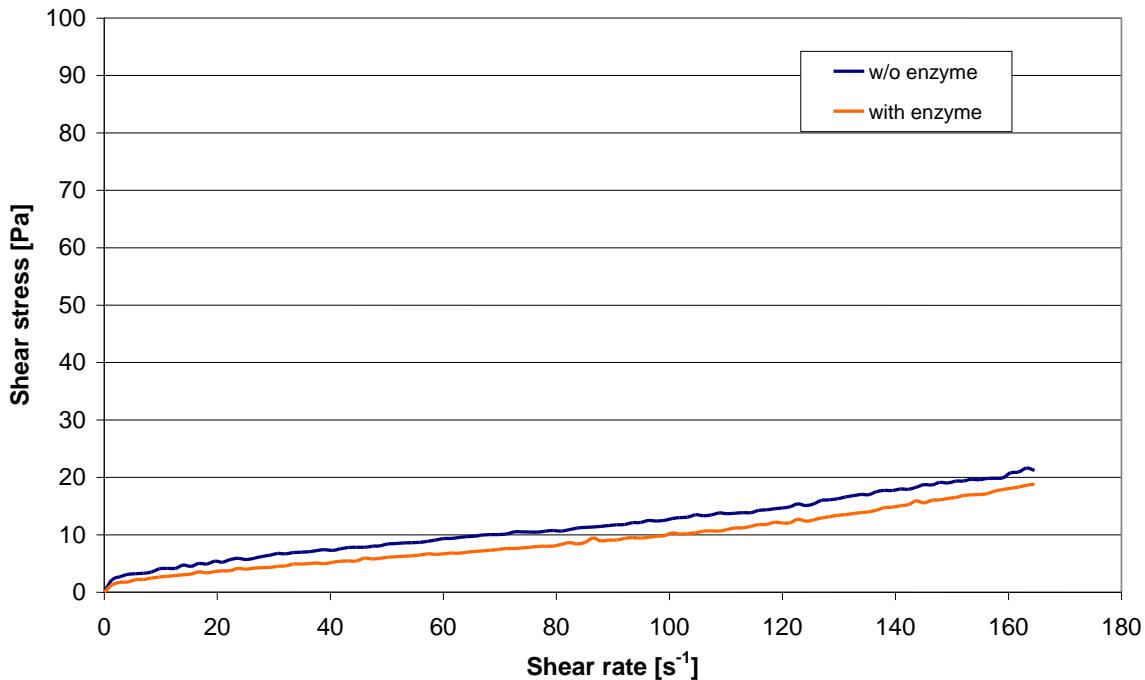


Figure 10 Shear stress of rough-ground rye untreated and treated with enzymes, mixed with liquid cattle manure at 20° C

Enzyme treatment led in most cases to a visible decrease in torque although mostly not significant, e.g. maize silage showed a decrease in viscosity from 2.1 ± 0.15 Ncm to 1.7 ± 0.08 Ncm at 40° C and 200 rpm, at the same conditions the values of feed residue changed from 4.6 ± 0.31 Ncm to 4.4 ± 0.20 Ncm. In the case of grass silage a decrease in torque could only be determined at 20° C (from 6.1 Ncm to 3.2 Ncm) whereas at 40° C torque slightly increased for each step in revs per minute (2.3 ± 0.24 Ncm to 2.5 ± 0.54 Ncm at 50 rpm; 3.1 ± 0.54 Ncm to 3.4 ± 1.08 Ncm at 100 rpm; 5.9 ± 0.75 Ncm to 6.2 ± 1.05 Ncm at 200 rpm). As the standard deviation also increased for these measurements we consider this as a superposition of effects and especially the difficulty resulting from the structural impact of this material on fluidity.

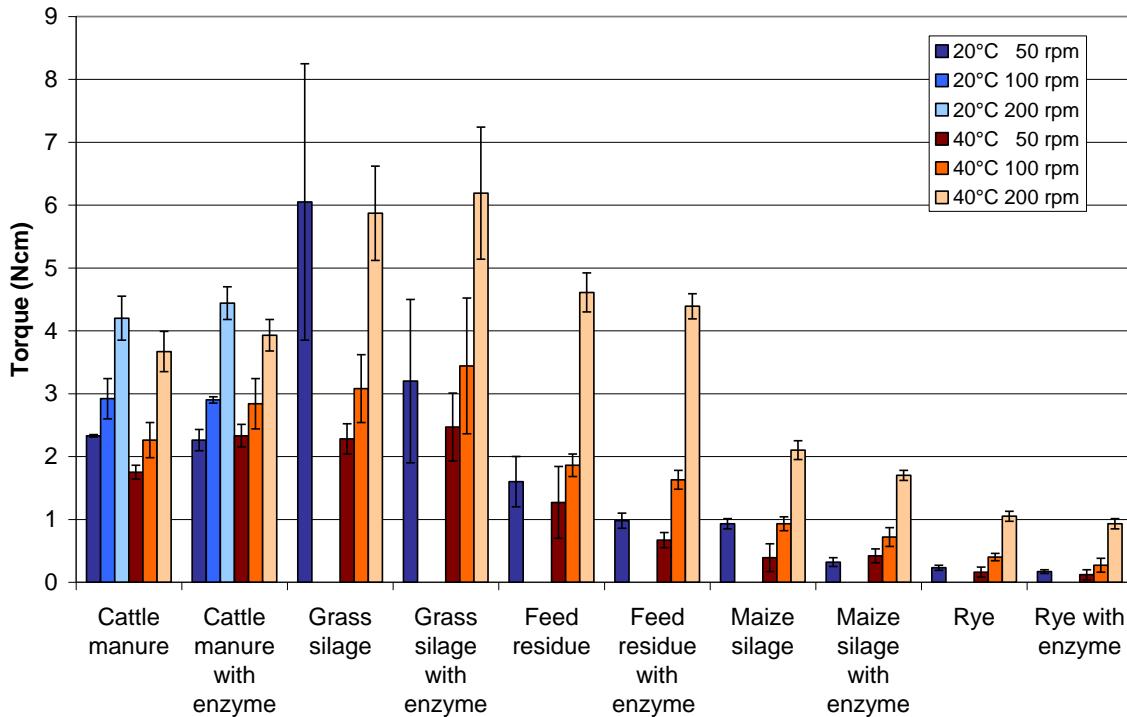


Figure 11 Torque of various materials untreated and treated with enzymes, mixed with liquid cattle manure at a set of revs per minute and temperatures, error bars represent standard deviation

4 DISCUSSION AND CONCLUSIONS

The applied measuring devices proved acceptable applicability and delivered in general comparable results. The fluidmeter appears to be a simple installation easy to use and delivering substantial results as long as the investigation is carried out by one person and on a series of reasonably different materials. Because of its simplicity it could be used for field comparisons of the digested residues of various biogas plants. The rheometer is a typical laboratory equipment. It is able to determine small differences in viscosity of a large scale of materials provided that the structure of these is not too coarse. It seems to be less appropriate for serial investigations of digested residues from various biogas plants which might show considerable heterogeneity. The torquemeter was able to measure the fluidity of all materials investigated and to differentiate the various influences. Although, in large-scale digesters the mixers are usually of propeller or paddle shape the torquemeter seems to be generally able to reflect the conditions in a real digester. At least, from the experiments one can conclude that it would be ideal to install devices measuring force or work at the stirrers in biogas plant.

The measurements of the untreated mixtures showed that the addition of materials like grass silage, feed residue, maize silage, and rough-ground rye does not change the basic thixotropic properties of liquid cattle manure. The main reason might be that these properties of liquid manure are already the result of components like feed residue and straw. Therefore, the measurements of the mixtures investigated here are comparable to each other and one can conclude that it is viable to observe probable effects of enzyme treatment on fluidity.

Although in most measurements there was a visible effect of enzyme treatment on the fluidity of the materials this effect was often not significant. Considerable differences could be seen for materials like maize silage and feed residue which have similar fluidity and variability as liquid manure itself but are quite susceptible to enzymatic cleavage of their structures. Whereas, material as grass silage is very inhomogeneous even after enzyme treatment and shows therefore little difference between treated and untreated variants. Finally, rough-ground rye has already a large effect on fluidity of the basic substrate and the enzyme treatment could not increase this effect significantly. Furthermore, increasing the temperature showed in most cases higher effects on fluidity than enzyme application. Although in practice it will generally not be possible to increase the temperature of the biogas process in large quantity, i.e. by 20° C.

Based on the results it can be recommended the application of enzymes in order to increase fluidity of feedstock mixtures containing considerable fractions of coarse or fibrous material and to decrease power consumption for mixing the digester content. Nevertheless, we expect it necessary to confirm lab-scale results from this study in large scale biogas plants. Only from these it will be possible to assess if the cost reduction for less mixing would compensate the costs for the enzyme application.

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