# Grubbens Deflaker model GLD 200 (from Cellwood Machinery AB)

## Pre-treatment of biomass for anaerobic digestion

**Test report**

**J.no.1003**

Version 6, April 17th 2012

# 1. Table of contents

# 2. Introduction

This test report describes the test results from tests according to the test plan for verification of a mechanical biomass treatment. The test was prepared according to the DTI- DANETV Test Centre Quality Manual

## 2.1. Verification protocol reference

J.no. 1003

## 2.2. Name and contact of vendor

**Producer (vendor)**
Cellwood Machinery AB, Box 65 SE- 571 21 Nässjö , Sweden

Contact:Olof Lekander , phone: +46 (0) 386 76093, e-mail: olof.lekander@cellwood.se

**Danish distributor**
Al 2- Agro Krøgebækvej 25, DK 8682 Hovborg
Contact: Preben Nissen, phone: +45 3169 6501, e-mail: pbn@al-2.dk

## 2.3. Name of centre/test responsible

Danish Technological Institute, Verification Centre, Life Science Division, Kongsvang Allé 29, DK-8000, Aarhus, Denmark.
Test responsible: B. Malmgren-Hansen (BMH), phone: +45 72201810, e-mail: bmh@teknologisk.dk.
Internal reviewer: Lars D.M. Ottosen (LDMO, phone: +45 72202194, e-mail: ldmo@teknologisk.dk.

## 2.4. Expert group

Thorkild Qvist Frandsen (TQF), Agrotech, phone: +45 87 43 84 68, e-mail. tqf@agrotech.dk
Kasper Stefanek (KPS), Agrotech, phone: +45 87438468, e-mail: kps@agrotech.dk

# 3. Test design

The test design is based on a comparison of methane potential of untreated biomass fibres and biomass fibres treated with the Deflaker GLD200 process.

The target of the process is:

- Methane yield of treated fibres in manure

The effects of the process are tested for:

- Change in methane yield from treated biomass compared to untreated biomass in the treatment period of interest in biogas plants (approx. 20 days for themophilic and-30 days for mesophilic in active growth)
  In the batch digestion experiments the methane yield of non treated fibres are compared with the methane yield of treated fibres

The test procedure is described in the following:

**Pretreatment of solid biomass**
In order to obtain a homogeneous biomass before the Deflaker treatment an extra pretreatment should be performed of biomasses containing thick and long particles (straw) in order to obtain fiber lengths of less than 3-5 cm. Such material was not part of this test.

**Storage of pretreated material**
Pretreated wet material must only be stored a few days to avoid decomposition.

**Cleaning**
Before the test of the Deflaker water was passed through the Deflaker for cleaning. An amount of 10 times the free volume of the Deflaker should be used according to test plan, but it was decided that less water for cleaning was sufficient.

**Mixing of material with liquid.**
The fibre material was mixed with water to obtain a pulp with good pumping characteristics. Typical VS in the pulp is expected to be 7-15 % from earlier tests. The mixing was performed in a 1 $m^3$ tank/reservoir using a mixer which produces a good mixing. Mixing was performed for a sufficient period to obtain good wetting and a homogeneous pulp. In an industrial application liquid manure may be used instead of water. However, it was decided to use water in the test to avoid a correction step for biogas produced from added manure.

**Test**
When starting up the test operational conditions were used which produces maximum mechanical stress on fibres (maximum rotation of motor).

Fibres were pumped from the stirred tank/reservoir at a rate that allowed treatment for at least 10 minutes. Samples were taken from inlet and outlet as fast as possible –at least 3 samples during the run (start/middle and end)- if possible 5 samples.

The samples were pooled to an average test sample which was subdivided to laboratory samples. See section 3.5.7.

3-5 separate samples from inlet and outlet were also analysed for DM (total Solids, dry matter),VS (Volatile Solids) to analyse the homogeneity before performing biogas analysis. See pretesting program appendix 4.

**Measurements of Methane potential**

The methane potential was measured according to method 1 or method 2 for measuring methane potential described in appendix 5.

The dry matter, DM, and volatile solids content of the samples was analyzed before performing biogas tests.

Tests were made with a total of 25 g VS/l all in at least triple measurements on the mixed fraction of input and output.

The result is a calculation of (l CH4 /VS of added biomass) for treated and non-treated biomass as function of time for mesophilic biogasification.

### 3.1. Test site

The test site was Bånlev Biogas

### 3.2. Type of site

The Deflaker was installed for tests at a commercial biogasplant which have the necessary facilities needed in the test.

### 3.3. Addresses

**Test site for Deflaker**

Bånlev Biogas, Bjergagervej 4, · Spørring, DK8380 Trige

**Biomass sources:**

***1: Cow manure from Hadbjergvej 133, 8370 Hadsten***

***2: Maize silage from Per Therkildsen Risvangsvej 12, 8530 Hjortshøj***

### 3.4. Descriptions

The Deflaker is a mechanical process for pretreatment of biomass fibres suspended in liquid. The purpose is to disintegrate the fibre structures resulting in improved bacterial anaerobic digestion, higher methane yields and faster production of methane

The principle is shown in Figure 1.

The system consists of two discs with teeth- One disk rotates and the other is stationary-(stator disc). The fibre pulp is pumped into the centre of the stationary disc passing the teeths which rip up the fibre structure and the fibrepulp is hurled to the outlet. Grubbens Deflaker types GLD 200 have fixed axial rotor discs, and the gap can therefore not be adjusted on this model.

The discs are made of hardened, acid-resistant steel with Brinell hardness of about 400 HB.

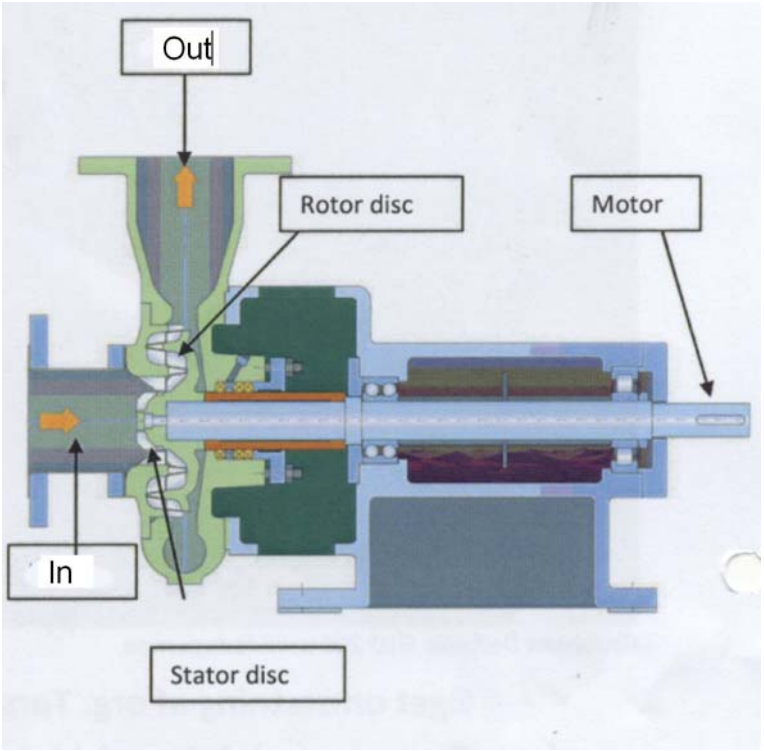

Figure 1 Scematic design of the Grubben Deflaker GLD 200


**Figure 2 Discs**

## 3.5. Tests

### 3.5.1. Test methods

The test methods, standards:
Methane potential are measured according to protocol method 1 in appendix 5 and/or method 2 in appendix 6.

### 3.5.2. Test staff

The test staff is:
B. Malmgren-Hansen Sampling and reporting and responsible for performing Methane potential incl. GC analysis (TI)
Dorthe Kvistgaard Laboratory Analysis (TI)

### 3.5.3. Test schedule

| Task | Timing |
|---|---|
| Application definition document | March 2011 |
| Verification protocol with test plan | April 2011 |
| Test | May 2011 |
| Analysis phase | May.-July 2011 |
| Test reporting | December 2011 |
| Verification | January 2012 |
| Verification report | February 2012 |

### 3.5.4. Test equipment

The test equipment includes sampling containers, buckets and equipment for measuring methane potential, dry matter and volatile solids.

**Type and number of samples**
The types and number of samples are summarized in the table below:

| | |
|---|---|
| **Methane potential**<br>1. Volume of methane produced | 3-6 replicates with 25 gVS fibres /l depending on inhomogeneity of nontreated and treated biomass pulp. |
| **Other analysis**<br>1. Total solids<br>2. ash content<br>3. Total nitrogen<br>4. Ammonium nitrogen<br>5. Total phosphorus<br>6. K | 2 samples for nontreated fibres of Total N, NH4-N,P,K for characterisation<br><br>For treated and non-treated fibre pulp at least 2 samples with analysis of DM,VS. |

### 3.5.5. Operational conditions

The operating conditions of the Deflaker will be treatment at maximum effect - rotation (3000 rpm)

### 3.5.6. Operation measurements

- Total power consumption during treatment will be recorded

### 3.5.7. Sampling

**Sampling of biomass (main sample) from inlet and outlet**

The main samples are taken from valves with large dimension (1") to ensure that no material is stuck in the valve. Before obtaining the sample 2 liters are discarded. A 10-15 liter bucket or a 1 liter PE bottle is used for sampling the main samples.

**Preparation of subsamples**

In all subdividing of samples care must be taken to produce representative subsamples as some fibre material may float or sink.

Depending on homogeneity of the biomass pulp (suspension of biomass particles in water) the subsamples will be obtained in the following way:

1) ***Homogenious pulps:*** a subsample is made by stirring main sample well during transfer to subsample
2) ***Inhomogenious pulps with floating layers or fast sinking layers***: Sieving of fibres is performed followed by propotional weigh of solid and liquid fractions into subsamples.

The most appropriate subsampling method will be decided when inspecting the main samples

**Handling of samples**

All fibre material is refrigerated if tests are performed within 2-3 days or freezed down for later analysis.

### 3.5.8. Product maintenance

The Grubben Deflaker system includes discs which will have a live time dependent on particles like sand in input.

Details are described in the manual:
The manual "Grubbens Labyrintha Deflaker Machine and Function Desciption GLD 200" describes 1: Description of functions including requirements of input (there must be no foreign objects like stones etc. which can damage discs. It is mentioned that large amounts of sand will shorten the disc wear time). 2: Machine description, 3:How to install the machine, 4: Start-up, 5: Maintenance (lubrication) and bearings, 6: Technical specifications, 7: Mounting and dismounting of parts

### 3.5.9. Health, safety and wastes

The manual describes how to operate and maintain the equipment. There is no waste of any kind exept when replacing lubrication oil.

# 4. Reference analysis

## 4.1. Analytical laboratory

Analytical laboratories providing analysis of any kind as part of the verification tests, within or outside the test centre body has the responsibility for:

- Maintaining an ISO 17025 accreditation with the quality management system required herein.
- Application of accredited analytical methods, where available
- Application of other methods according to both international standard methods or in-house methods that are validated as required for accredited methods

**The used analytical laboratory are shown below**
Analysis of total and ammonium nitrogen, total phosphorus, total and volatile solids are performed by Eurofins Steins laboratory, Hjaltesvej 8, DK-7500 Holstebro, phone: +45 7022 4286, website: www.eurofins.dk

Determination of biogas volume and methane concentration and additional measurements of total and volatile solids at laboratory scale are performed by: DTI Life science division, Kongsvang Allé 29, DK-8000 Aarhus C, Denmark phone +45 72201000, contact: B.Malmgren-Hansen.

## 4.2. Analytical parameters

See 4.3

## 4.3. Analytical methods

| Analytical parameters | Standard |
|---|---|
| Methane potential | Bioprocess Control method described in Appendix 5 |
| Total solids | EØF 103°C |
| Total volatile solids (Loss on ignition) | DS 204 |
| Total nitrogen | Kjeldahl |
| Ammonium nitrogen | 71/393/EØF |
| Total phosphorus | ICP-OES: (ISO/DS 11885, 2009) |
| K | ICP-OES: (ISO/DS 11885, 2009) |
| S | ICP-OES: (ISO/DS 11885, 2009) |

## 4.4. Analytical performance requirements

See 4.3.

## 4.5. Preservation and storage of samples

Samples are stored in labelled 1 l PE bottles. All samples are freezed if analysis are not performed within one day. Transfer to analytical laboratory may be performed directly using

cooled transport wessel. Samples for batch testing of methane potential are freezed immediately after sampling until testing.

# 5. Data management

## 5.1. Data storage, transfer and control

The data to be compiled and stored are summarized in table below. Analytical raw data were filed and archived according to the specifications of the laboratories quality management systems.

| Data type | Data media | Data recorder | Data recording time | Data storage |
|---|---|---|---|---|
| Test plan and report | Protected pdf | Test responsible | When approved | DTI protected data storage |
| Test details at laboratory and full scale | Excel, word etc. | Test staff at test site | During Test | DTI protected data storage |
| Calculations | Excel | Test responsible | During calculation | DTI protected data storage |
| Analytical reports | Protected pdf, paper | Test responsible | When received | DTI protected data storage |

# 6. Quality assurance

## 6.1. Test plan review

Internal review of the test plan will be done by LDMO
External review of this test plan is describe in 1.4

## 6.2. Performance control – reference analysis

***Batch testing of methane potential:***
The incubation temperature 35 +/- 1 ˚C (mesophile fermentation) is controlled by logging the temperature in the incubation chamber.

A test of possible leakage is performed by comparing the triplet measurements of methane potential (the standard deviation shall be small).

## 6.3. Test system control

Interlaboratory calibration has earlier been made between the method used on separated fibres from swine production between Aarhus University and the DTI laboratory. Comparative study of method 1 and 2 described in appendix 5 has been performed by DTI showing no significant difference between the two methods. The ON-line measuring with automatic data collection is evaluated to increase the quality control/security.

### 6.4. Data integrity check procedures

All transfer of data from printed media to digital form and between digital media are checked by spot check of not less than 5 % of the data. If errors are found in a spot check, all data from the transfer are checked.

### 6.5. Test system audits

Supervision of methane tests by laboratory leader Paul Lyck Hansen

### 6.6. Test report review

Internal review of the test report will be done by LDMO, phone + 45 72202194, e-mail: ldmo@teknologisk.dk
External review of the test report will be done by the experts groups in 1.4.

# 7. Test results

The test report will be included as an appendix in the verification report according to the DANETV Centre Quality Manual.

## 7.1. Test Performance summary

The purpose of the present verification was to show an effect on biogas potential from mechanical treatment of different biomasses using Cellwood Deflaker GLD 200.

**Operational data**
The performance of the plant was evaluated and the current consumption during the test was measured.

**Data for Fibre Material**
The analysis of fibre material is primarily based on estimation of total solids and volatile solids.

**Biogas tests**
Biogas test were performed on untreated and treated fibres at mesophilic conditions. The accumulated methane production was calculated after 30 days active growth of the methanogens. The start of the active growth period is defined as the point where the methane production increase dramatically (just after the lag phase) as shown in the figures in appendix 5. There was no significant lag phase in the tests.

## 7.2. Test measurement summary

Target and measured values of tested parameters.

| Parameters | Target | Measured value | Method/comment |
|---|---|---|---|
| **Overall performance** | | | |
| Capacity | | (approximately 20 m3/h) | Based on Set flow. |
| **Chemicals** | | None | |
| | | | |
| **Energy** | | | |
| Electricity consumption | | 35 kw | Based on measurement during test on cow manure (9%DM) and maize silage 9.3% in water |
| **Treatment effects** | | | |
| Increase in Methane production % (maize silage) | | 9.5 | Methane potential (mesophilic 35˚C) after 30 days active methane production at 12.3 gVS/l. |
| Increase in Methane production % (cow manure) | | No increase | Methane potential (mesophilic 35˚C) after 30 days active methane production at 25 gVS/l |

It is concluded for Cellwood Deflaker GL200 system:

- there is a positive effect of approximately 10% on methane production when treating Maize silage
- there is no statistical significant effect on methane production for the tested cow manure

## 7.3. Test quality assurance

Selected tests of methane potentials on biomasses with known methane potential

## 7.4. Deviations from test plan

The test plan was followed

# 8. Appendix 1 Terms and definitions used in the test plan

Terms and definitions used in the protocol are explained in Table 8.1.

**Table 8.1 Terms and definitions used by the DANETV test centres.**

| Word | DANETV | Comments on the DANETV approach |
|---|---|---|
| Analytical laboratory | Independent analytical laboratory used to analyse test samples | The test centre may use an analytical laboratory as subcontractor |
| Application | The use of a product specified with respect to matrix, target, effect and limitations | The application must be defined with a precision that allows the user of a product verification to judge whether his needs are comparable to the verification conditions |
| DANETV | Danish centre for verification of environmental technologies | |
| (DANETV) test centre | Preliminary name for the verification bodies in DANETV with a verification and a test sub-body | Name will be changed, when the final nomenclature in the EU ETV has been set. |
| Effect | The way the target is affected | The effect could be concentration reduction, decrease in treatment period, pH increase etc. |
| (Environmental) product | Ready to market or prototype stage product, process, system or service based upon an environmental technology | The product is the item produced and sold and thus the item that a vendor submit for verification |
| Environmental technology | The practical application of knowledge in the environmental area | The term technology is covering a variety of products, processes, systems and services. |
| Evaluation | Evaluation of test data for a technology product for performance and data quality | None |
| Experts | Independent persons qualified on a technology in verification | These experts may be technical experts, QA experts for other ETV systems or regulatory |

| Word | DANETV | Comments on the DANETV approach |
|---|---|---|
| | | experts |
| Matrix | The type of material that the product is intended for | Matrices could be soil, drinking water, ground water etc. |
| Method | Generic document that provides rules, guidelines or characteristics for tests or analysis | An in-house method may be used in the absence of a standard, if prepared in compliance with the format and contents required for standards. |
| Performance claim | The effects foreseen by the vendor on the target (s) in the matrix of intended use | None |
| Performance parameters | Parameters that can be documented quantitatively in tests and that provide the relevant information on the performance of an environmental technology product | The performance parameters must be established considering the application(s) of the product, the requirements of society (regulations), customers (needs) and vendor claims |
| Procedure | Detailed description of the use of a standard or a method within one body | The procedure specifies implementing a standard or a method in terms of e.g.: equipment used |
| Producer | The party producing the product | None |
| Standard | Generic document established by consensus and approved by a recognized standardization body that provides rules, guidelines or characteristics for tests or analysis | None |
| Target | The property that is affected by the product | Targets could be *e.g.*. contaminant concentration |
| Test centre, test sub-body | Sub-body of the test centre that plans and performs test | None |
| Test centre, verification sub-body | Sub-body of the test centre that plans and performs the verification | None |
| Test/testing | Determination of the performance of a product for parameters defined | None |

| Word | DANETV | Comments on the DANETV approach |
|---|---|---|
| | for the application | |
| Vendor | The party delivering the product to the customer | Can be the producer |
| Verification | Evaluation of product performance parameters for a specified application under defined conditions and adequate quality assurance | None |

## Appendix 2 References (verification protocols, requirement documents, standards, methods, existing data)

1. DANETV. Centre Quality Manual, DTI 2009.
2. European Parliament and Council. Directive 2006/42/EC of the 17th May 2006 on machinery and amending Directive 95/16/EC (recast).
3. European Council: Directive 89/655/EEC of 30 November 1989 concerning the minimum safety and health requirements for the use of work equipment by workers at work (amended 2007/30/EC).
4. ISO 12100-2:2003: Safety of machinery - Basic concepts, general principles for design - Part 2: Technical principles.
5. Measurement protocol for biogas potential measurements for ETV tests at DANETV. (Method 1 and 2)
6. Unpublished results from test on deflaked manure fibres and straw, Århus University, (Maibritt Hjort,Research centre Foulum) given in spread sheet.
7. Cellwood machinery (2008) Information about the Deflaker.http://www.cellwood.se/Templates/ArticleProduct.aspx?PageID=2888cd67-ae71-4893-b424-0dcc18d5397c [assessed 23 September 2008]
8. Sundin, Anna Maria (2008), DISINTEGRATION OF SLUDGE - A WAY OF OPTIMIZING ANAEROBIC DIGESTION. 13th European Biosolids & Organic Resources Conference & Workshop,Käppala Association, Lidingö, Sweden. (http://www.kappala.se/admin/bildbank/uploads/Dokument/Processutveckling/Sundin,_A-M._

Disintegration_of_Sludge__A_way_of_Optimizing_Anaerobic_Digestion.pdf)

**Appendix 3 References methods**

**Appendix 4 In-house test methods**

***Pretesting program***

In the test of the Deflaker it is planned to test a number of biomasses with different degree of inhomogeneity. As the biomasses are treated by making a pulp of water /biomass fibres there may be flotation and sedimentation of fibre particles which produces an inhomogeneous pulp. This can lead to large standard deviations in measurements of biogas potential which makes a verification uncertain.

To evaluate the possible variations a pretesting program was performed in the following way on samples of treated biomass:

3-5 separate samples are taken of input and output during the test with the Deflaker. Further a mixed sample of the separate samples was prepared.

Samples were analysed for DM, VS. Mean and standard deviation is calculated.

Because the VS is correlated to the produced amount of methane a study of standard deviations will give some information of the expected standard deviations in biogas experiments.

Roughly if a difference in methane yield between treated and non-treated material is expected to be 10 % then the relative standard deviations of VS should be less than approximately 10% /2 = 5% in order to be able to conclude that there is an effect.

If the results of the pretesting program shows larger relative standard deviations than the expected effect the biogas test should be avoided.

## Appendix 5 In-house analytical methods

Measurement protocol for methane potential measurements for ETV tests at DANETV:

### *Method 1:* Measurement protocol for biogas potential measurements for verification tests (ETV, CBMI)

**First version: 12-5-09 revision v6 16/5-11**

*B. Malmgren-Hansen and Lars Ditlev Mørck Ottosen, Danish Technological Institute*
*Revised by Thorkild Quist Frandsen/Kasper Stefanek, Agrotech Henrik B.Møller,DJF*

The protocol v5 16/5-09 was developed as part of the CBMI project subproject 05 Test, certification and declaration, www.cbmi.dk . The present version is slightly modified

**Purpose**

The purpose is to make a common work protocol for performing batch fermentation on biomass used for mesophilic or thermophilic biogasification.

The protocol is based on methods used at DJF, Agrotech and DTI. DTU methods have also been evaluated.

**Description of test**

The test is a modified version of ISO 11734 [1)]

The test is based on performing batch biogasification with degassed inoculum from a biogas plant and added media with recording of produced gas amounts and content of methane.

The biogasification is performed for

- test material
- inoculum (blank test)
- reference material
- varying concentrations of added test material (inhibition test)

The test on test material and blanks are performed as at least a triplicate test. In case of high inhomogeneity the number of replicates can be increased.

**Conditioning of test material**

Samples must be representative of the biomass to be tested and with a homogeneous structure allowing for taking representative subsamples. Procedures for correct conditioning of biomass (test material) and subsampling must be described elsewhere as it will depend on the structure of the biomass.

**Handling and storing of samples**

Test material (fibre samples/liquid) samples are taken in e.g. 1 litre PE bottles, filled only 80% allowing for freezing.

If testing cannot be performed immediately, the samples are frozen.

**Materials**

- Infusion bottles which can withstand a pressure of 2 bar (volume ½-1 litre)
- Butyl rubber stoppers+ Al Crimps
- Measurement device for measuring volume of produced gas (volume measurement or pressure measurement)
- Reference substrate

**Conditions**

Incubation at 35 +/- 1 ˚C (mesophilic) or 52 +/- 1˚ C (thermophilic)
The incubation temperature must be verified in the thermostating equipment within at least +/- 1˚C using calibrated temperature measurement devices.
When infusion bottles are removed for gas volume measurement, the period of storage outside the incubation chamber should be minimized (<1 hr).

**Inoculum**

Manure from biogas plant degassed 2 weeks at temperature of interest (mesophilic or thermophilic).
The NH4-N content shall be below 4 g/l unless a special test condition is chosen. pH must be between 6.5 and 8.5. For mesophilic biogasification, inoculum from thermophilic reactors may be used, as the mesophilic culture exists in such media, however, at a lower concentration.

**Trial period**

The test period may be up to 90 days. However the test period may be shortened if the period of interest is lower. In normal operations of biogas plants the period of mesophilic operation is approx 30 days and thermophilic operation approx. 20 days. In this case 45 days of test is sufficient. See also figure 1 and 2 later.
Sufficient measurement points on the curve (10-15) should be made to calculate the biogas potential at least after 20 or 30 days and after the total number of days in the test.
If a lag phase in methane production is observed, the days in the lag phase should be added to the test period.

When running comparisons of products/process treatments etc. the same manure batch should be used as inoculum to decrease uncertainty from blank subtraction.

**Biogas potential test in infusion bottles**

Inoculum of known volume/weight and test samples were added to the infusion bottles.

There must be 40-60% free space in bottles allowing for accumulation of gas.

*Addition of inoculum:*
Preferred conditions:

- 500 ml infusion bottles : 200 ml inoculum (measured with 0.1% accuracy)
- 1000 ml infusion bottles : 400 ml inoculum (measured with 0.1% accuracy)

*Addition of test material (biomass):*

DM and VS was measured/known on test material before addition.

Test materials was added within a range that gives sufficient sensitivity and no inhibition.
The exact concentration must be estimated in an inhibition experiment.

Typical concentrations of test material was in the range
1 -30 g VS per liter inoculum.

The added amount was measured with 0.1% accuracy.

The test samples were flushed with $N_2$- 4 minutes before testing.

Tests were made in triplicate

**Blanks**
Tests were performed on inoculum (triplicate) for each new batch of inoculum.

The blanks were flushed with N2 for 4 minutes before testing.

**Reference**
A test compound (like sodium benzoate/cellulose powder or known fiber mix) should be run in inoculum (double or triplicate) for each new batch of inoculum.

The reference samples were flushed with N2 for 4 minutes before testing.

**Inhibition**
Inhibition from different substances may occur.
For NH4-N, inhibition may occur at levels of approx. 4g/l in the inoculum/test material mixture.

If no inhibition occurs, the same volume methane/g VS should be obtained for different values of added VS after complete fermentation.

To verify whether inhibition is present at test conditions, tests are performed with at least two concentrations of added VS etc. 100% and 30-50% and followed for at least 45 days.

**Produced gas**
Volume is calculated as pressure increase (ISO 11734) in headspace or measured directly with a volume collection tube (syringe or waterfilled gas collecting cylinder).
Efforts must be made to ensure no loss of process gas (ensuring gastight connections by pressure test).

**CH4/CO2**
Measured by GC for each measurement point during test (Method description in Biomass and Energy v26, 2004, p.487). GC must be calibrated using reference gas each day.

**pH**
pH is measured on inoculum batch before test.
pH is measured in test samples after finished biogasification as control of inhibitory acidification. (The measurement may be reduced to 1 pH measurement of triplicates showing same biogas production curves).

**Result**
For each measurement point, the ml methane amount is calculated.

Blank tests are subtracted.

A sum curve of produced (net) nml methane/gVS as function of time is calculated and plotted using correction for T,P.

All raw data on produced gas volume and methane should be available upon request.

**Typical methane production curves**
In Figure 1 is shown a typical curve for accumulated methane production at mesophilic biogasification of fibres separated from the slurry. Figure 2 shows the production rate for methane. In this test there is a lag phase during the first 10 days with the major production of methane from day 15 to 30. The lag phase is a delay in production while microorganisms are adapting to the biomass. It may be observed for some biomasses.

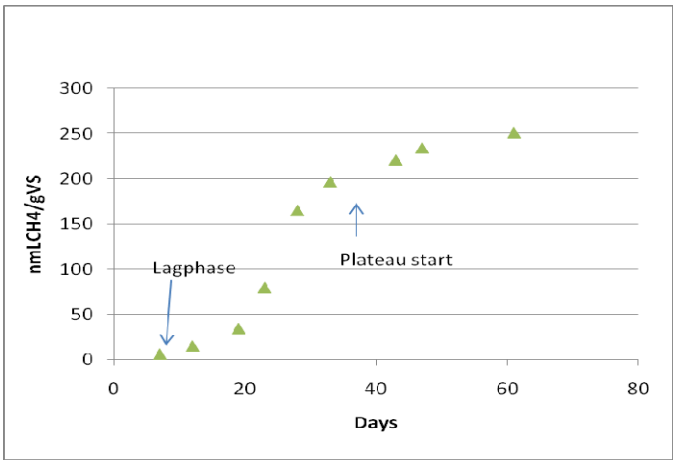

Figure 1. Accumulated methane production

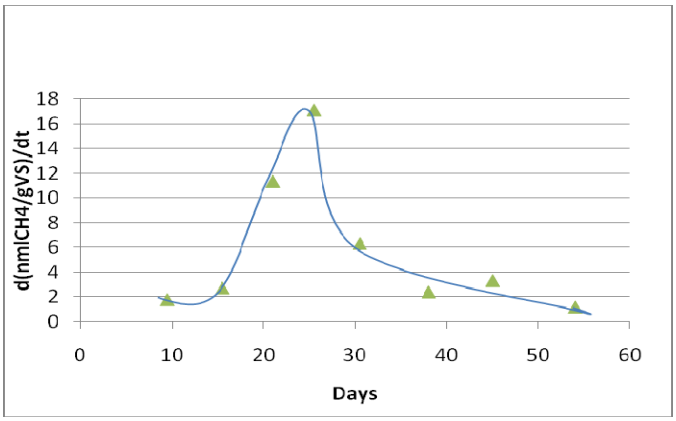

Figure 2. Methane production rate

**1) The test is a modification of ISO 11734 including a simple inhibition test like required in Angelidaki Water sci. & Tech p.927, 2009. Additional nutrient medium is omitted – standard fermentet manure is used as reference (including sufficient nutrients and bacteria adapted to anaerobic fermentation at such circumstances).**

***Method 2:Measurement using the bioprocess control system***

**Purpose**

The purpose is to make a common work protocol for performing methane potential tests with biomass using mesophilic or thermophilic anaerobic fermentation
The protocol is based on an automated biogas measurement method from bioprocess control.
The method has been intercalibrated with method 1 on separated fibres from swine.

**Description test**

The test is a modified version of ISO 11734 [1)]

The test is based on performing batch fermentation with degassed inoculum from a biogas plant and added media with recording of produced bioggas and methane volume

The anaerobic fermentation is performed for

- test material
- inoculum (blank test)
- reference material
- varying concentrations of added test material (inhibition test)

The test on biomass for test and blanks are performed as at least a triplicate test. In case of high inhomogeneity the number of replicates can be increased.

**Conditioning of test material**

Samples must be representative of the biomass to be tested and with a homogeneous structure allowing for taking representative subsamples. Procedures for correct conditioning of biomass (test material) and subsampling must be described as it will depend on the structure of the biomass.

**Handling and storing of samples**

Test material (fibre samples/liquid) samples are taken in e.g. 1 litre PE bottles, filled only 80% allowing for freezing.

If testing cannot be performed immediately, the samples are frozen.

**Materials**

- Complete measurement setup from bioprocess control

**Conditions**

Incubation at 35 +/- 1 ˚C (mesophilic) or 52 +/- 1˚ C (thermophilic)
The incubation temperature must be verified in the thermostating equipment within at least +/- 1˚C using calibrated temperature measurement devices.

**Inoculum**

Manure from biogas plant degassed 2 weeks at temperature of interest (mesophilic or thermophilic).

The NH4-N content shall be below 4 g/l unless a special test condition is chosen. The pH must be between 6.5 and 8.5. For mesophilic fermentation, inoculum from thermophilic reactors may be used, as the mesophilic culture exists in such media, however, at a lower concentration.

**Trial period**
The test period may be up to 90 days.

However the test period may be shortened if the period of interest is lower. In normal operations of biogas plants the period of mesophilic operation is approx 30 days and thermophilic operation approx. 20 days. In this case 45 days of test is sufficient. See also figure 1 and 2 later.
Sufficient measurement points on the curve (10-15) should be made to calculate the biogas potential at least after 20 or 30 days and after the total number of days in the test.
If a lag phase in methane production is observed, the days in the lag phase should be added to the test period.

When running comparisons of products/process treatments etc. the same manure batch should be used as inoculum to decrease uncertainty from blank subtraction.

**Biogas potential test in infusion bottles**
Inoculum of known volume/weight and test samples are added to the infusion bottles. The bottles are placed in a heating box. Produced biogas is cleaned for $CO_2$ in a acid trap and the volume of total gas and cleaned gas is measured automatically. The system is developed for on-line measurements of low methane flows produced from the anaerobic digestion in laboratory scale tests.

**Laboratory setup for measurement of biogas and methane production of different substrates.**

*Addition of inoculum:*
Preferred conditions:

- 500 ml infusion bottles : 300-400 ml inoculum (measured with 0.1% accuracy)

*Addition of test material (biomass):*

DM and VS shall be measured/known on test material before addition.

Test materials are added within a range that gives sufficient sensitivity and no inhibition.
The exact concentration must be estimated in an inhibition experiment.

Typical concentrations of test material are expected to be in the range
1 -30 g VS per liter inoculum.

The added amount is measured with 0.1% accuracy.

The test samples are flushed with $N_2$- 2-4 minutes before testing (using an $N_2$ amount of 10 times the free volume of filled flasks).

Tests are made at least in triplicate.

**Blanks**
Tests are performed on inoculum (triplicate) for each new batch of inoculum.

The blanks are flushed with $N_2$- 2-4 minutes before testing (using an N2 amount of 10 times the free volume of filled flasks).

**Reference**
A test compound (like sodium benzoate/cellulose powder) or reference fibres with known biogas potential should be run in inoculum (double or triplicate) for each new batch of inoculum.

The reference samples are flushed- 2-4 minutes before testing (using an N2 amount of 10 times the free volume of filled flasks).

**Inhibition**
Inhibition from different substances may occur.
For NH4-N, inhibition may occur at levels of approx. 4g/l in the inoculum/test material mixture.

If no inhibition occurs, the same amount of ml methane/g VS should be obtained for different added amounts of VS after complete fermentation.

To verify whether inhibition is present at test conditions, tests should be performed with at least two concentrations of added VS etc. 100% and 30-50% and followed for at least 30 days of active biogas production.

**Produced amount of Methane**
The bioprocess control system removes Carbon dioxide by washing with NaOH and measures Methane directly

**pH**
pH is measured on inoculum batch before test.
pH is measured in test samples after end of fermentation as control of inhibitory acidification. (The measurement may be reduced to 1 pH measurement of triplicates showing same biogas production curves).

**Result**
For each measurement point, the volume of methane (in ml) is calculated.

Blank tests are subtracted.

A sum curve of produced (net) nml methane/gVS as function of time is calculated and plotted using correction for T,P.

All raw data on produced volume of methane should be available upon request.

**Typical biogas production curves**
In Figure 1 is shown a typical curve for accumulated methane production at mesophilic biogasification of fibres separated from the slurry. Figure 2 shows the production rate for methane. In this test there is a lag phase during the first 10 days with the major production of methane from day 15 to 30. The lag phase is a delay in production while microorganisms are adapting to the biomass. It may be observed for some biomasses.

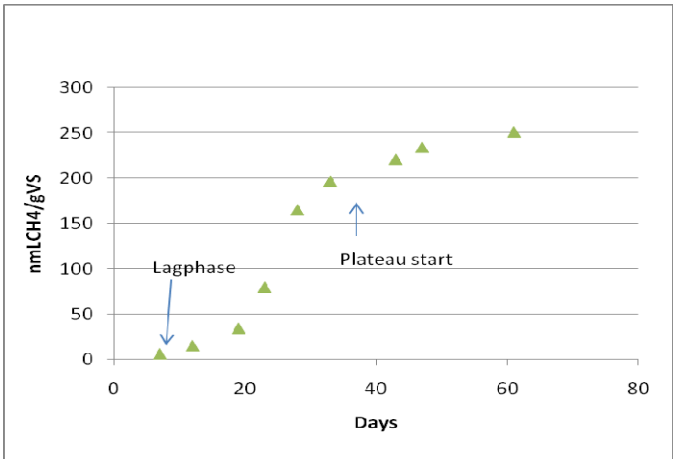

Figure 1. Accumulated methane production

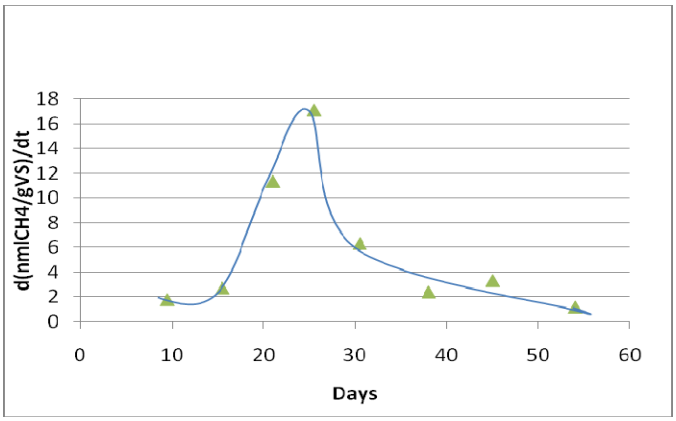

Figure 2. Methane production rate

**Appendix 6 Data reporting forms**

Not specified here. All necessary data must be given in tables.

## Appendix 7 Test data report
**Test**

The test was performed as described in the test plan on May 31th 2011.

***Calibration***

Before test the weight for measuring biomass addition was tested using a constant weight of 4,350 kg weighed on a calibrated weight at Danish Technological Institute. The used weight at the test site was measuring correctly with no deviation on 2 decimals.

The used current clamp was tested together with a calibrated current clamp showing correct current measurements.

***Tests performed***

In the tests the biomass was added to a stirred 1 $m^3$ tank with amounts shown in Table 2.
The inner diameter of the tank was measured to 120 cm and the height at the position were the tank was full=90 cm corresponding to a volume of 1.01 $m^3$.
The tank is shown in Figure 3 and the Deflaker in Figure 4.

**Figure 3 Stirred tank for biomass addition in test**


**Figure 4 Deflaker GLD 200 Input sample valve in the middle**

The operating conditions for Deflaker GLD 200 was a flow of 20 $m^3$/h or 333 litre per minute and a rotational speed of 3000 revolutions/min. During the test output was removed to the biogas plants drainage system. As the flow of the Deflaker is large the test period was limited to approximately 3 minutes. It was considered possible to take at least 3 samples during the test as required in test plan.

During the test the current consumption of the Deflaker was measured using a current clamp In Table 2 are shown the amounts of biomass and water used and the power consumption for each phase of 3*390 V.

**Table 2 conditions in test and measured current consumption**

| Biomass/medium | Biomass kg | Water kg | Current A |
|---|---|---|---|
| Water | 0 | 1000 | 22 |
| Cow manure | 600 | 400 | 30 |
| Maize Silage | 92.64 | 1000 | 30 |

The power consumption was thus 390*30*3=35.1 kW

The cow manure is shown in Figure 5 while input of maize silage is shown in Figure 6 and treated maize silage in Figure 7.
Sampling of input was made through the valve marked IN in Figure 4 while output was sampled directly from outlet tube –see sampling of treated cow manure in Figure 8. Approximately 5 samples of 0.8 litres were taken of input and output during each trial.

**Figure 5 Cow manure in mixing tank**

**Figure 6 Maize silage**

**Figure 7 Treated maize silage**

**Figure 8 Sampling of treated cow manure**

## Analysis results

### *Analysis of composition of added and treated fibres*

Table 3 shows analysis results for the used cow manure after addition of water while Table 4 shows result from maize silage taken from a sample directly from the big bag ( Figure 6).

**Table 3 Analysis results cow manure**

| Parameter | Input | Output |
|---|---|---|
| Total N g/kg | 4.08 | 4.17 |
| NH4-N g/kg | 2.31 | 2.38 |
| P g/kg | 0.55 | 0.55 |
| K g/kg | 2.7 | 2.8 |
| S g/kg | 4.7 | 4.9 |
| DM % | 8.81 | 9.02 |
| VS % [1] | 81 | 81 |

1 VS :Volatile Solids
Analysis by EuroFins

Original concentrations in cow manure was 1000/600* concentration measured etc Total N= 10/6*4.08=6.8 g/kg. The table shows that analysed parameters of input is comparable to output as expected.

**Table 4 Analysis results maize silage added fibres**

| | Input |
|---|---|
| **Parameter** | |
| Total N g/kg | 3.3 |
| NH4-N g/kg | 0.4 |
| P g/kg | 0.48 |
| K g/kg | 2.4 |
| S g/kg | 1.2 |
| DM % | 21.2 |
| VS % [1] | 92 |

1 VS :Volatile Solids
Analysis by EuroFins
Analysis of DM ,VS for input and output liquids with suspended maize silage fibres is shown in table 5.

In Table 5 are shown analysis results of samples of cow manure taken during the test. The tests are based on two different samples and results in a small standard deviation of 1.3 % relative on input and 0.9% relative on output. From the small standard deviations it is concluded that no further treatment is necessary to minimize standard deviation and that biogas tests can be performed.

**Table 5 Analysis results of input and output cow manure (Average and Standard Deviation)**

| | **Input Cow manure Average** | **Input Cow manure Std.dev** | **Output Cow manure Average** | **Output Cow manure Std.dev** |
|---|---|---|---|---|
| **Parameter** | | | | |
| DM % | 9.12 | 0.12 | 9.04 | 0.08 |
| VS % | 81.9 | 0.55 | 81.9 | 0.44 |

Analysis by Danish Technological Institute

The dry matter of the original cow manure can be calculated to 9.1*1000/600=15.2 %DM

Maize silage was very inhomogenius with a liquid phase and a solid phase with large particles (flakes) of maize separation from the liquid phase very fast. As it is difficult to take representative samples of a system where the phases separate from each other fast it was decided to use a proportional weighing method.

*Subsampling without proportional weighing*
The output samples was more homogeneous than the input samples due to the deflaking. To evaluate the standard deviation obtained without subsampling a test with analysis of DM was performed for sample 1,3,5 of output without using proportional weighing. The result was an average of DM of 2.0 % with a standard deviation of 0.18 corresponding to a relative standard deviation of 9%.

*Subsampling using the proportional weighing method*
The 5 samples of input and 5 samples output were divided into a solid and liquid part using a 1 mm sieve to be able to obtain representative samples and for subdividing for further tests.

The 5 liquid and 5 solid samples of input and output samples were mixed into 2 “mix samples” producing 8 fractions to be used for biogas tests and analysis:

Solid input mix 1, solid input mix 2
Liquid input mix 1, liquid input mix 2
Solid output mix 1, solid output mix 2
Liquid output mix 1, liquid output mix 2

The fractions were analyzed individually for DM and VS and later addition to biogas tests was made by proportional weighing. DM and VS is shown in Table 6.

**Table 6 Data for samples divided into a liquid and filtered (solid) part (Maize silage)**

| | Propotional weiging part | DM | DM | VS | VS |
|---|---|---|---|---|---|
| **Parameter** | **%** | **%** | **Std.dev** | **%** | **Std.dev** |
| Input liquid | 88.7 | 0.617 | 0.003 | 87.95 | 0.23 |
| Input solid | 11.3 | 14.37 | 0.64 | 96.45 | 0.49 |
| Output liquid | 89.6 | 0.81 | 0.01 | 91.19 | 1.09 |
| Output solid | 10.4 | 11.63 | 0.31 | 97.09 | 0.03 |

The measured DM of output using the proportional weighing method was DM=1.94%, with standard deviation= 0.033). This is in agreement with the DM measured without using the proportional weighing method: DM=2.0 standard dev. =0.18 within one standard deviation.

The mass balance of the test in terms of VS can be calculated as follows for 100 grams input and output:

gVS in input/100 g= 88.7*0.617/100*87.95/100+11.3*14.37/100*96.45/100=2.047 gVS
gVS in output/100 g=89.6*0.81/100*91.19/100+10.4*11.63/100*97.09/100=1.836 gVS

There is a 10.3% lower VS amount in the samples of output than in the input. This is assumed to be based on errors in sampling of input as the sampling of input (with large fibres) was made through a valve connected to a T (see Figure 4) while output was sampled directly from outlet (Figure 8) and therefore should be more representative for the original amount of VS in the mixing tank.

A T- connection where part of the fluid passes through a valve and the rest through the main tube to the Deflaker may produce another concentration of large particles in output than in the input The maize silage flakes has a longitudinal dimension of 3-5 cm which is close to the dimension of the valve.

However, the produced amount of biogas must be calculated for the actual measured concentration of VS in the samples from input and output and when the concentration difference is small as here it will not influence the measured amount of accumulated methane/g of VS.

## Methane potential

The nontreated and treated (deflaked) fibres were tested according to method 1 (appendix 5) and/or method 2 (appendix 6)

Cow manure was tested using method 2 with some additional measurements using method 1 and maize silage was tested using method 1

When using method 1 the temperature of the climate chamber was 35 ˚C+/-0.5 ˚C except for small periods of approximately 1 hour when bottles was removed for volume measurement as seen in Figure 9. When using method 2 the temperature of the climate chamber was 35 ˚C+/-0.5 ˚C during the complete test. The temperature was verified using a calibrated thermometer.

Tests with cow manure was initiated 22-8-11 using manure was from Bånlev Biogas reactor 2 running at mesophilic conditions retrieved on 10-8-11. The ammonia content was 4.4 g/l which is above the limit were some inhibition occur described in method 1 and 2. To reduce the ammonia to below 4 g/l the concentration was reduced by adding 1 part water to 4 parts manure.

**Table 7 Cow manure preparation of flasks for biogas test method 2 (400 g)**

| Test | DM % | VS % | gVS/L | g cow manure | g inoculum | g demin water |
|---|---|---|---|---|---|---|
| Cow manure in | 9.11 | 81.9 | 25.0 | 134.03 | 212.78 | 53.19 |
| Cow manure out | 9.04 | 81.9 | 25.0 | 135.07 | 211.95 | 52.99 |

Tests with Maize silage was initiated 14-11-11 using manure from Bånlev Biogas reactor 1 running at mesophilic conditions retrieved on 1-11-11. The ammonia content was 4.6 g/l which is above the limit were some inhibition arise described in method 1 and 2. In the tests the ammonia content is diluted by the water present in the maize silage.

**Table 8 Maize silage preparation of flasks for biogas test method 1**

| Test | gVS/l | g liquid fase | g solid phase | g inoculum | g dem water [1] |
|---|---|---|---|---|---|
| Maize silage in | 12.4 | 107.68 | 13.65 | 66.67 | 12.00 |
| Maize silage in | 6.2 | 53.84 | 6.83 | 66.67 | 72.67 |
| Maize silage out | 12.3 | 119.42 | 13.91 | 66.67 | 0.00 |
| Maize silage out | 6.1 | 59.71 | 6.95 | 66.67 | 66.67 |

1 demineralized water was added to obtain 200 gram liquid/solids in each 500 ml flask at either 12.4 or 6.1 gVS/l and with 66.7 g inoculum in each flask.

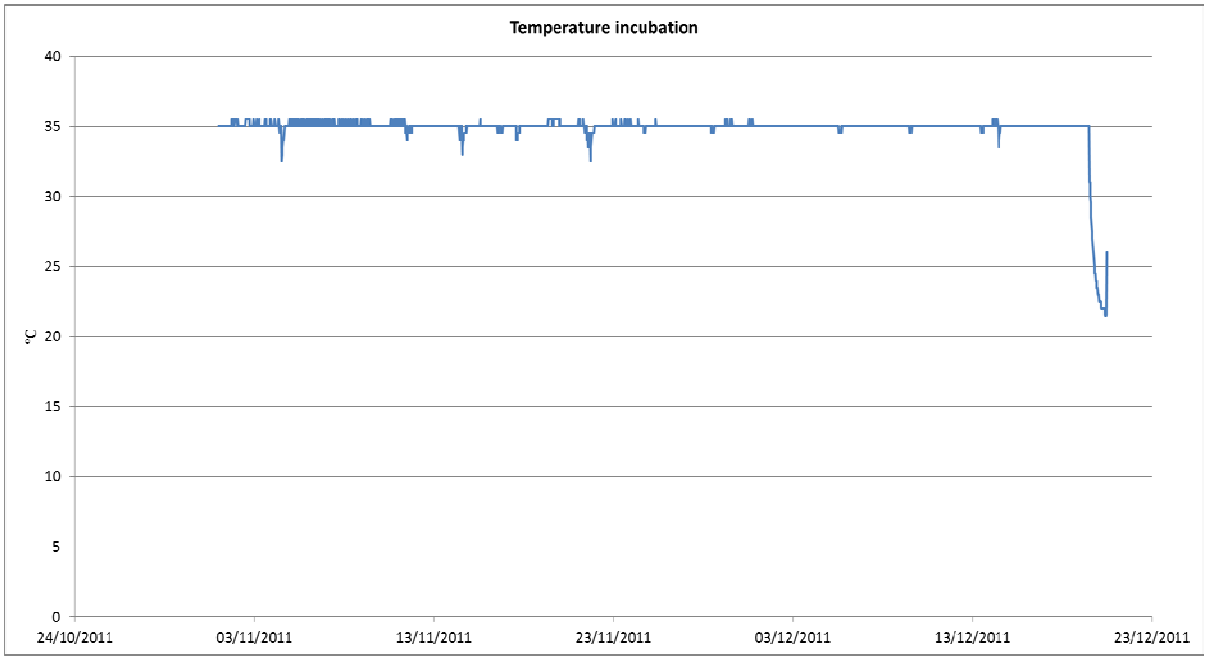


**Figure 9 Temperature during biogasification of maize silage (method 1)**

In Figure 9 is shown the logged incubation temperature during biogasification of maize silage using method 1. The temperature is at 35 ºC +/-1 ºC.

At regular intervals gas volume was measured and the gas composition was analysed.

In method 1 the Methane content was measured with a gas chromatograph.
Calibration on a synthetic biogas with known amount of methane and carbon dioxide was performed each measurement day before running the tests.

In method 2 only the volume of methane is measured as carbon dioxide is removed by a washing step.

## Results

### *Cow Manure*

Methane potential of cow manure was measured using method 2 with (Figure 10) and method 1 (Figure 11)

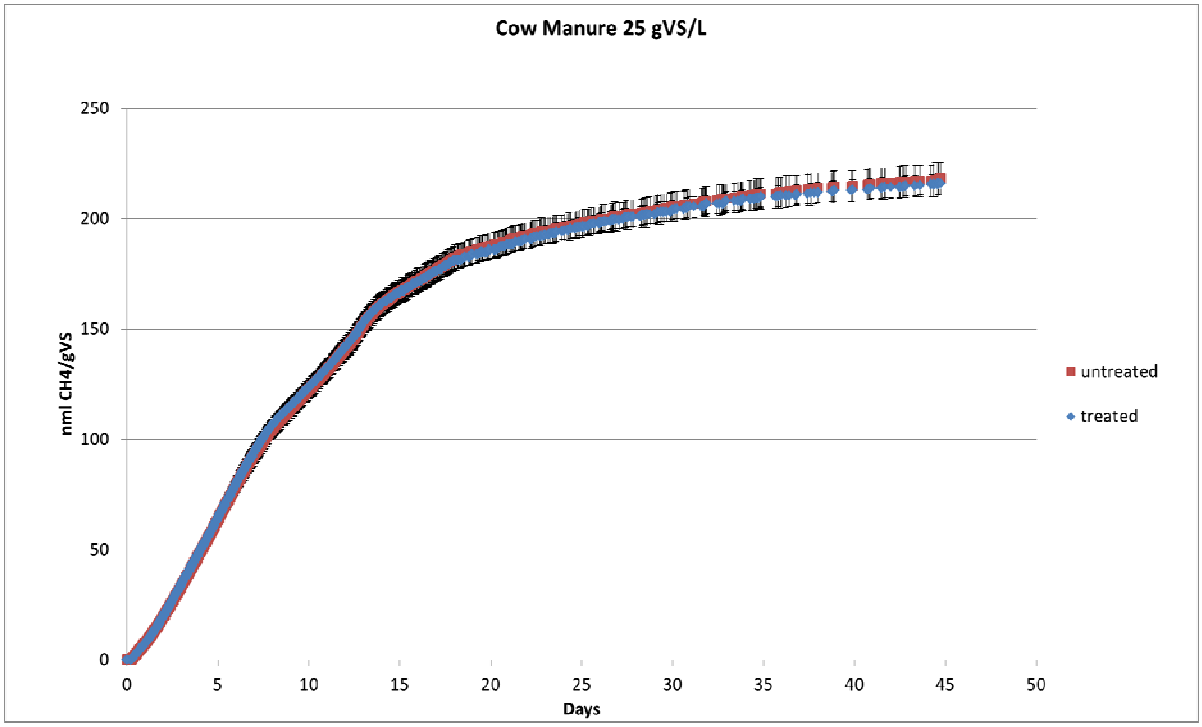


**Figure 10 Produced accumulated methane using 5 replicates for input and 4 replicates for output, 25 gVS/l and method 2.**

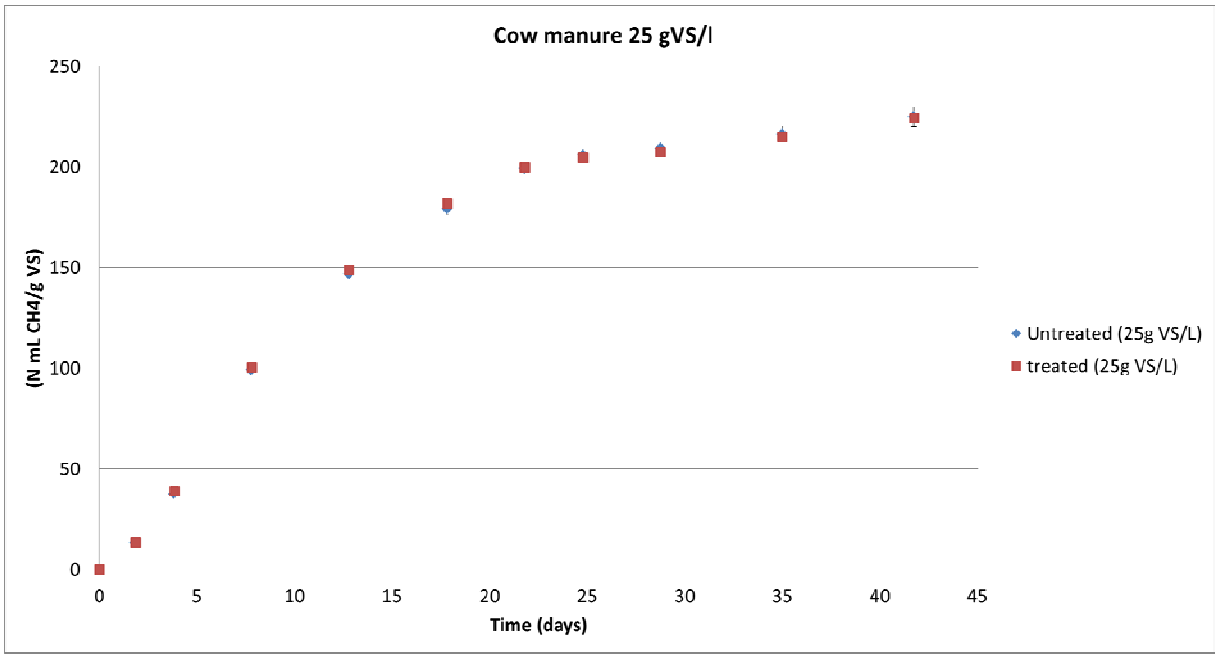


**Figure 11 Produced accumulated methane using 3 replicates 25 gVS/l and method 1.**

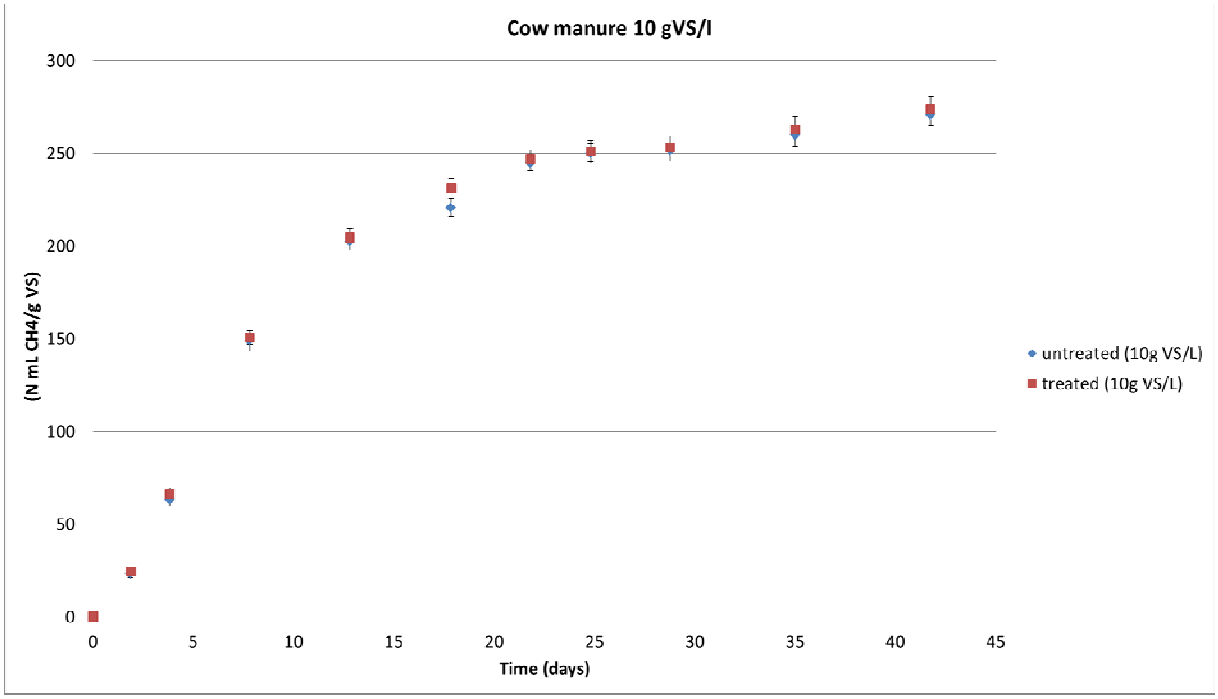


**Figure 12 Produced accumulated methane using 3 replicates 10gVS/l and method 1.**

The figures show no relative difference in produced amount of methane between input and output with a production of approximately 200 ml methane/gVS for 25 gVS/l after 30 days.

**Table 9 biogas output for cow manure at 25 gVS/l (method 1)**

| day | Input Nml CH4/gVS | Input Std.dev. | Output Nml CH4/gVS | Output Std dev. |
|---|---|---|---|---|
| 28.8 | 209.0 | 3.1 | 207.2 | 2.3 |
| 35.0 | 216.0 | 4.1 | 214.9 | 2.5 |

**Table 10 biogas output for cow manure at 10 gVS/l (method 1)**

| day | Input Nml CH4/gVS | Input Std.dev. | Output Nml CH4/gVS | Output Std. dev. |
|---|---|---|---|---|
| 28.8 | 251.4 | 5.5 | 253.0 | 6.1 |
| 35.0 | 259-7 | 6.2 | 262.6 | 7.1 |

As reference a test was used with 25 gVS/l and method 1 based on separated fibres from swine manure. The test showed approximately 190 ml methane produced /g VS after 30 days “active production” which is comparable with earlier results using the same reference.

***Maize Silage***

Biogas potential of maize silage was measured using method 1 with 5 replicates for 12.3 gVS/l. Further the produced volume of methane was measured at 6.1 gVS/l using 2 replicates as a control of the effect of adding a lower concentration.

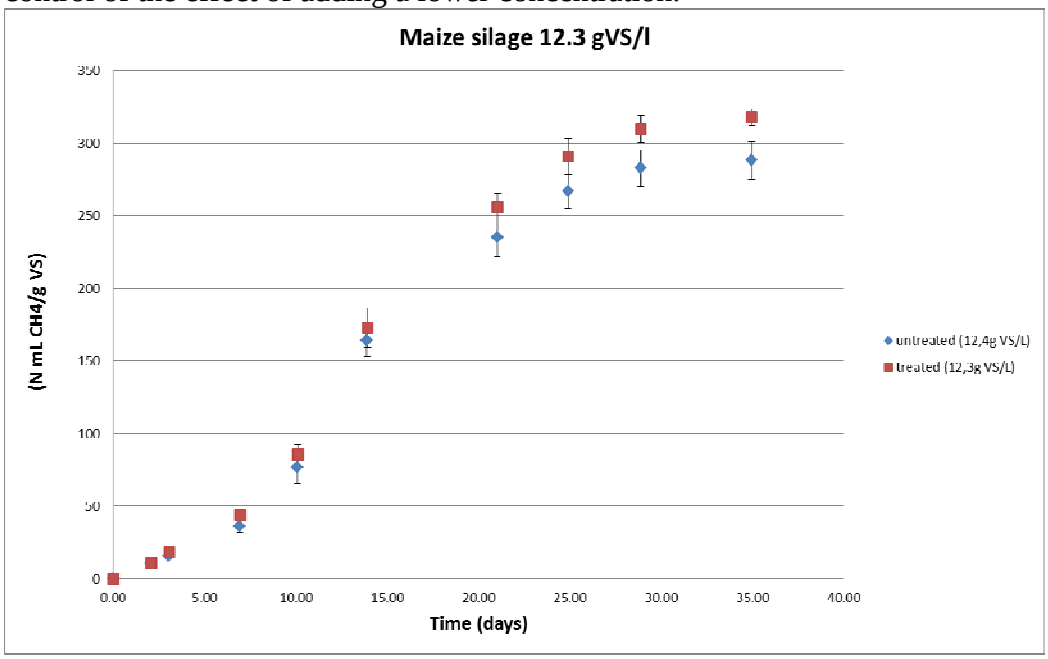


**Figure 13 Produced accumulated methane using 5 replicates 12.3gVS/l and method 1**

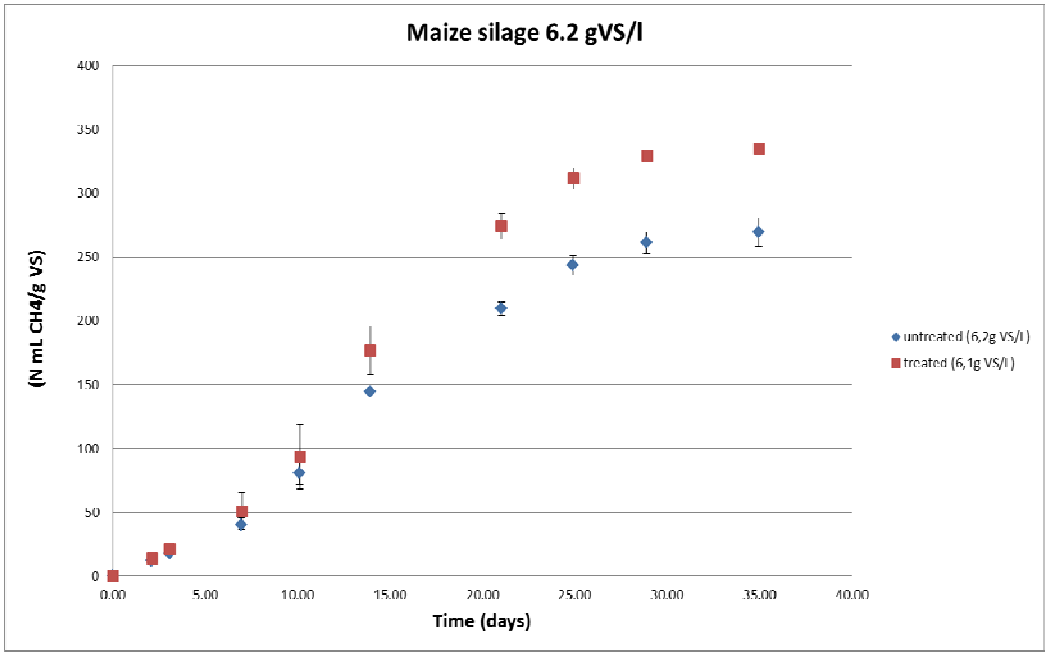


**Figure 14 Produced accumulated methane using 2 replicates 6.1gVS/l and method 1.**

The curves show an effect on improving the methane volume at approximately 30 days.

**Table 11 improved biogas output for maize silage at 12.3 gVS/l**

| day | Input Nml CH4/gVS | Input Std.dev | Input rel. Std.dev % | Output Nml CH4/gVS | Output StdAfv | Output Rel. Std.dev % | improvement % |
|---|---|---|---|---|---|---|---|
| 28.9 | 283.0 | 12.5 | 4.4 | 309.5 | 9.4 | 3.0 | 9.4 |
| 35.0 | 288.3 | 12.9 | 4.5 | 317.7 | 5.8 | 1.8 | 10.2 |

**Table 12 improved biogas output for maize silage at 6.1 gVS/l**

| day | Input Nml CH4/gVS | Input Std.dev | Input rel. Std.dev% | Output Nml CH4/gVS | Output Std.dev | Output Rel. Std.dev % | improvement % |
|---|---|---|---|---|---|---|---|
| 28.9 | 261.1 | 8.5 | 3.3 | 329.3 | 4.2 | 1.3 | 26.1 |
| 35.0 | 269.3 | 11.2 | 4.2 | 334.9 | 0.7 | 0.2 | 24.3 |

The effect seems to depend on the concentration of maize fibres.

## Discussion and conclusion

The cellwood Deflaker GLD 200 showed no measurable improvement in methane potential when treating cow manure within the uncertainty.

For Maize silage there was a significant increase in methane production under laboratory conditions. The increase was 9.5% at 12.3 gVS/l based on a 5-double measurement of methane after 30 days of active biogas production. When using a lower concentration of 6.2 g/l the effect seems to be larger but this is only based on a double measurement. .
The method used for measuring methane production deviates from the conditions in full scale on the following major points:

- The test is a batch test with a one time feeding and not a continuous feeding as in a full scale plant