Upgrading of dairy cattle manure as monosubstrate for biogas production
Final report part 2 CSTR experiments

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Summary

Thermo-chemical treatment NiX improved by 48% the methane yield of the second step of a 2-step CSTR biogas process and by 16% the total methane yield of the overall 2-step biogas process. The results from CSTR experiments were confirmed by batch tests.

This project studied the effect of treatments to improve the efficiency of a 2-step CSTR biogas process digesting cattle manure. A thermo-chemical treatment (based on patented NiX treatment) and a combined thermo-chemical (NiX) followed by biological treatment were tested.

The treatments were applied to the solid fraction (fibres) of the effluent from the first step (thermophilic digester) of the biogas process. The effluent from the first step was separated into the solid fraction (fibres) and the liquid fraction. The solid fraction was treated with NiX treatment at 140 °C, NaOH 4.3% w/w TS, 20 minutes. The treated solid fraction was then mixed with the liquid fraction and this mixture was used as feed for the second step (mesophilic digester).
NiX treatment resulted in 48% higher methane yield of the mesophilic digester compared to the yield of the mesophilic digester without NiX treatment. The overall effect of NiX treatment on the 2-step biogas process was 16% methane yield increase.
The combined treatment NiX followed by biological treatment did not increase the methane yield.

Batch tests confirmed the methane yield increase caused by NiX treatment. With the batch tests, the methane yield increase registered for the mixture of solid fraction and liquid was 69%. The batch tests showed that NiX treatment increased by73-82% the methane yield of the solid fraction alone.

The biogas production increase occurred two days after the application of NiX treatment and it reached a constant level at steady state operation of the 2-step CSTR biogas process. Thus, NiX treatment at the treatment conditions tested proved to be suitable to increase the methane yield for long-term operation (steady state) or to temporarily boost the biogas production. The low dosage of NaOH makes it attractive for commercial-scale applications.

Contents

1 Introduction

The project was run in collaboration between Xergi and Novozymes.

The original plan of this project included three parts:

1. lab-scale tests (batch) to screen different microorganisms to be used for biological treatment;
2. pilot-scale tests (CSTR) of the treatments selected during part 1;
3. full-scale tests.

This part of the report focuses on part 2, the pilot-scale CSTR tests. The tests included thermo-chemical treatment (based on patented NiX) and combined thermo-chemical NiX and biological treatment. Part 2 lasted for 16 months, from December 2009 to March 2011 included.

1.1 Choice of thermo-chemical treatment (NiX)

The treatment conditions to be used for the thermo-chemical treatment had to fulfill the following:

1. compatible with Xergi's NiX patent;
2. methane yield improvements proved at lab-scale.

Because at the time of the choice of the thermo-chemical treatment (June 2010) there were no reliable results yet on the effect of the patented NiX treatment on the methane yield of fibres from digested cattle manure, the thermo-chemical treatment chosen for this project was a combination of patented NiX and other treatments tested at lab-scale.

A thermo-chemical treatment that had been tested in batches by Xergi (EBRU), using fibres from cattle manure as substrate, was modified to fulfill the requirements of the NiX patent. This modified thermo-chemical treatment is called NiX in this report and is described in Appendix B: NiX treatment.

1.2 Choice of biological treatment

The success criterion of the previous part 1 of this project (25% improvement) was not met, however some effects due to biological treatment were observed. For this reason, part 2 (combined thermo-chemical and biological treatment at pilot-scale CSTR) was run.
For part 2, Xergi (EBRU, APJE, SBG) and Novozymes (PNIE and MISA) chose treatment with fungal strain Coprinus as biological treatment (Appendix C: combined NiX and biological treatment).
Details about part 1 can be found in the first part of the report and in
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2 Procedure

Xergi's pilot-scale plant *Anlæg2* was used for the experiments. A 2-step biogas process with first step thermophilic (52 °C) and second step mesophilic (37 °C) digested cattle manure. The treatments were applied to the solid fraction of the effluent from the thermophilic digester (Figure 1).

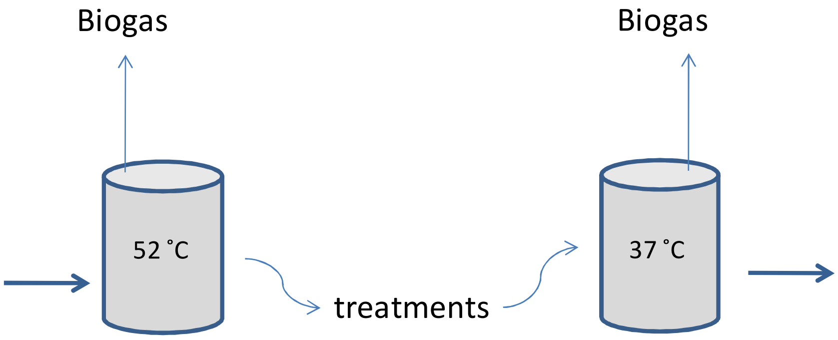

Figure 1. 2-step biogas plant and treatment.

The hydraulic load of the two digesters was maintained constant during the project, while the characteristics of the substrate and the process configuration were modified during the phases described below. The project was divided into the following phases:

Dec 2009 – Jan 2010: phase 0, no treatment
Feb – July 2010: phase 1, baseline
July – Oct 2010: phase 2, NiX treatment
Nov – Dec 2010: phase 3, baseline
Jan 2011 – Mar 2011: phase 4, NiX treatment + biological treatment
Mar 2011 – Apr 2011: phase 5, NiX treatment

The phases are described in 2.2 CSTR process configurations.

2.1 Operation of the digesters

2.1.1 Substrate

The substrate was a mixture of solid fraction of cattle manure and water 1:3 w/w WW (1 kg wet weight of solid fraction from cattle manure into 3 kg of water). The cattle manure was obtained from Århus University, Faculty of Agricultural Sciences and it was separated into the solid and the liquid fractions with a centrifuge GEA Westfalia Separator Umwelttechnick GmbH (model UCD-305-00-02).
The solid fraction of cattle manure was collected in two batches of 1300 kg each, it was divided into portions of 70 kg and it was maintained frozen at -10 °C until use. The solid fraction of cattle manure contained TS 26.3 ± 3.3%, VS 22.3 ± 2.8% (average of 13 measurements from June 2010 to March 2011), NH_4-N 2.31 g/kg (one measurement February 2011).

The substrate (mixture of solid fraction from cattle manure and water) contained TS 6.6 ± 0.8%, VS 5.6 ± 0.7%, NH4-N 0.58 g/kg.

2.1.2 Thermophilic digester

The thermophilic digester was maintained at constant conditions during the 16-month project: temperature 52 °C, volume 150 L, feed 8.4 ± 0.4 kg-WW / d.
The resulting HRT (hydraulic retention time) and OLR (organic loading rate) were 18 d and 3.1 kg-VS / (m^3 d), respectively (VS is the organic matter content of the substrate, calculated from a moving average of the last three VS measurements).

2.1.3 Mesophilic digester

The mesophilic digester was maintained at constant temperature 37 °C, volume 200 L, feed 7.1 ± 0.4 kg-WW / d and HRT 27.5 ± 2.7 d. During the different phases of the project, the process configuration was modified as described in detail in 2.2 CSTR process configurations.

2.2 CSTR process configurations

2.2.1 Phase 0

A 2-step biogas process based on two CSTRs in series was used for this phase (Figure **2**, Figure 3). The purpose of Phase 0 was to establish a stable biogas process. When steady state was reached, the experimental set-up was modified and Phase 1 – baseline started.

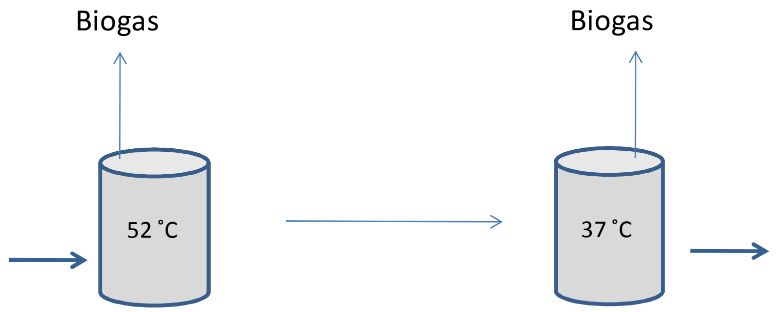

Figure 2. Process configuration during Phase 0**.**

Figure 3. Pilot-scale biogas plant anlæg 2 during Phase 0.

2.2.2 Phase 1 – baseline

The experimental setup of Phase 0 was modified introducing the two intercept barrels IB1 and IB2 between the thermophilic and the mesophilic digester (Figure 4, Figure 5).

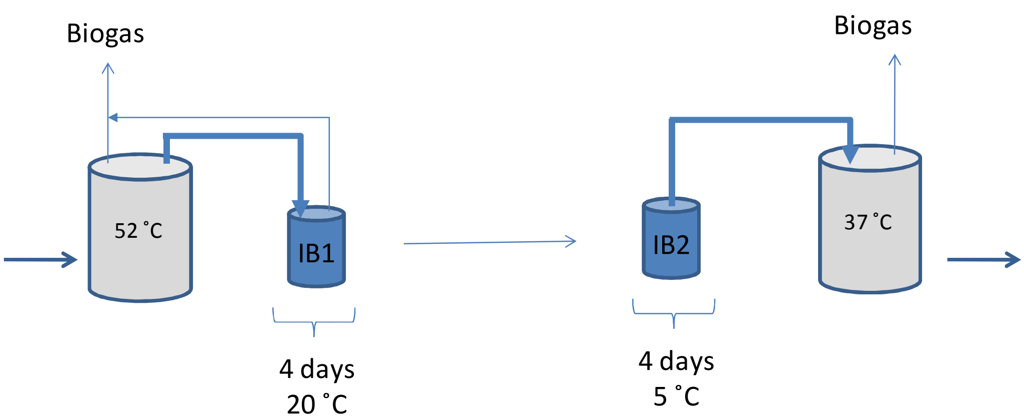

Figure 4. Process configuration during Phase 1 – baseline.

Figure 5. Pilot-scale biogas plant “anlæg 2” during Phase 1 – baseline**.**

The barrel IB1 received the effluent from the thermophilic digester. IB1 was at room temperature and it was not stirred. The biogas produced by IB1 was collected and counted together with the biogas produced by the thermophilic digester. Twice per week, the content of IB1 was manually transferred into the barrel IB2. IB2 was stirred and maintained at 5 °C. The biogas produced in IB2 was not collected (batch tests showed that the biogas production from IB2 was negligible). The material from IB2 was used as feed for the mesophilic digester. The average residence time of the material in IB1 or IB2 was 4 days.

The process configuration of Phase 1 – baselinePhase 1 was maintained until steady state was reached. Phase 1 – baseline (together with Phase 3 – baseline) was used as reference baseline during the project.

2.2.3 Phase 2 – NiX treatment

Separation and NiX treatment were introduced with Phase 2 – NiX treatment (Figure 6).

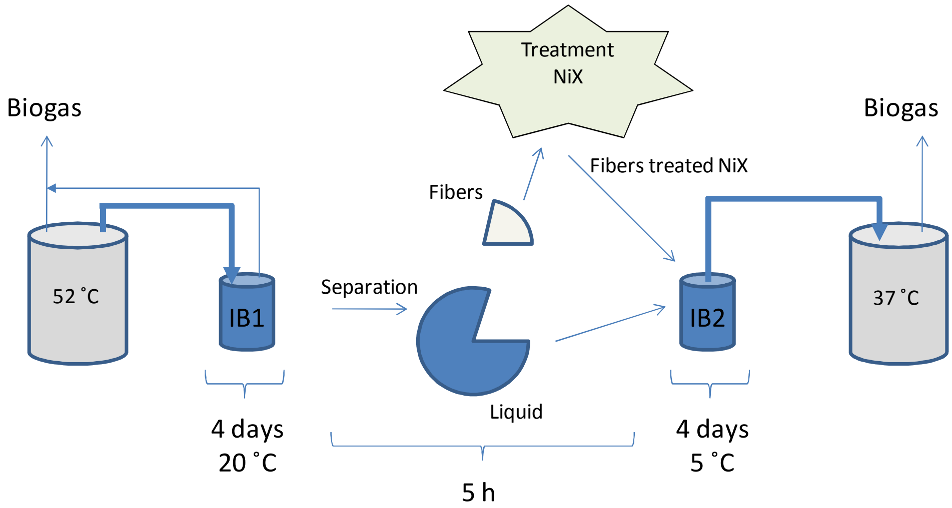

Figure 6. Process configuration during Phase 2 – NiX treatment**.**

The solid and the liquid fractions of the material contained in IB1 were separated with the press described in s

Appendix A: separation. The liquid fraction (TS 1.4 ± 0.2%) was kept in a barrel for approximately 5 h, while the solid fraction was being treated. The solid fraction (fibres, TS 16.5 ± 2.3%) was treated with NiX (Appendix B: NiX treatment).
After the treatment, the treated solid fraction and the liquid were mixed in IB2 and were used as substrate for the mesophilic digester.
The duration of Phase 2 – NiX treatment was 109 days, corresponding to 4.0 HRT.

2.2.4 Phase 3 – baseline

The process configuration during Phase 3 – baseline was the same as during Phase 1 – baseline (Figure 4, Figure 5). These two phases were used as reference baseline. Phase 3 – baseline was introduced to prove that after a period with treatment (Phase 2 – NiX treatment), the system could come back to the same performance as it was before the treatment (baseline, Phase 1 – baseline).

2.2.5 Phase 4 – combined treatment

The process configuration during Phase 4 – combined treatment is shown in Figure 7. After separation with the press, the solid fraction was treated with the combined NiX and biological treatment. For details about the combined treatment, see Appendix C: combined NiX and biological treatment.

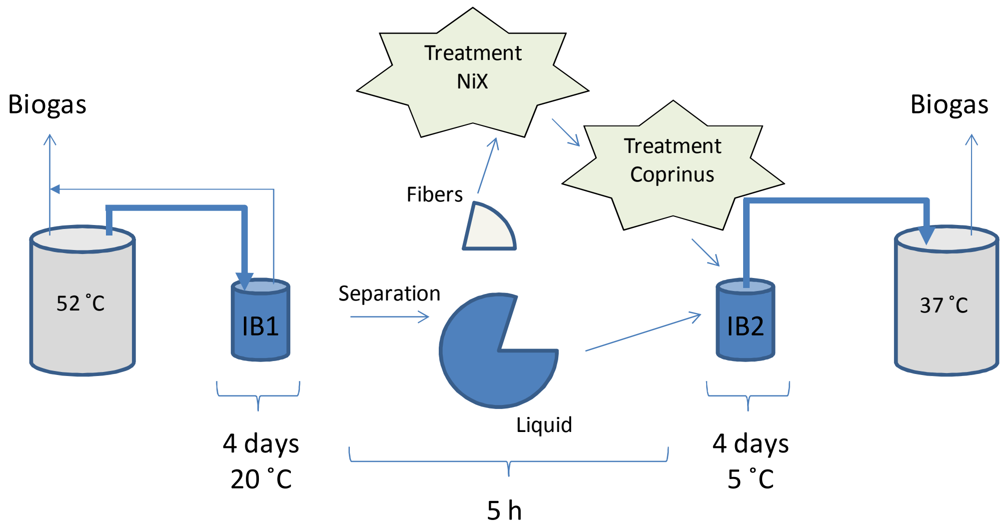

Figure 7. Process configuration during Phase 4 – combined treatment.

2.2.6 Phase 5 – NiX treatment

Phase 5 – NiX treatment was a repetition of Phase 2 – NiX treatment (Figure 6). Phase 5 – NiX treatment was introduced to prove that the results obtained during Phase 2 – NiX treatment could be repeated.

2.3 Batch tests

The substrates for the batch tests were sampled during the CSTR experiments. Details about the preparation of the batches are given in Appendix D: batch experiments.
Two series of batch tests were made on 27 October 2010 and on 14 March 2011.

3 Findings

Treatment with NiX improved the methane yield of the mesophilic digester by 48% and the total methane yield (thermophilic + mesophilic digester) by 16%. The results from CSTR experiments were confirmed by batch tests.
Combination of NiX and biological treatment with Coprinus resulted in the same methane yield as during the baselines.
The methane yield from the thermophilic digester (not affected by the treatments) was constant during the 16-month experiment.

3.1 Effect of NiX treatment

3.1.1 Effect on yield

The effect of NiX treatment was tested with Phase 2 – NiX treatment and with Phase 5 – NiX treatment.
The yield during these phases was compared to the yield during Phase 1 – baseline and Phase 3 – baseline (baselines). Details about the treatment are given in Appendix B: NiX treatment. Phase 3 – baseline was introduced to prove that after a period with treatment (Phase 2 – NiX treatment), the system could come back to the same performance as it was before the treatment (Phase 1 – baseline).
Phase 5 – NiX treatment was run to confirm that the methane yield increase caused by NiX treatment during Phase 2 – NiX treatment could be repeated.

During Phase 2 – NiX treatment, the methane yield of the mesophilic digester was improved by 48% when the substrate was treated with NiX, from 65 ± 7 L-CH_4 / kg-VS (average of yield during baselines) to 96 ± 7 L-CH_4 / kg-VS (VS is the organic matter content of the solid fraction of raw cattle manure used as feed for the thermophilic digester).
The overall methane yield improvement of the 2-step biogas process was 16%, from 180 ± 13 L-CH_4 / kg-VS (average of yield during baselines) to 209 ± 15 L-CH_4 / kg-VS (Figure 8, Figure 9).

During Phase 5 – NiX treatment, the methane yield of the mesophilic digester was improved by 74% when the substrate was treated with NiX, from 65 ± 7 L-CH_4 / kg-VS (average of yield during baselines) to112 ± 7 L-CH_4 / kg-VS.
The overall methane yield improvement of the 2-step biogas process was 31%, from 180 ± 13 L-CH_4 / kg-VS (average of yield during baselines) to 236 ± 7 L-CH_4 / kg-VS (Figure 8, Figure 9).

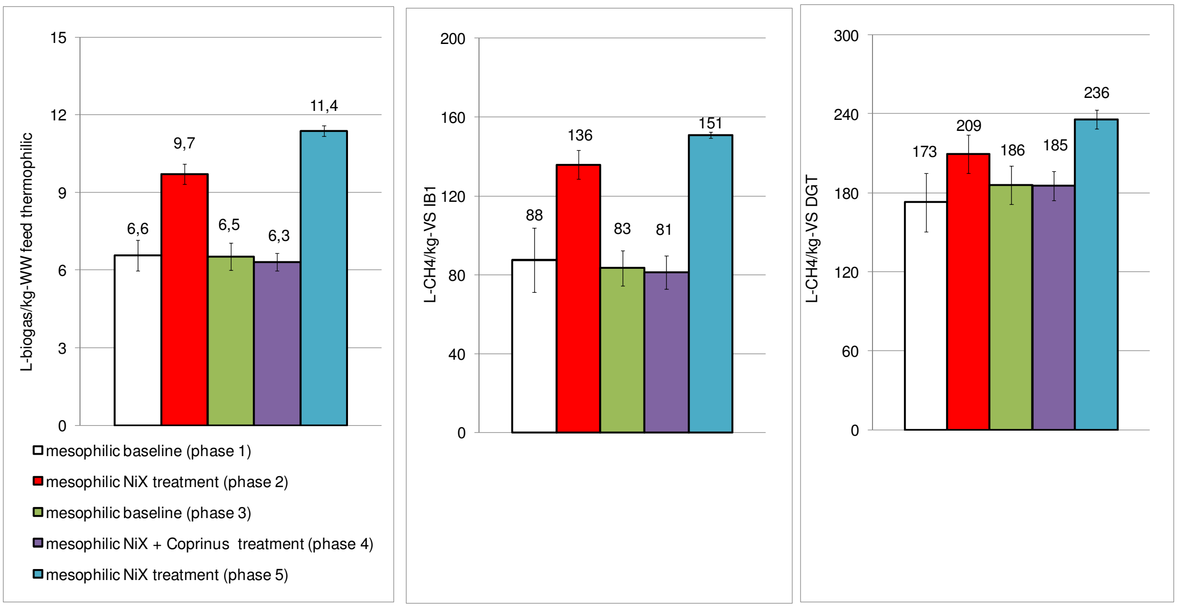

Figure 8. Effect of NiX treatment (*WW feed thermophilic* is the wet weight of the substrate of the thermophilic digester, *VS IB1* is the organic matter content of the material in IB1, *DGT* is solid fraction of cattle manure used to prepare the substrate of the thermophilic digester).

Phase 2 – NiX treatment and Phase 5 – NiX treatment resulted in different yield. The two phases allow to conclude that NiX treatment increases the methane yield.
Because Phase 2 – NiX treatment lasted for 4.0 HRT, it can be concluded that the digester had reached steady state. Therefore, the methane yield registered at the end of Phase 2 – NiX treatment is considered the one obtainable with NiX treatment.

Phase 5 – NiX treatment confirmed that NiX treatment was the reason for the yield improvements registered during Phase 2 – NiX treatment, but it does not allow to conclude that the yield obtained during Phase 5 – NiX treatment can be maintained with long-term operation at steady state.

The effect of NiX treatment can be observed already after two days after the beginning of the treatment (Figure 9). Therefore, NiX treatment can be used to boost the biogas production when digesting substrates with a composition similar to the one of fibres from digested cattle manure.

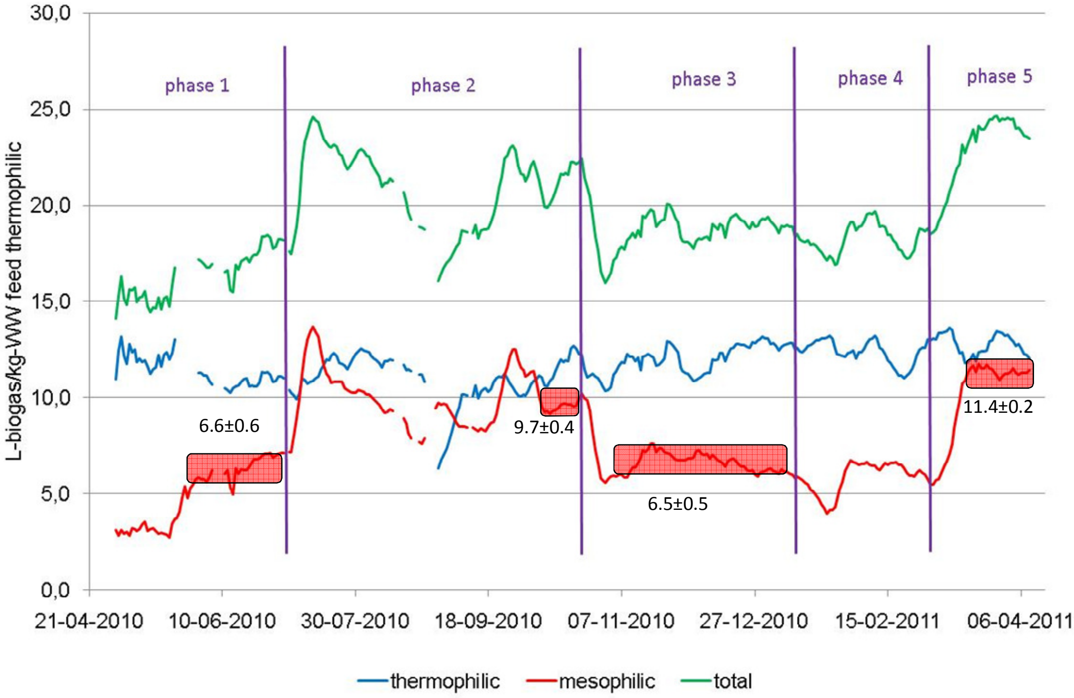

Figure 9. Biogas yield mesophilic digester phase 2 and 5 (NiX treatment) compared to baseline (phase 1 and 3) (*WW- feed thermophilic* is the wet weight of the substrate of the thermophilic digester).

During the first days of operation of Phase 2 – NiX treatment, the yield of the mesophilic digester showed a decreasing trend after a strong peak. This may be due to the dynamics of the populations of microorganisms in the digester.

When the highly digestible NiX-treated material started to be introduced in the mesophilic digester at the beginning of Phase 2 – NiX treatment, the concentration of the microorganisms in the digester increased. As a consequence of the increased microbial activity, part of the undigested substrate present in the digester since Phase 1 – baseline was converted into biogas. This resulted in an extra biogas production. As the days passed, the extra material was consumed and the digester reached a steady state.

This trend for the biogas production (peak followed by decreasing trend) was not observed during Phase 5 – NiX treatment, suggesting that the dynamics of the microorganisms populations were different from those of Phase 2 – NiX treatment. The two phases were started following two different phases (a baseline for Phase 2 – NiX treatment and a treatment phase for Phase 5 – NiX treatment), therefore it is reasonable to expect different dynamics of the populations.

The low biogas yield of the mesophilic digester during Phase 1 – baseline until 27 May 2010 was due to a gas leak.

The technical problems that occurred during Phase 2 – NiX treatment affected the biogas production (see Appendix E: technical problems during Phase 2). The biogas production was restored as soon as the technical problems were fixed. The conclusions of this report do not take into account the periods when the biogas production was fluctuating because of the technical problems.

3.1.2 Effect on process parameters

The methane percentage in the biogas (50%), the NH4-N (0.55 g-N / kg-WW) and tot VFA concentration in the mesophilic digester (< 100 mg / kg-WW) were not affected by NiX treatment, or the variations were too small to be detected.

Although NiX treatment was expected to remove NH4-N, no effect was detected. The NaOH dosage may be too low to result in significant NH4-N removal. However, because NiX treatment was applied only to the solid fraction of the effluent of the thermophilic digester (fibres, 20% of the effluent wet weight) and the NH4-N concentration in the effluent of the thermophilic digester was low (0.67 g-N / kg-WW) any effect may have been below the detection limit.

No effect on the pH of the mesophilic digester was observed. During the baselines Phase 1 – baseline and Phase 3 – baseline, the pH was 6.9 ± 0.1 and 7.0 ± 0.4, respectively. During Phase 2 – NiX treatment and Phase 5 – NiX treatment, the pH was 7.0 ± 0.2 and 7.3 ± 0.1, respectively.

No effect on the TS and VS content of the mesophilic digester was observed.

3.2 Effect of NiX + Coprinus combined treatment

3.2.1 Effect on yield

The effect of combined NiX treatment followed by biological treatment with Coprinus was tested with Phase 4 – combined treatment. The yield during Phase 4 – combined treatment was compared to the yield during Phase 1 – baseline and Phase 3 – baseline. Details about the treatment are given in Appendix C: combined NiX and biological treatment.
Combined treatment with NiX and Coprinus resulted in same methane yield as during the baseline (Figure 10).

Treatments with Coprinus were made twice per week and each lasted for 14 days. The first treatment with Coprinus started on 11 January 2011. During the 14 days of treatment, the barrel IB2 was filled with the effluent of the mesophilic digester itself (effluent recirculation). This lasted for 14 days, until 25 January 2011, when the first batch of fibres treated with Coprinus was available.
In the period from 11 January 2011 to 25 January 2011, the biogas yield from the mesophilic digester decreased as the result of the effluent recirculation. During this period, the biogas yield was lower than during the baselines (Phase 1 – baseline and Phase 3 – baseline).

After 25 January 2011, the biogas yield increased and reached the same level as during the baselines. This level was constant until 08 March 2011 and was considered the final yield for the combined NiX and Coprinus treatment.
In the period from 11 January 2011 to 10 February 2011, the liquid fraction was kept at room temperature during the 14-day treatment of the solid fraction. To avoid VS consumption by microorganisms present in the liquid fraction, from 10 February 2011 until the conclusion of Phase 4 – combined treatment, the liquid fraction was maintained frozen at -10 °C during the 14-day treatment of the solid fraction. However, this did not result in higher methane yield from the mesophilic digester.

The combined treatment (NiX treatment and Coprinus treatment in series) did not increase the yield, although NiX treatment alone resulted in increased yield. Probably, the easily degradable compounds released with NiX treatment were consumed by Coprinus during the 14-day Coprinus treatment with the overall result of same yield as during the baselines.

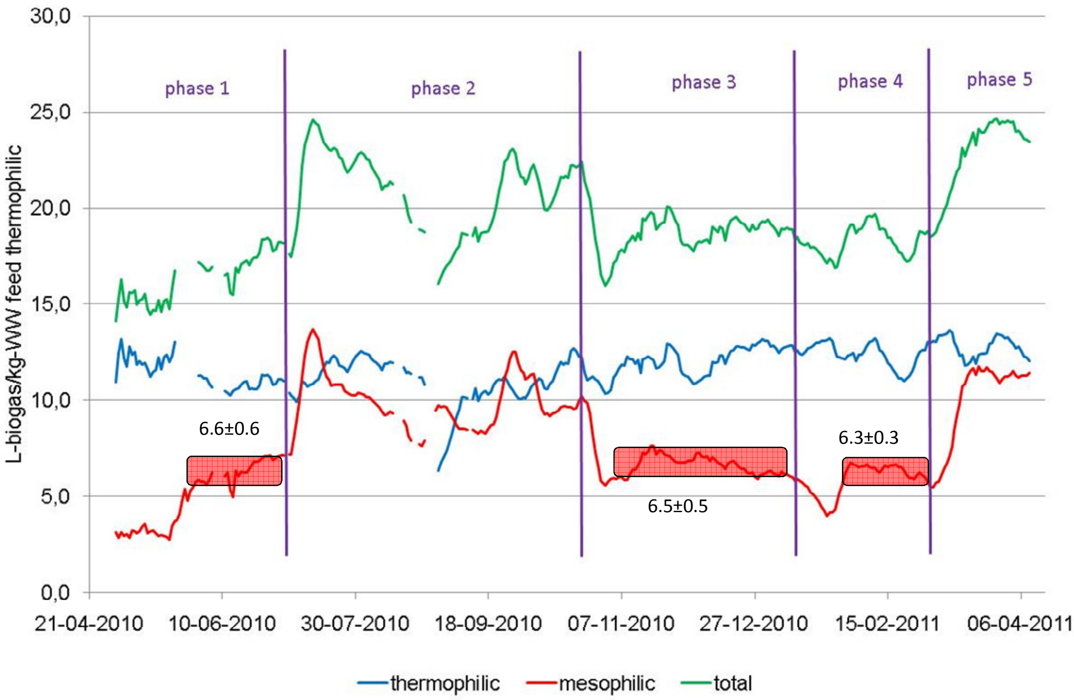

Figure 10. Biogas yield mesophilic digester phase 4 (combined NiX and biological treatment) compared to baseline (phase 1 and 3) (*WW feed thermophilic* is the wet weight of the substrate of the thermophilic reactor).

3.2.2 Effect on process parameters

The methane percentage in the biogas (50%), NH4-N (0.55 g-N / kg-WW) and tot VFA concentration in the mesophilic digester (< 100 mg / kg-WW) were not affected by NiX treatment or the variations were too small to be detected.

No significant effect on the pH of the mesophilic digester was noticed, as during Phase 4 – combined treatment the pH was 7.3 ± 0.1 (during the baselines Phase 1 – baseline and Phase 3 – baseline, the pH was 6.9 ± 0.1 and 7.0 ± 0.4, respectively).

3.3 Thermophilic digester and IB1

The methane yield of the thermophilic digester was constant at 11.9 ± 0.9 L-biogas / kg-WW (kg-WW is kg wet weight of substrate thermophilic digester). The TS and VS content of the material in IB1 (effluent from the thermophilic digester) were TS 4.5 ± 0.4% and VS 3.8 ± 0.4%.
The total VFA concentration in the thermophilic digester was < 200 mg / kg-WW.
The pH of the thermophilic digester was 7.1 ± 0.1.

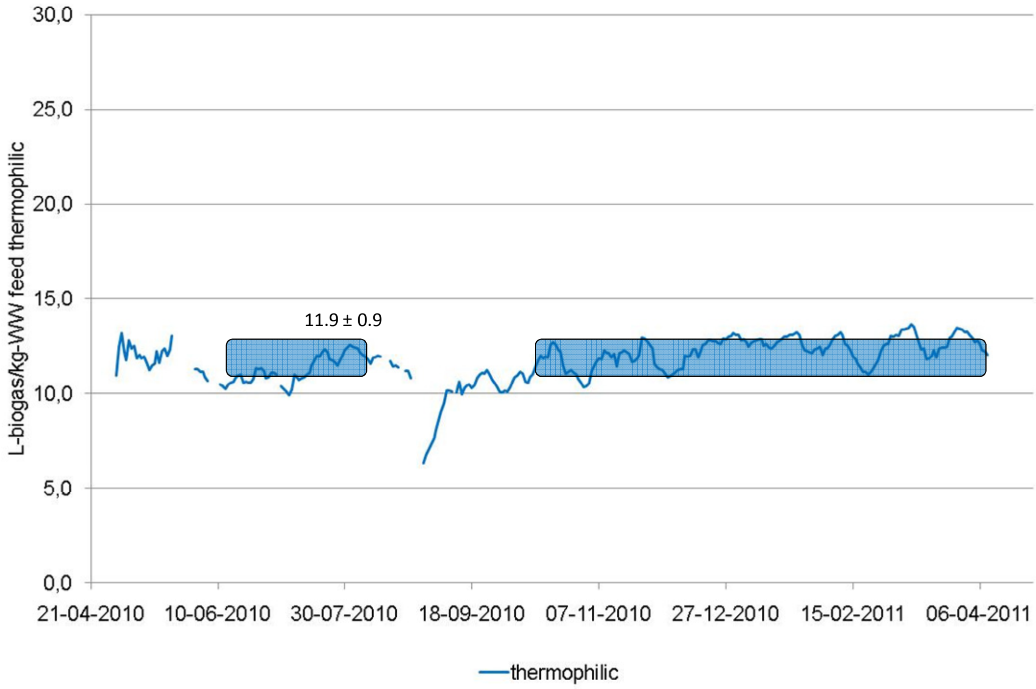

Figure 11. Biogas yield thermophilic digester.

3.4 Batch tests

Two series of batch tests were made, on 27 October 2010 and on 14 March 2011. The results are given in Table 1. Details about the preparation of the batches are in Appendix D: batch experiments.

Table 1. Results of the batch tests

Date batch start	Name batch	Methane yield ($L\text{-}CH_4$ / kg-VS)
27 October 2010	IB1	145 ± 26
	IB2 (during NiX treatment)	245 ± 27
	Fibres untreated	97 ± 10
	Liquid	128 ± 34
	Fibres treated NiX	176 ± 12
	Solid fraction cattle manure	199 ± 10
	Cellulose	386 ± 35
14 March 2011	Fibres untreated	113 ± 6
	Fibres treated NiX	195 ± 3
	Fibres treated NiX + Coprinus	149 ± 5
	Coprinus	377 ± 6
	Solid fraction cattle manure	222 ± 8
	Cellulose	376 ± 12

3.4.1 Batch tests: effect of NiX treatment

The effect of NiX treatment was measured in both batch tests (27 October 2010 and 14 March 2011). The batch tests from 27 October 2010 are part of CSTR experiments Phase 2 – NiX treatment, while the batch tests from 14 March 2011 are part of CSTR experiments Phase 5 – NiX treatment.
Details about the preparation of the batches are given in Appendix D: batch experiments.

The batch tests of 27 October 2010 showed that NiX treatment increased the methane yield of the solid fraction fibres by 82%, from 97 ± 10 to 176 ± 12 $L\text{-}CH_4$ / kg-VS. The batch tests of 14 March 2011 showed that NiX treatment increased the methane yield of the solid fraction fibres by 73%, from 113 ± 6 to 195 ± 3 $L\text{-}CH_4$ / kg-VS.

Lower methane yields for untreated fibers and for fibers treated with NiX were registered with the batch tests of 27 October 2010 than with the batch tests of 14 March 2011. This was probably due to the lower methane yield of solid fraction of cattle manure used during Phase 2 – NiX treatment compared to the quality of solid fraction of cattle manure used during Phase 5 – NiX treatment (Table 1). However, the methane yield increase due to NiX treatment was in the range 73-82%, slightly higher during Phase 2 – NiX treatment than during Phase 5 – NiX treatment. This suggests that the methane yield improvement caused by NiX treatment is stronger for substrates with low methane yield.

The material from IB2 (feed of the mesophilic digester) had methane yield 69% higher than the material from IB1. Thus, NiX treatment resulted in 69% methane yield improvement in batch tests.

The improvement registered with CSTR experiments (Figure 8) comparing Phase 1 – baseline with Phase 2 – NiX treatment was 55%, from 88 ± 16 to 136 ± 7 L-CH_4 / kg-VS IB1 (where g-VS IB1 is the organic matter content of the material in IB1).

For the mesophilic digester, the yields registered with CSTR tests were 58% of the yields registered with batch tests. This is acceptable, considering that this was achieved with a 1-step CSTR at the given HRT and low conversion kinetics due to mesophilic conditions and recalcitrant substrate. During the baselines, the overall 2-step CSTR biogas process achieved 173 and 186 L-CH_4 / kg-VS DGT, corresponding to 87% and 84% of the 199 and 222 L-CH_4 / kg-VS DGT registered with batch tests (*DGT* is the organic matter content of the solid fraction of cattle manure).

3.4.2 Batch tests: effect of combined NiX and Coprinus treatment

The effect of the combined treatment NiX followed by Coprinus was measured in batch tests on 14 March 2011.

The methane yield of the fungal strain Coprinus (including the medium used to suspend it) as it was received from Novozymes was 377 ± 6 L-CH_4 / kg-VS.
Fibers treated with combination of NiX and Coprinus resulted in methane yield 149 ± 5 L-CH_4 / kg-VS. This was higher than the methane yield of untreated fibers, but it was lower than the methane yield of fibers treated only with NiX treatment.

The lower methane yield of fibers treated with combined treatment compared to the methane yield of fibers treated only with NiX treatment may be due to the consumption of easily degradable organic matter or to the release of inhibitory compounds by Coprinus.
In CSTR experiments, the higher methane yield of fibers treated with combined treatment compared to untreated fibers could not be detected, as the baselines and Phase 4 – combined treatment resulted in the same methane yield.

4 Conclusions

NiX treatment improved the efficiency of the 2-step biogas process digesting cattle manure.

The methane yield of the mesophilic digester was increased by 48%, from 65 ± 7 L-CH_4 / kg-VS (average of yield during baselines) to 96 ± 7 L-CH_4 / kg-VS (VS is the organic matter content of the solid fraction of cattle manure).

The total yield, (thermophilic + mesophilic digester) was increased by 16%, from 180 ± 13 L-CH_4 / kg-VS (average of yield during baselines) to 209 ± 15 L-CH_4 / kg-VS. The methane potential of the solid fraction measured with batch tests was increased by 73-82%, with higher effect on untreated fibers with lower methane yield.

Combined treatment with NiX and Coprinus resulted in same methane yield as during the baseline (CSTR experiments), but the batch tests showed that the fibers treated with combined treatment NiX and Coprinus had higher methane yield compared to the untreated fibers.

The yield of the thermophilic digester (not affected by treatment) was constant during the project.

5 Recommendations

Because the composition of fibres from digested cattle manure is similar to the composition of many other lignocellulosic compounds, the effects registered during the tests described in this report may be obtained with biogas processes digesting lignocellulosic substrates other than cattle manure.

The low NaOH dosage and the possibility to use NiX treatment for long term operation or temporarily to boost the biogas production make NiX treatment attractive for commercial-scale applications. However, lab-scale and CSTR tests on the specific substrate will be needed.

6 Appendices

6.1 Appendix A: separation

A stainless steel press (Figure 12) was realized at Xergi to separate the material from IB1 into its liquid fraction and solid fraction (fibres).

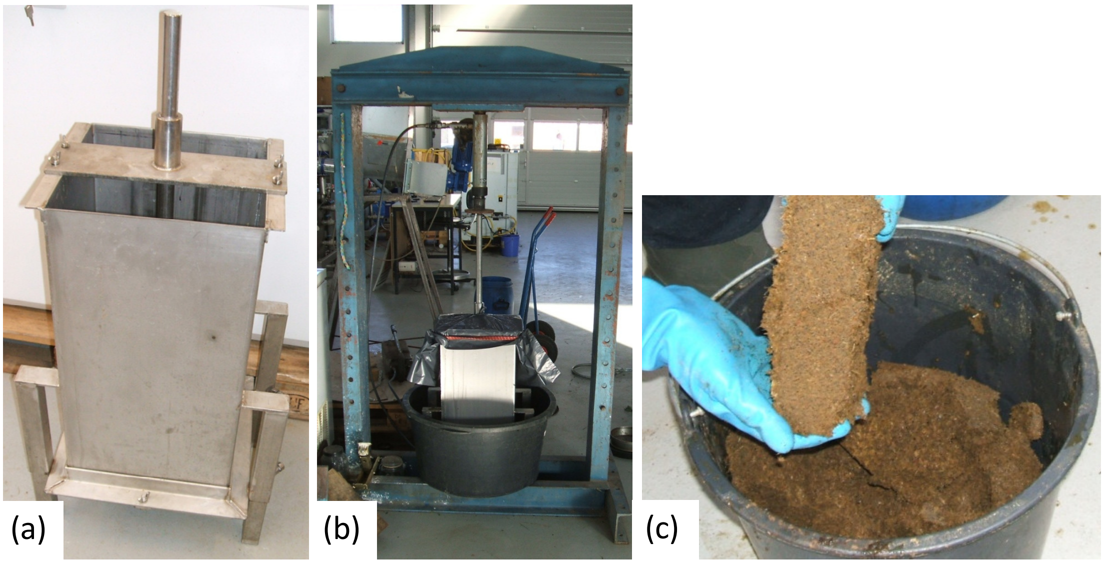

Figure 12. Press (a) + (b) for separation of material IB1 into liquid and solid fraction (fibres) (c)

The material from IB1 was poured into the stainless steel press and was pressed by a piston actuated by a hydraulic press. The liquid fraction passed through a 8-mm sieve placed at the bottom of the stainless steel press.
The solid fraction (fibres) was collected inside the stainless steel press, between the piston and the 8-mm sieve. The liquid fraction was further filtered with a 2-mm sieve and the retentate was counted as solid fraction (fibres). Data about the separation are given in Table 2 (averages and standard deviations based on the 79 measurements made during the experiments). No relevant mass losses were observed (mass balance within -4.2 % and +11.3 %, at an average of +2.4%).

More details about the procedure for separation can be found in
S:\Process Research\Projects\Research Projects\10030 Novozymes EUDP\guidelines separation and treatment

Table 2. Separation with press

	% of Kg-WW	TS	VS
IB1	100 %	4.5 ± 0.4 %	3.8 ± 0.4 %
Solid fraction (fibres)	21 %	16.5 ± 2.3 %	14.5 ± 2.2 %
Liquid fraction	79 %	1.4 ± 0.2 %	1.0 ± 0.3 %

6.2 Appendix B: NiX treatment

NiX treatment was applied with Xergi's thermo-chemical treatment unit (Figure 13). The substrate for NiX treatment was the solid fraction (fibres) separated from the material contained in IB1.

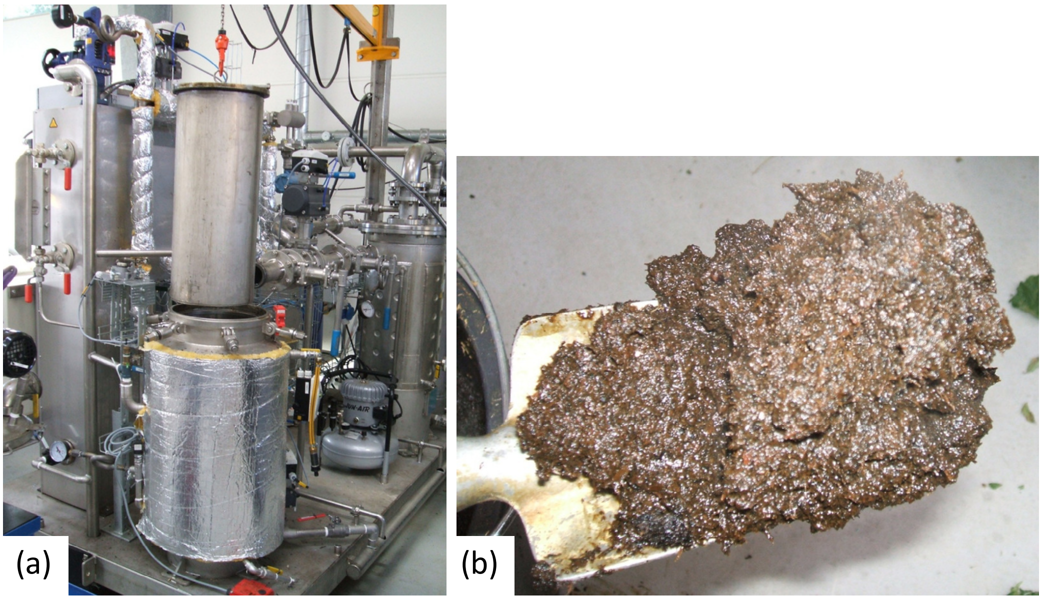

Figure 13. Xergi's thermo-chemical treatment unit for NiX treatment (a), fibres after treatment (b)

Steam at 8-12 bar was injected into a chamber containing a mixture of substrate (fibres) and catalyst (NaOH) in the proportions given below. When the set-point relative pressure (4 bar, corresponding to approximately 140 °C inside the chamber) was reached, it was maintained for 20 minutes. The sample was mixed during the treatment.

A pre-heating of the external walls of the chamber and injection of steam on these walls during NiX treatment was made to minimize steam condensation inside the chamber during the treatment. At the end of the treatment, the pressure was released in approximately 15 minutes avoiding mass losses. The treatment conditions are summarized below:

Sample: solid fraction (fibres) from IB1
Catalyst: 4.3 ± 0.6 % NaOH w/w TS (percentage by TS weight of untreated fibres)
Treatment: 4 bar (140 °C), 20 min, mixing

NiX treatment was applied twice per week. Table 3 gives the average amounts and characteristics of the substrates:

Table 3. NiX treatment, average of treatments.

	TS	VS
Untreated solid fraction (fibres)	16.5 ± 2.3 %	14.5 ± 2.2 %
Treated solid fraction (fibres)	13.8 ± 2.3 %	10.8 ± 2.4 %

The decrease of TS content and the increase of mass WW (data not shown) were due to steam condensation. The mass balance on TS basis did not reveal any relevant TS loss.
A detailed description of the procedure for NiX treatment and operation of the thermo-chemical treatment unit can be found in
S:\Process Research\Projects\Research Projects\10030 Novozymes EUDP\guidelines separation and treatment

6.3 Appendix C: combined NiX and biological treatment

Biological treatment with fungal strain Coprinus was applied in series after NiX treatment.
The solid fraction (fibres) from the content of IB1 was treated with NiX. Then, the NiX-treated material was treated with Coprinus.

Once per week, Coprinus was delivered by Novozymes in 5-litre portions. Coprinus was dosed in the NiX-treated fibres 3:1 w/v (3 kg of fibres and 1 L Coprinus).
The mixture was distributed into containers to obtain a layer 2-3 cm thick (Figure 14).
The containers were covered with a cloth and incubated at 30 °C for 14 days. Containers with water were kept in the incubator to maintain a high degree of humidity. Oxygen was supplied pumping atmospheric air with an aquarium pump.

Dosage: 3:1 w/v
Incubation: 14 days, 30 °C, with aeration

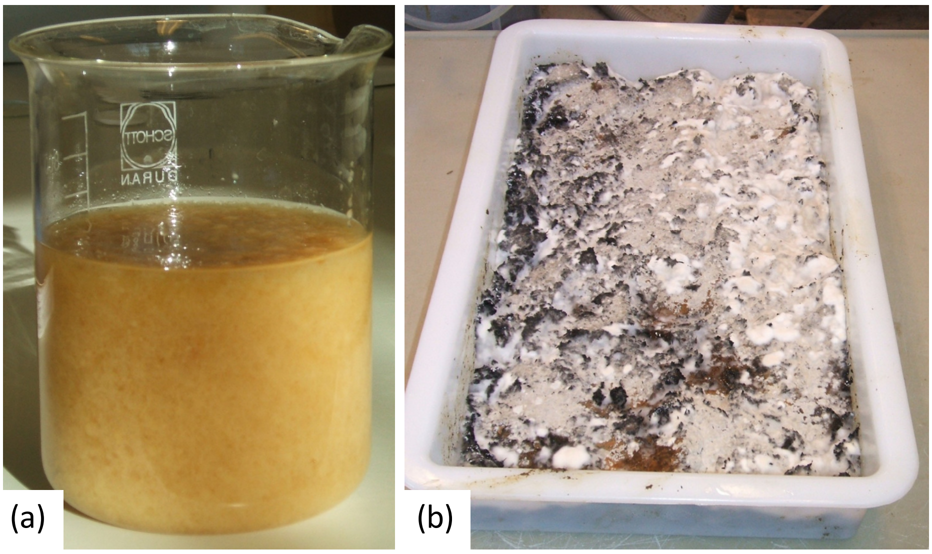

Figure 14 fungal strain Coprinus (a), solid fraction fibres after 14-day Coprinus treatment (b)

6.4 Appendix D: batch experiments

The methane potential was determined in batches, with infusion bottles of 543 mL total volume, at 52 °C. Anaerobic digested effluent from a thermophilic biogas plant (52 °C) was used as inoculum for the batch assays.

Inoculum (200 mL) and substrate were added into the bottle in the amounts given in Table 4. Control batches with pure cellulose as substrate (for examination of inoculum viability) and blank batches without substrate (to measure the methane production from the inoculum) were included.

The assays were made in triplicates.

Table 4. Batch experiments: batch preparation and methane yield

Date batch start	Name batch	Substrate WW (g)	Substrate TS% (%)	Substrate VS% (%)	Methane yield ($L\text{-}CH_4$ / kg-VS)	($L\text{-}CH_4$ / kg-WW)
27 October 2010	IB1	15.09	4.5	3.8	145 ± 26	5.6 ± 1.0
	IB2 (during NiX treatment)	15.01	4.2	3.2	245 ± 27	7.8 ± 0.9
	Fibres untreated	10.00	15.0	13.2	97 ± 10	12.8 ± 1.4
	Liquid	30.01	1.4	1.0	128 ± 34	1.2 ± 0.3
	Fibres treated NiX	8.07	12.7	9.3	176 ± 12	16.4 ± 1.1
	Solid fraction cattle manure	5.04	21.3	18.3	199 ± 10	36.5 ± 2.2
	Cellulose	0.50	100	100	386 ± 35	386 ± 35
14 March 2011	Fibres untreated	10.30	21.9	19.5	113 ± 6	22.1 ± 1.2
	Fibres treated NiX	5.33	15.5	13.2	195 ± 3	25.6 ± 0.4
	Fibres treated NiX + Coprinus	8.50	16.1	13.2	149 ± 5	19.6 ± 0.6
	Coprinus	33.77	3.2	3.0	377 ± 6	11.3 ± 0.2
	Solid fraction cattle manure	5.27	25.9	22.9	222 ± 8	50.9 ± 1.7
	Cellulose	0.50	100	100	376 ± 12	376 ± 12

6.5 Appendix E: technical problems during Phase 2

During Phase 2 – NiX treatment, technical problems occurred and affected the gas production of the thermophilic and of the mesophilic digester (Figure 15).

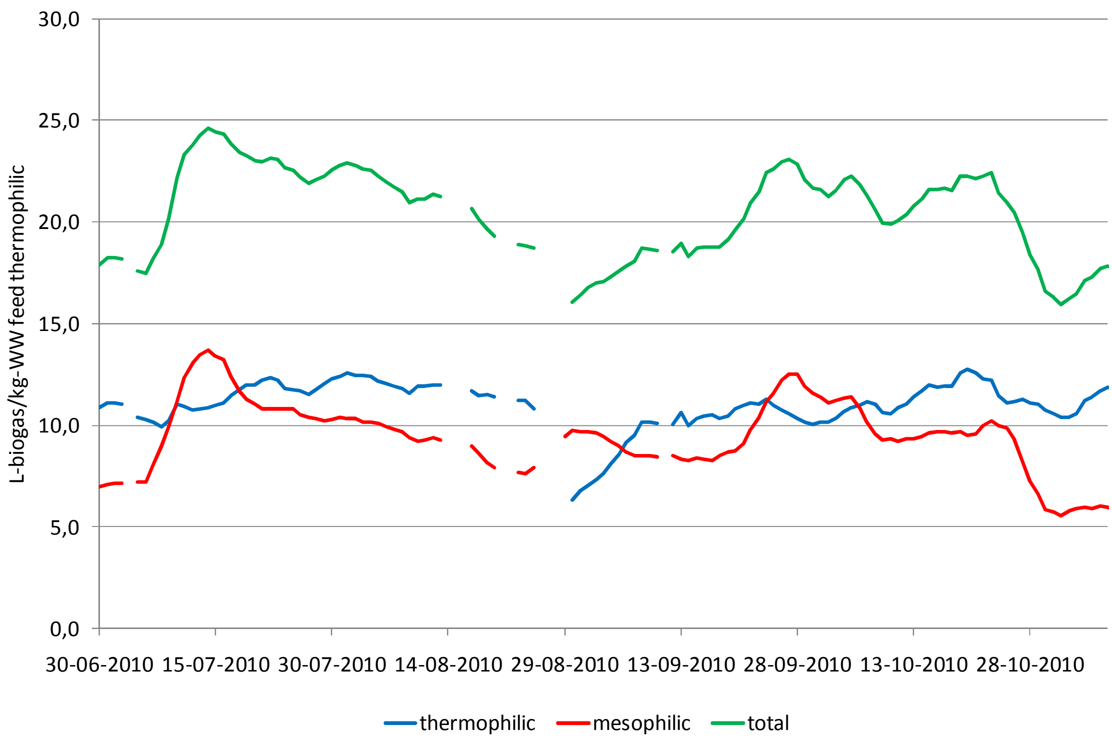

Figure 15. Technical problems during Phase 2 – NiX treatment

The fluctuations of the biogas production were due to:

<u>14-16 Aug</u>: computer stop (no feed, no effluent, no heating)
<u>17 Aug</u>: no electricity (no heating)
<u>22 Aug:</u> pump stop (no feed, no effluent)
<u>26-30 Aug</u>: computer stop (high temperature 65 °C in thermophilic digester)
<u>11 Sept.:</u> pump stop (no feed)

On 26 August, a computer problem caused a temperature increase to 65 °C in the thermophilic digester for 5 hours. For the next three days, the thermophilic digester was not fed to avoid overloading.

On 30 August, 25 kg of inoculum of the thermophilic digester (17% of the working volume 150 L) were replaced with effluent from the mesophilic digester. Normal feeding of the thermophilic digester started on 30 August after replacing the inoculum.