

**Upgrading of dairy cattle manure as monosubstrate for biogas production
Final report part 1 batch experiments**

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Summary

This part of the report summarizes the results of the exploration of enhanced biogas potential from dairy cow manure by microbial treatment with commercially available products from Novozymes Biologicals.

This part of the project was a screening in batches of biological treatments on fibers from anaerobically digested cattle manure.

The best results in the lab-scale trials were obtained with fungal strain *Coprinus* on autoclaved fibers and resulted in methane potential increases of 30-40 mL-CH₄/g-VS.

The success criterion for moving from lab-scale (part 1) to pilot-scale (part 2) was that 25% of the theoretical methane potential of fibers from anaerobically digested dairy cattle manure was converted to methane in batch tests, compared to batch testing of water-treated fibers. In lab-scale batch testing, this corresponded to an increase of 100 – 113 mL-CH₄/g-VS in methane yield compared with water control, assuming a theoretical methane yield of cow manure of 400 – 450 mL-CH₄/g-VS.

The results from batch tests described in this report (part 1 of the project) revealed a potential synergistic effect between the thermo-chemical treatment NiX and a microbial treatment, but did not meet the success criterion.

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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 1, the lab-scale batch tests and is a collaboration between Xergi, Novozymes (NZ) and Novozymes Biologicals (NZB).

Part 1 of the project deals with the development of a microbial treatment of the insoluble fraction (fibers) of anaerobically digested dairy cow manure with the purpose of improving the utilisation of the recalcitrant fibres for biogas production.

The success criterion for moving from lab-scale (part 1) to pilot-scale (part 2) was that 25% of the theoretical methane potential of mechanically separated fibers from anaerobically digested dairy cow manure was converted to methane in batch tests, compared to batch testing of water-treated fibers. In lab-scale batch testing, this corresponds to an increase of 100 – 113 mL-CH₄/g-VS in methane yield compared with water control, assuming a theoretical methane yield of cow manure of 400 – 450 mL-CH₄/g-VS.

Preliminary results from NZB have consistently shown an 80 – 100 mL-CH₄/g-VS increase when applying NZB commercial products to fibers from Five Star Dairy, Elk Mound, Wisconsin. The first attempt to verify these results at NZ and Xergi however, were unsuccessful. In these experiments, it was not possible to identify an increase in methane production between the microbial-treated and untreated fibers. Therefore a new investigation was made and it is described in this part of report.

2 Procedure

The substrate fibers were obtained from Microgy. The methane potentials were determined in batches.

The experiments were run as a triangle between Xergi, NZ and NZB to detect the effects of:

1. differences between the inocula;
2. differences between the fibers treatments;
3. differences between the methods applied for suspending microorganisms from the product bran formulation;
4. lack of stability and/or variations in the microbial product used at NZB, Xergi and NZ.

2.1 Treatments

Three concurrent treatments of fibers treated with identical NZB product lots were set up at Xergi, NZ and NZB.

The treated fiber samples were exchanged between Xergi, NZ, NZB. Anaerobic batch digestions were subsequently set up with different inocula from local sources to determine the methane potential of the treated samples. Each member of the project performed anaerobic digestion studies of their own samples along with samples treated by the other project group members.

Due to delivery problems beyond our control the NZB treated samples destined for NZ were delayed two weeks and have not been included in this summary.

In the following it is important to distinguish between the terms listed below.

Term	Explanation
Microbially treated samples	Fiber samples incubated 14 days at 30 °C in the presence of 1008 or New consortium A
Water control	Fiber samples incubated 14 days at 30 °C without added microorganisms.
Treated samples	Microbially treated samples and water control
Untreated samples	Fibers that have not been incubated and not treated with water or microorganisms
AD negative control	Inoculum used for the batch tests
Avicell cellulose Positive control	Cellulose added to the biogas inoculum to verify activity of inoculum

“New consortium A” is a microbial product based on the commercial product 1008. Consortium A is composed of seven bacterial strains.

Biological treatments were made at Xergi and NZ also on autoclaved fibers (121 °C, 20 minutes). These treatments were applied at 14% and 20% TS, with three different fungal strains (Coprinus, Kernia, Chrysosporium). To measure the methane yield of the fungi, batch tests digesting autoclaved fungi were made.

2.2 Preparation of batches

The methane potentials were determined in batches, with infusion bottles of 543 mL total volume, at 52 °C. Effluent from a thermophilic biogas plant (52 °C) was used as inoculum for the batch assays. Inoculum (200 mL) and substrate (approximately 0.4 g-VS) were added into the bottle. 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 bottles were flushed with nitrogen gas and sealed with rubber stoppers and aluminium screw lids.

3 Findings

3.1 Batch tests Xergi with substrate treated by Xergi and NZB

The tests made at Xergi did not show significant effects on the net methane production of the microbially treated fiber fraction compared to the water controls (Figure 1, Figure 2).

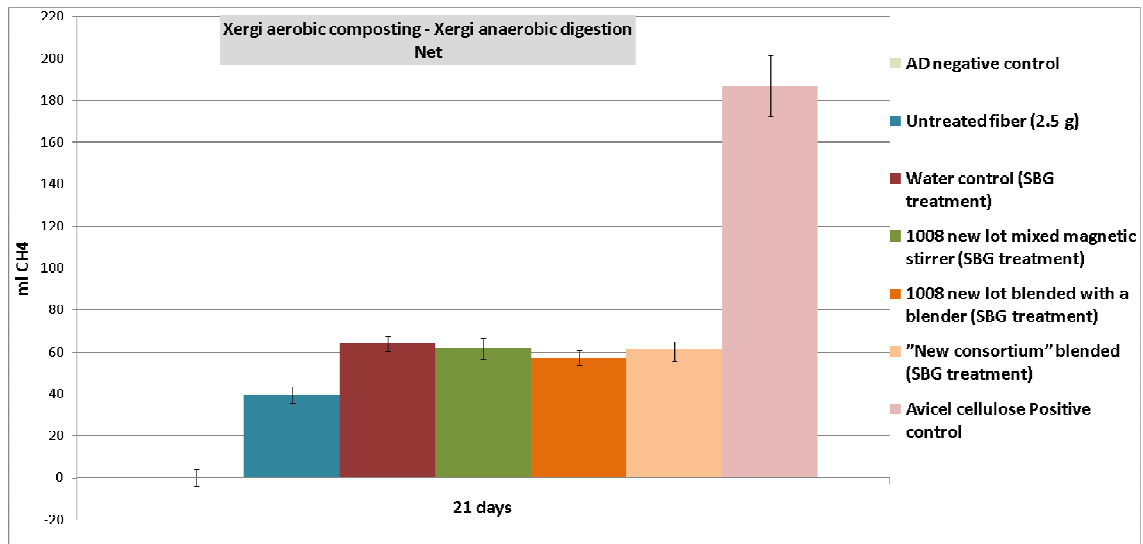


Figure 1 net CH₄ production. Batches Xergi, substrate Xergi

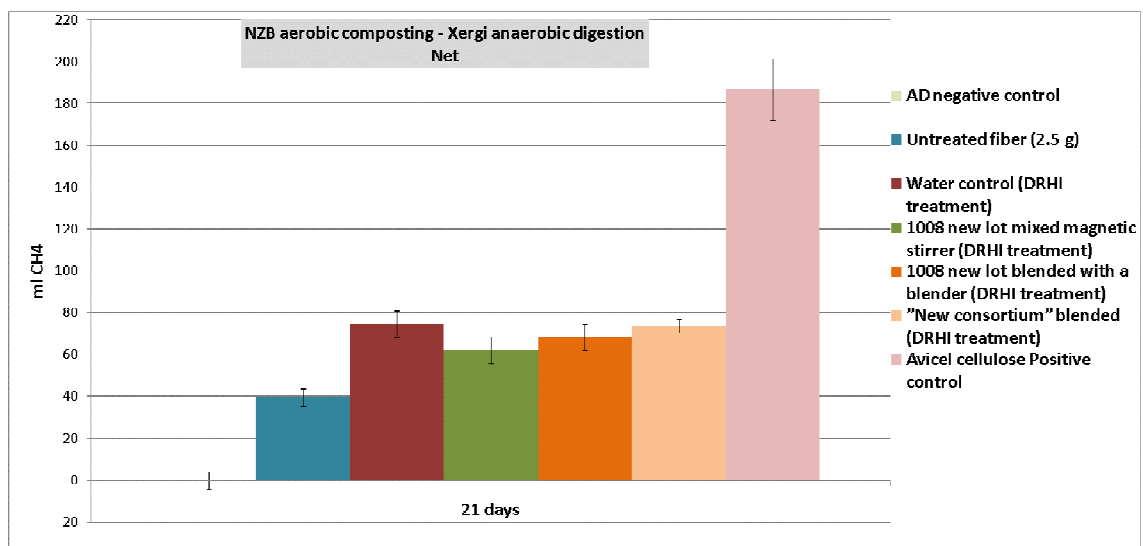


Figure 2 net CH₄ production. Batches Xergi, substrate NZB

At Xergi, treatment with fungal strain Coprinus on autoclaved fibers resulted in methane potential increases of 30-40 mL-CH₄/g-VS. However, the batch tests on autoclaved fibers did not result in

significantly different methane yield of the treated fibers compared to the untreated fibers (Figure 3).

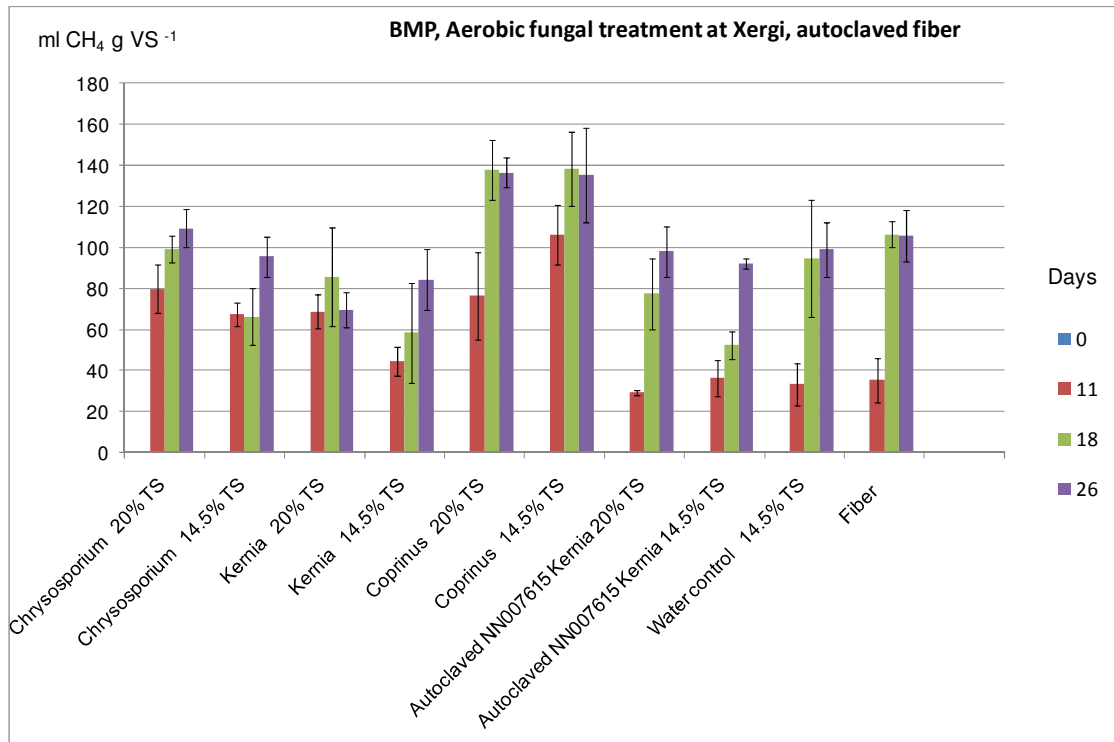


Figure 3 CH₄ yield from autoclaved fibers (batches Xergi)

3.2 Batch tests NZB with substrate treated by Xergi, NZ and NZB

The tests made at NZB resulted in increased methane production of the microbially treated fiber fraction as compared to the water control (Figure 4, Figure 5, Figure 6). The enhanced CH₄ production was 70-110 mL-CH₄/g-VS.

The methane levels achieved in the NZB digestions are in the same range as those obtained at Xergi. Hence, microbial treatment is necessary in the NZB digestions to reach the same levels as both the water controls and microbially-treated samples achieved in the Xergi digestions.

If enhanced CH₄ production is dependent on the anaerobic inoculum, the treatment is not generically applicable for improving biogas production in practice.

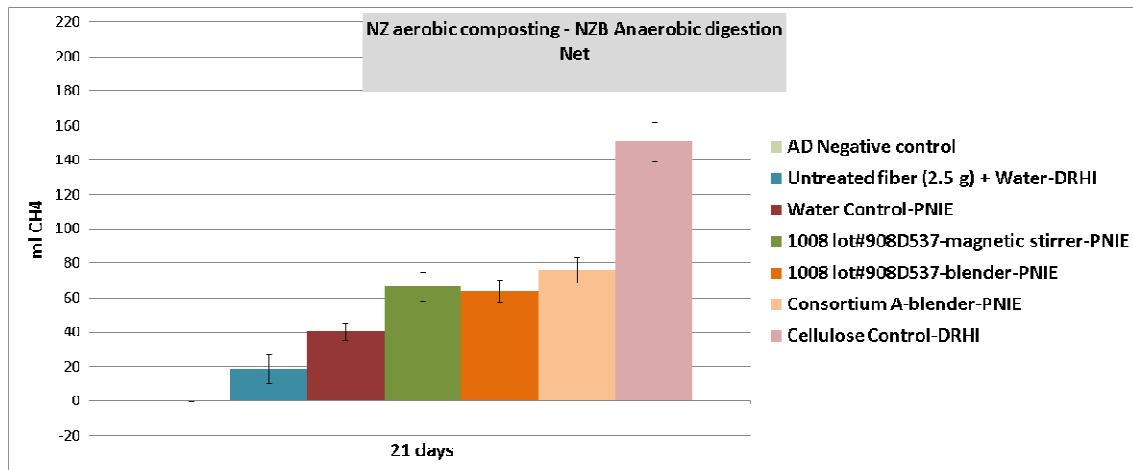


Figure 4 net CH₄ production. Batches NZB, substrate NZ

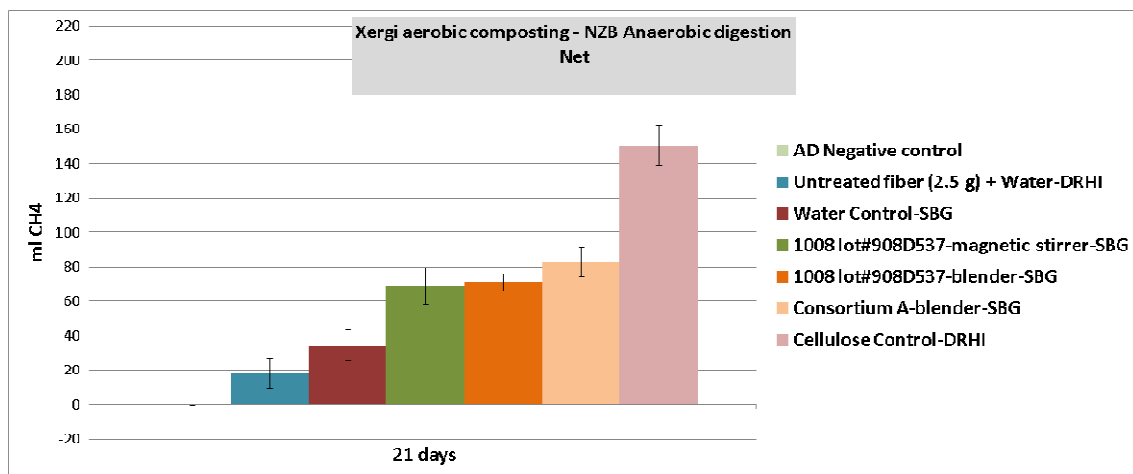


Figure 5 net CH₄ production. Batches NZB, substrate Xergi

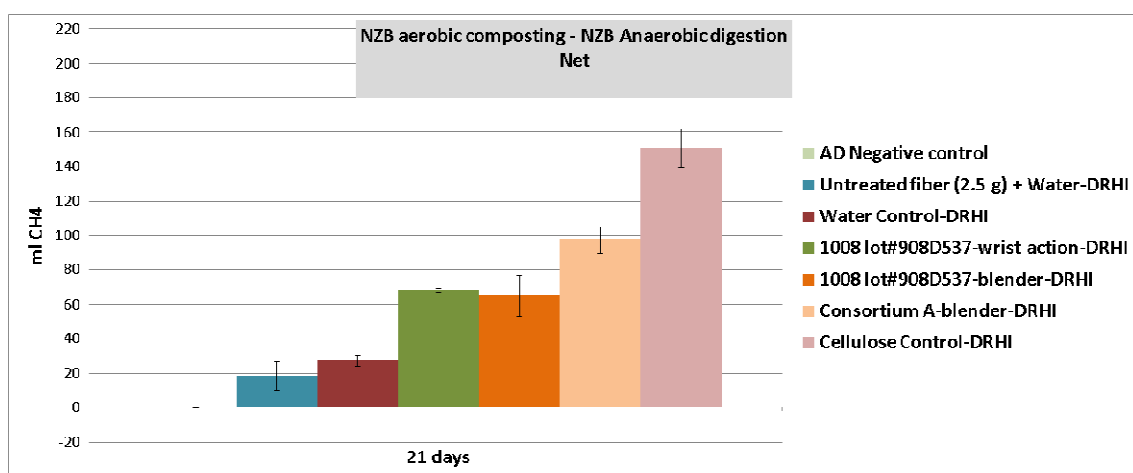


Figure 6 net CH₄ production. Batches NZB, substrate NZB

3.3 Batch tests NZ with substrate treated by NZ

The tests made at NZ do not allow any conclusion (Figure 7).

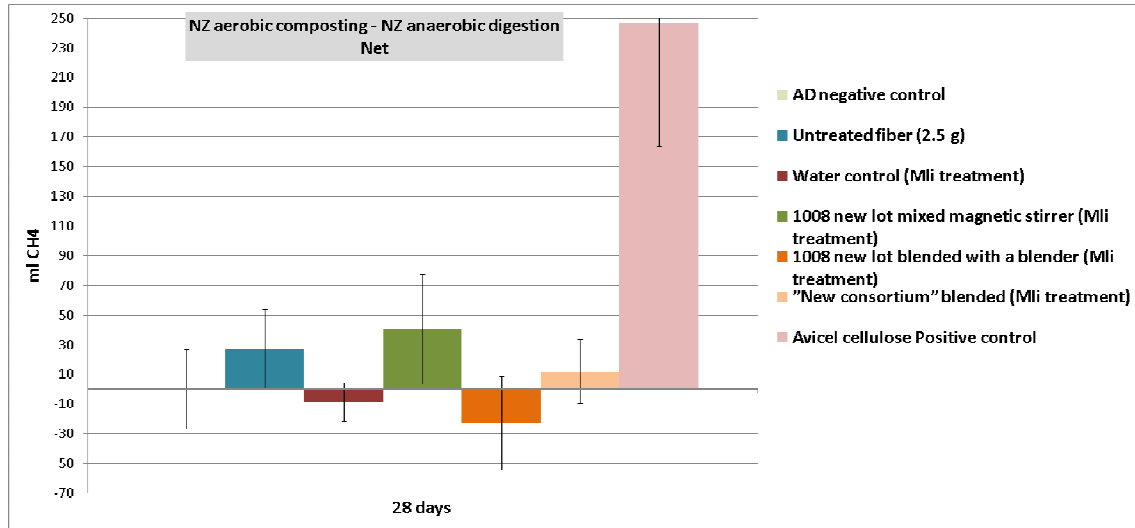


Figure 7 net CH₄ production. Batches NZ, substrate NZ

Also at NZ, treatment with fungal strain Coprinus on autoclaved fibers resulted in increased methane potential compared to the untreated fibers. However, the increase was not significant (Figure 8).

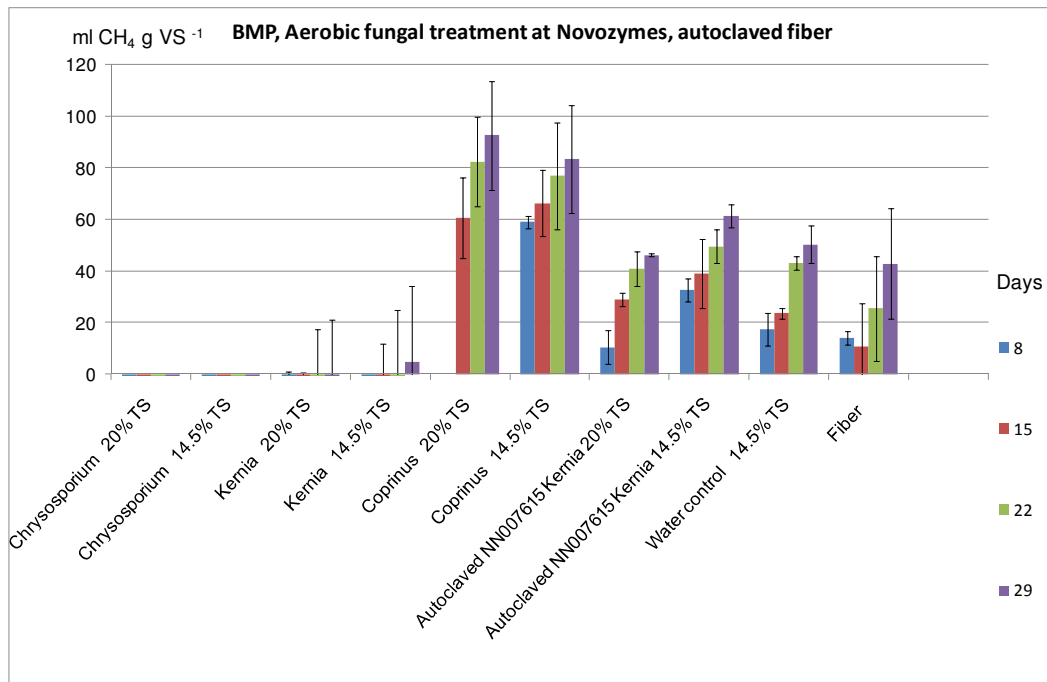


Figure 8 CH₄ yield from autoclaved fibers (batches NZ)

4 Conclusions

Although the results from part 1 did not meet the criterion for moving to pilot-scale testing (part 2), the presence of a synergistic effect between autoclaving a microbial treatment with *Coprinus* was apparent.

It has not been possible, at Xergi, to show that addition of microorganisms to a degassed dairy cow manure fiber fraction resulted in increased methane production as compared to the water control. In terms of the original success criterion, the methane levels achieved in the NZB digestions are 70-110 NmL CH₄/g VS as compared to the water control. In the Xergi digestions there is no increase in methane production as compared to the water control.

However, it is important to notice that when digestions are performed at NZB, the methane produced above the AD background in microbially treated fiber samples is similar to what is achieved at Xergi in all treated samples (water control as well as microbially treated). Hence, when digestions are performed at NZB, it seems that addition of microorganisms is necessary to achieve the same methane levels which are obtained in the Xergi digestions only without the addition of microorganisms.

4.1 Autoclaved fibers

Comparing the results from autoclaved fibers (Figure 3 and Figure 8), the effect of fungal strain *Coprinus* on the methane yield is apparent. This may be the synergistic effect between autoclaving and treatment with *Coprinus*. However, the methane yield increases were not significant. The methane yields of untreated fibers and of fibers treated with water were different at Xergi and at NZ.

4.2 Not autoclaved fibers

Figure 1 and Figure 2 (Xergi) indicate that there is no significant difference between the treated samples (compare water control, both 1008 treatments and the "New consortium A" treatments) neither for the Xergi treated samples nor for the NZB treated samples. The absolute methane production for the treated samples is in the area 60-70 mL above the AD background. The untreated fibers show a residual methane potential of around 40 mL. The AD background is 100 mL (data not shown).

Figure 4, Figure 5 and Figure 6 (NZB) indicate that there is a significant difference between the treated samples (compare Water control with both 1008 treatments and the "New consortium A" treatment) for both the Xergi treated samples, the NZ treated samples and the NZB treated samples. The absolute methane production for the microbially-treated samples is in the area 65-80 mL except for the NZB treated "New consortium A", which shows a net methane production of about 100 mL. The water control, in the NZB case, reaches a methane production of 30-40 mL. The un-

treated fibers show a residual methane potential of around 20 mL. The AD background is 30 mL (data not shown).

Figure 7 (NZ) is inconclusive.

The methane production level in the NZB and Xergi digestions of the microbially-treated fiber samples are similar (65-80 mL vs. 60-70 mL).

The methane production level in the NZB digestions of the water controls, and the methane production level in the Xergi digestions of the water controls are dissimilar (25-40 mL vs. 60-70 mL). The background productions of methane in the NZB digestions and the Xergi digestions are dissimilar (30-40 mL vs. 100 mL).

For comparison purposes, Figure 9 shows the methane productions presented above in terms of achieved methane per gram VS added to the batch bottles (yield).

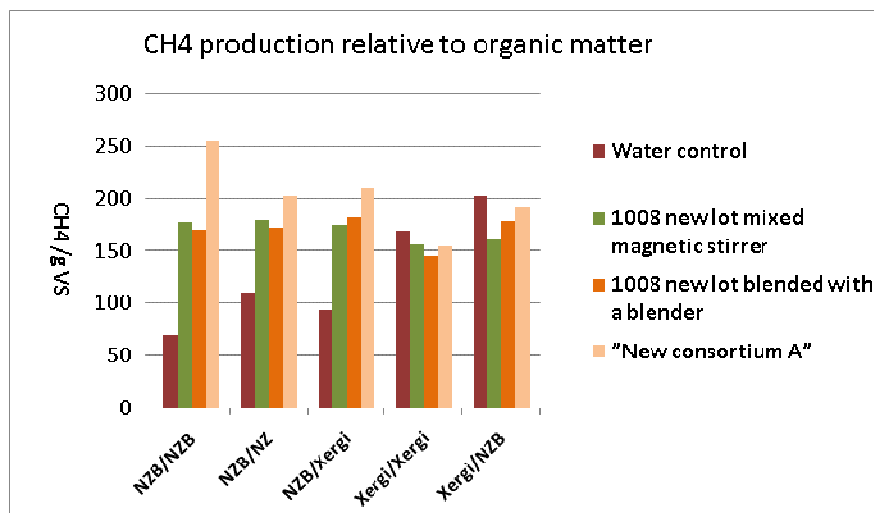


Figure 9 Summary of methane yields from Figure 1-Figure 7

All digestions which have not been supplied with microbially-treated fibers perform at a lower level at NZB than at Xergi. This may be seen by comparing the AD negative control, the untreated fiber, the water controls and the positive controls in the NZB digestions with the Xergi digestions.

The results obtained at NZB, when performing anaerobic digestions on NZ treated fiber, Xergi treated fiber, and NZB treated fiber are comparable. As are the results from the digestions performed at Xergi on NZB treated fiber and Xergi treated fiber.