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PROJECT FINAL REPORT

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Project acronym: BIOMAN

Project title: Economically efficient biogas production from manure fibres and straw

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Content

4.1 Final publishable summary report

4.1.1 An executive summary

The objective of the BIOMAN project was to promote more sustainable as well as economically feasible biogas production from low value substrates such as manure and 2nd generation biomasses. To achieve this, BIOMAN was based on the assumption that more biogas can be retrieved from the fibre fraction of manure by specifically treating the fibre fraction. A concept termed the "Re-Injection Loop" was formulated; consisting of a solid separation of the digested fibre fraction followed by a series of enzymatic and physical treatments on the digested fibre fraction alone following a reinjection into the anaerobic digester.

A survey was conducted to identify the potentially available biomasses and the corresponding quantities. Based on the survey, eight substrates were included in the further work with BIOMAN, and these substrates were characterized chemically and by biomethane potential measurements (BMP). The substrates encompassed both types of manure (cow, pig and chicken) as well as wheat straw and green biomasses. Primary focus was on manure (pig and cow) and lab-scale experiments were carried out on these two manure types. The optimal conditions for solid-liquid separation, enzymatic and ultrasound treatment were identified alone and in combination. This was evaluated based on biomethane potential measurements (BMP) and economical data to ensure a net positive effect of the treatments. Pilot scale studies were initiated in 30 L reactors and operated initially based on lab-scale results. The feed from the HTN biogas plant was used for the pilot scale studies. Initially, the feed consisted mainly of cow manure, but during the course of the project, the percentage of other easily degradable substrates such as organic waste was significantly increased. As a consequence, the possible increment of the Re-Injection Loop was significantly reduced due to the decrease of recalcitrant substrates.

Several attempts were initiated to overcome this, and pilot scale reactors were operated for a longer time than initially planned. The configurations of the pilot scale reactors investigated were as follows: no reinjection; reinjection from solid liquid separation; enzymatic treatment; and the combination of solid liquid separation and enzymatic treatment.

Initially, the Re-Injection Loop was supposed to be implemented at the HTN biogas plant in Spain. Possible installation of the Re-Injection Loop at HTN was initiated, but it was decided that more pilot scale experiments were needed to ensure the possible effect of the concept. However, the business foundation of biogas production in Spain changed significantly during the course of the project. HTN biogas plant was producing the maximum allowed electricity (fixed number) prior to the installation of the Re-Injection Loop. Therefore, producing more biogas would not provide more income to HTN and the heat from the process cannot be sold. Moreover, HTN was provided with subsidies for receiving various types of organic waste to be used for bio-gasification as mentioned above. Because of this, HTN had no interest in implementing the Re-Injection concept as the capacity of the plant, and thus the provided subsidies, would decrease. This is the main reason why the concept was not implemented at HTN during the project. However, the Re-Injection Loop consisting of solid liquid separation and enzymatic treatment could potentially be implemented at biogas plants operating only on manure and other recalcitrant substrates.

4.1.2 Summary description of project

There is still a need to identify low-cost methods that ensure additional biogas yield from low recalcitrant substrates as manure and 2^{nd} generation substrates. The European livestock production and agriculture need more advanced and economical methods for handling manure and slurry, and several EU projects dealing with these challenges have been granted. Both enhanced biogas yields and recycling of resources back to the agricultural system, as fertilizer and soil organic carbon enhancer present in the hardly degradable fibre fraction, are of major importance. Dedicated strategies of the European Community are focusing on the green energy market and one of the highest priorities is to reduce the dependency on fossil fuels. This is accomplished by securing safe and stable energy supplies to the industrial and private sector. Not only electricity and heat, but also fuel for the transport sector is interesting.

The Re-Injection Loop concept could potentially contribute to reducing emissions of the greenhouse gasses CH_4 and CO_2. The contribution comes from two sides: 1) Reduced CH_4 emission from manure and slurry storages, which is lowered due to the immediate need for controlled anaerobic digestion; 2) The produced biogas will replace fossil fuels and lead to net-reduced CO_2 emissions. Successful completion of the BIOMAN project would result in a technology package that could be installed on existing biogas plants operating on manure and energy crops. The Re-Injection Loop technology is specifically directed at the recalcitrant fibre fraction. Consequently, the possible biogas increment of the Re-Injection Loop is significantly lowered when the fibre content is low in the digesters.

The objectives of BIOMAN was to identify potential substrates available in the EU for the Re-Injection Loop concept, and a survey was conducted. Ten potential substrates consisting of different manures, wheat straw and green substrates were identified and subsequently chemically characterized. In order to conduct the Re-Injection Loop, separation of manure fibres was a prerequisite. Three common separation methods were investigated; centrifuge, screw press and bow screen. The separation equipment types differ in acquisition cost and maintenance costs ranging from the most expensive (centrifuge) to the cheapest technology (bow screens). Based on the following treatments in the Re-Injection Loop, bow screen separation was chosen as the cheapest and most suitable technology.

The subsequent technologies to be applied in the Re-Injection Loop following separation were enzymatic and ultrasound treatments. Experiments were carried out on lab-scale alone and in combination to identify the most optimal conditions. Experiments were conducted on different manure types identified earlier. For the enzymatic treatments, different enzymes were investigated and additional cost-effective blends from Enzyme Supplies Ltd. were formulated during the project. Optimal treatment conditions, as pH, temperature, hydrolysis time, enzyme concentrations were investigated on lab-scale and the most promising candidates were applied in pilot-scale experiments. The economical focus on enzyme price and dosage was of high priority and several tailor-made blends were made.

Ultrasound treatment was conducted on different manure fibres and straw. Optimal condition of specific energy yield and substrate concentrations were identified and significant increments of soluble COD and BMP were observed. However, the costs associated with the ultrasound operation exceeded the potential benefit in terms of BMP yield. Since the ultrasound treatment alone proved to have a negative impact on the economical balance of the Re-Injection Loop, the ultrasound technology was not investigated in pilot-scale.

For the pilot-scale experiments, three different unitary operations were proposed initially: solid-liquid separation (bow screen, screw press), ultrasound and enzyme treatment. Finally, three unitary operations were tested in WP3: solid-liquid separation by bow screen, solid-liquid separation by screw press and enzyme treatment. All treatments were applied separately on the fibre fraction or similar substrates and the operating conditions were optimized. The effect of re-injection after solid-liquid separation using bow screen (fibre fraction with 8% TS) resulted in 9% methane yield increment, whereas with screw pressing, simulating a decanter or screw press (fibre fraction 30-40%TS), increments between 21-33% methane yield were observed. These values are based on mean values and do not consider standard deviations. The effect of enzyme addition to the fibre fraction had visible effects with high fibre contents, pH adjustment and higher dosages. However, in the HTN feed there was only a medium fibre content (manure), representing around 60-70% of the mixture. When the enzyme was a multicomponent fungal cellulase with addition of xylanase, there was a significant increase on the methane yield (33-35%), when the fibre concentration in the substrate was high (feeding 5% straw). That was based on mean values not considering standard deviations.

The economic situation of the Spanish full-scale partner HTN changed during the course of the project. The regulations related to feed-in tariff in Spain were downgraded and decreased from 10 cents to 4 cents per kWh. In addition, the HTN biogas plant had a contract to sell a fixed amount of electricity, so there was no benefit in producing more from the Re-Injection Loop setup as the plant already operated at maximum electricity production. A high income at the HTN biogas plant originated from waste management payment, where processing of organic waste (different dairy products) is used in the digester. The results was that the HTN plant now operated on easily degradable organic waste streams (approx. 25%), which masked the effect on gas production from the fibre treatment. A consequence was that the Re-Injection Loop was not economically feasible to implement at the HTN biogas plant.

The feed-in tariff and subsidiaries changed throughout Europe, not only in Spain. For instance, at the beginning of the project the more than 6,000 German biogas plants operating on maize as the primary substrate were not potential purchasers of the Re-Injection Loop concept. However, the subsidiaries for these biogas plants changed, and therefore other potential low-value substrates could be introduced, increasing the demand for technologies directed at low-value substrates. Re-injection of the digestate fibre fraction was an interesting and fairly simple strategy to increase the methane yield by 9-33%, without altering the fresh feed rate. The enzymatic treatment showed an effect only if the fibre content was high, indicating that other biogas plants only operating on manure could still obtain an economical benefit from implementing the Re-Injection Loop concept. Based on the preliminary business plan, the most economical design of the Re-Injection Loop would consist of separation without subsequent treatment (enzymatic/ultrasound).

Description of the main S&T results/foregrounds

4.1.3 WP1: Description of chemical properties and variation of manure and energy crops

The objective of WP1 was to identify a range of agricultural biomass substrates, which have a significant potential as feedstock for biogas production in Europe and on which the implementation of the Re-Injection Loop concept would have a significant impact.

This was achieved in a survey of biomass available for biogas plants in the sector, with special focus on substrates applicable for the Re-Injection Loop. The results of this survey are available in [D1.1] and shortly summarized in the below section:

The most important biomass types, described in the survey, are shown in Figure 2 and Figure 1. Figure 2 shows the total amounts (European level) of the biomass, and Figure 1 suggests estimates of available amounts.

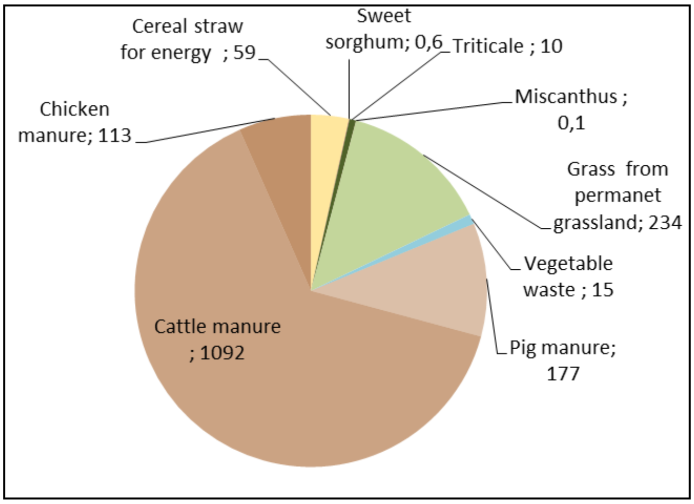

Figure 2: Total amounts (million ton) of biomass applicable for biogas plants with Re-Injection Loop at European level

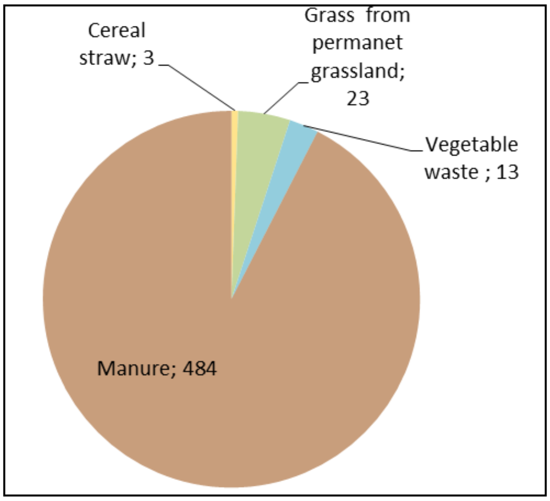

Figure 1: Estimation of available amounts (million ton) of substrates applicable for biogas plants with Re-Injection Loops at European level

The selection of substrates for further characterization was based on the following criteria:

- Applicability in the Re-Injection Loop. The main focus in the BIOMAN project was to improve the biogas yield for substrates after first fermentation and solid/liquid separation. Therefore, the selected substrates should preferably be applicable for biogas production and have a biochemical structure resulting in a sub-optimal biogas yield through a “normal” first fermentation.
- Availability. Of highest interest were substrates, which are available in large quantities in Europe, so that European biogas plants can benefit from the project results immediately. However, other substrates may also be interesting, for instance, if the availability is expected to increase in future, or if there is a potential market for the partner SMEs in other parts of the world, where “non-European substrates” may represent a large potential.
- Other special features. Other features can be taken into consideration as well, primarily in order to meet the needs and interests of the partner SMEs.

Based on the above criteria, eight substrates were selected. These substrates were analysed in order to identify the theoretical biogas yield of each biomass and to tailor the physical, chemical and enzymatic (pre) treatment within the Re-Injection Loop to the specific biomass characteristics. The results of this characterization are available in [D.1.2] and milestone [MS1], see Table 1.

Table 1. Overview of the chemical characterization of the selected substrates.

Substrate	**Tot-N**	**Tot Solids**	**Vol Solids**	**Cellulose**	**Hemicell.**	**Lignin**	**C /N**	**Biogas Potential**
	g/100 g biomass	g/100 g biomass	g/100 g TS	g/100 g TS	g/100 g TS	g/100 g TS		mL CH_4 /g VS
Triticale	1.25	46.25	87.71	21.38[1]	12.85[1]	14.36[1]	15.23	216
Sweet Sorghum	Nd	26,16	94,89	34,14	19,08	15,14	30.9	256
Festulolium (Rajsvingel)	Nd	85.71	85.48	22.36	21.51	21.02	Nd	240
Wheat straw	0.35	93.51[1]	95.84[1]	4.42[1]	27.22[1]	14.04[1]	109.25	228
Cow manure after AD – separated	10.06	27.28[1]	75.04[1]	16.25[1]	16.11[1]	27.80[1]	15.62[1]	104
Chicken deep litter after AD	0.85	35.34[1]	89.27[1]	18.89[1]	19.63[1]	27.52[1]	16.01	74
Wheat straw after AD	0.57	4.40	64.30	29.37	28.72	21.94	6.92	140
Mixed animal slurry after AD-separated (HTN)	0.69	30.82[1]	64.73[1]	15.46	12.98	25.27	15.5	66

Crystalline cellulose	Nd	96.64[1]	100.05[1]	96.87	99.94[1]	Nd	Nd	375

[1)]The figures are mean values from the analyses carried out by two/more RTDs. Nd: Not determined.

4.1.4 WP2: Lab-scale investigation on the Re-Injection Loop

The objectives of this work package was to evaluate the solid-liquid separation and the physical and enzymatic (pre)treatment separately and in combination. The potential effect of the enzymatic treatment was also evaluated on the microbial composition of the digesters.

Task 2.1. Evaluation of solid-liquid separation techniques on the digested fibre fraction output.

The three common separation techniques were tested, namely: centrifuge, screw press and bow screen. The separation equipment types differ in acquisition cost and maintenance costs ranging from the most expensive (centrifuge) to the cheapest technology (bow screens). The experiments were conducted at Morsoe biogas plant (centrifuged fibre from Morsoe Bio Energy, substrate 5). The results of the evaluation are available in [D 2.1], an overview of the data obtained from the separation studies are listed in Table 2. As seen from the table, the centrifuge is superior in terms of efficiency; however, the bow screen is less expensive and the dry matter content of the resulting fibre is more suitable for the subsequent treatment.

Table 2. Overview of data from separation experiments.

Separation method	Inlet (%w/w)	Solid fraction Fibre (%w/w)	Liquid fraction Reject (%w/w)	VS fibre %	% of total VS in fibre fraction	Potential methane increase %
Centrifuge (Day 1)	5.8	27	2.9	75.17	62	37
Screw press (Day 2)	5.1	31.3	3.7	84.0	39	20
Bow Screen (Day 1)	5.8	8.2	4.4	77.9	58	35

Task 2.2. Evaluation of enzyme addition on the digested fibre fraction output

In the initial screening of 11 different enzyme blends, with respect to increasing the biogas yield of wheat straw, the enzyme blend ES-CX900T supplied by Enzyme Supplies Ltd. showed the highest increase in the biochemical methane potential (BMP) of wheat straw. Based on this screening, the enzyme blend ES-CX900T and other enzyme blends with presumably high effect at low costs, supplied by Enzyme Supplies Ltd., were selected for further optimization and testing on digested manure fibres (DMF).

Improving enzyme treatment of digested manure fibres

Several screenings of enzymatic treatment were performed on DMF during the final period of the project to identify the most suitable enzyme blends and process parameters for implementing enzymatic treatment in the Re-Injection Loop concept, on the one hand to achieve a high increase of the biogas yield and on the other, to keep the implementation costs low. The screening and further optimization for enzymatic treatment of DMF included the following parameters:

- Testing the most potential enzyme blends and additional cost-effective blends from Enzyme Supplies on DMF from different biogas plants
- Reducing the enzyme dosage to a more economical level of 0.1 – 0.5% (g-enzymes/g-TS)
- Comparing the effect of the enzyme treatment at pH 5 with the effect of enzyme addition at the pH of the digested fibre fraction (pH 7-8)
- Reducing the retention time in the enzymatic treatment to 0.5-1 hour
- Testing the enzymatic treatment at different temperatures
- Adjusting the total solids (TS) concentration in the enzymatic treatment to a realistic value of 10%

In a first screening of the enzymatic treatment of DMF, the effect of the enzyme blends was tested on different samples of digested manure fibres separated from the digestate of three different biogas plants (Task 2.1), namely from HTN, Spain, from a biogas plant close to AINIA/Valencia, Spain, and from Morsø biogas plant, Denmark. The addition of enzymes was based on an economically feasible process operation of the Re-Injection Loop (see also 2.2.5), namely enzymatic treatment at pH 7 with low enzyme dosage (0.1 and 0.5% g-enzymes/g-TS), short retention time (0.5-1.0 h), and at a dry matter concentration of the DMF obtained (10% TS). Wheat straw was included as control for comparison with the results of the initial screening. The results showed that the methane yield of the untreated DMF (80-110 L-CH_4/kg-VS) was much lower than that of the raw wheat straw (280 L-CH_4/kg-VS), indicating that DMF has a much higher content of recalcitrant organic matter. The increase in the biogas yield after enzymatic treatment was for all three samples of DMF significantly higher than that of the wheat straw, indicating that a higher fraction of unused carbohydrates in the DMF could be made bioavailable through the enzymatic treatment. The low cost enzyme blend ES-3000L also showed a significant increase in methane yield of the DMF that would make the enzymatic treatment cost efficient in the Re-Injection Loop concept. In the following screenings, the effect of pH adjustment, temperature and treatment time of the enzymatic treatment on the biogas yield was investigated using ES-3000L as reference blend and modifications of this blend and other potential enzyme blends provided by Enzyme Supplies.

<u>Evaluation of enzymatic treatment at various pH</u>

Enzymatic hydrolysis was performed with pH adjustment at pH 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 and without pH adjustment, resulting in pH 8-8.5. Without pH adjustment during enzymatic hydrolysis, no distinct effect on the methane yield could be detected and mainly adjustment to pH 5 had an increasing effect. The course of the methane yield during the BMP test revealed that the lower the pH adjustment, the longer the lag phase in the BMP test, whereas the higher the pH, the lower the effect of the enzyme addition.

In another screening, with adjustment at pH 5 and pH 7 and without pH adjustment, the final methane yield was in most cases higher when pH was adjusted to pH 7 during the enzymatic hydrolysis step (Figure 3). For the low dosage, only the addition of the BG-X enzyme blend showed a positive effect and pH adjustment did not improve the effect. Addition of xylanase activity in the enzyme blend (ES-3000LX) did not improve the performance. A higher enzyme dosage was not necessarily translated into an increase of the final methane yield.

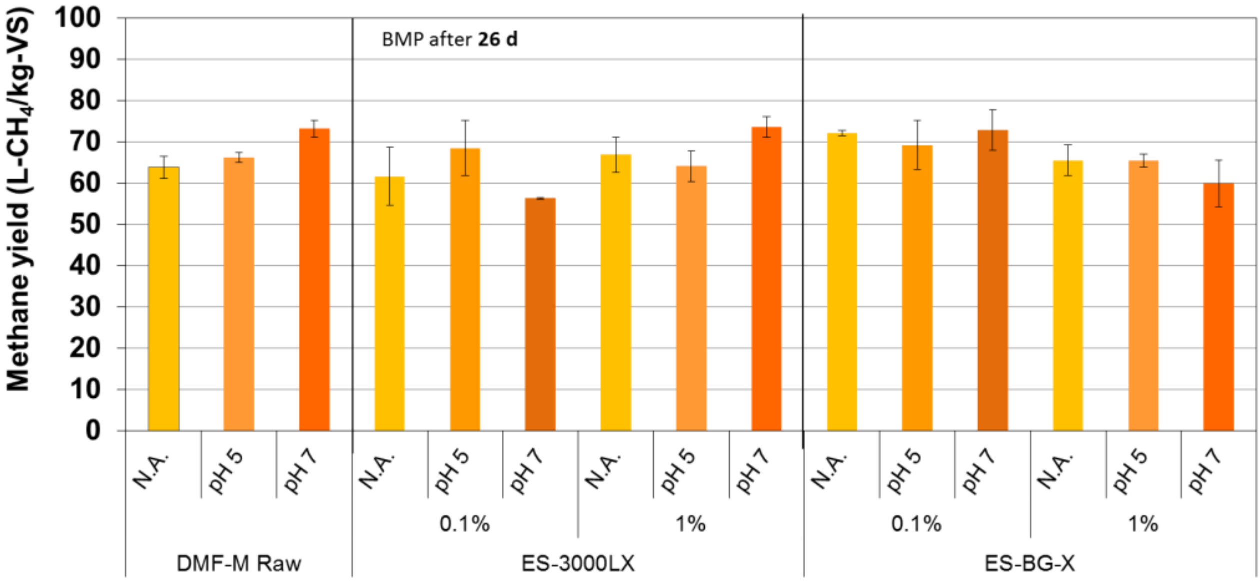

Figure 3. Methane yield after enzymatic treatment with ES-3000LX and ES-BG-X for 1 h at 42°C at different pH conditions (No pH adjustment, N.A. = pH 8.5) using two different enzyme dosages (0.1% and 1%)

Evaluation of enzymatic treatment at different temperature and treatment time

The difference in the optimum temperature of the enzymatic hydrolysis and the anaerobic digestion process is a challenge when using enzymes for the treatment of DMF in the Re-Injection Loop concept. The optimum temperature of the enzyme blends is typically around 50°C, whereas the temperature of the digestate from mesophilic biogas plants is around 40°C. The addition of enzymes with sufficient efficiency at 40°C would give a reduction in costs for additional heating and simplification of the process. Therefore, another screening with enzymatic treatment of DMF from Morso biogas plant (DMF-M) at 42°C and 50°C was made before anaerobic digestion was performed. Two different enzyme blends were used, ES-3000L and ES-ACx-8000P, an acid cellulase with a 2.5 times higher cellulase activity than ES-3000L. In addition, controls without enzyme addition were added at the respective temperatures to evaluate the effect of the thermal treatment before anaerobic digestion. All experiments were performed by using digestate from Hashøj biogas plant for dilution of DMF to a final TS value of 10%, resulting in pH 8 in the treatment.

Increasing the treatment temperature from room temperature (22°C) to 42°C or 50°C showed a thermal effect without addition of enzymes, especially for 72 h treatment, as the methane yield was increased from 66 L-CH_4/kg-VS to 82 and 84 L-CH_4/kg-VS, respectively (figure 2.2.2). For the treatment at 50°C (for 1 h), the methane yield increased to 78 L-CH_4/kg-VS. A significant further increase of the methane yield by addition of enzymes was only observed for 72 hours of enzymatic treatment before anaerobic digestion. For all experiments, no clear difference in the effect of applying 42°C or 50°C during the enzymatic treatment could be identified.

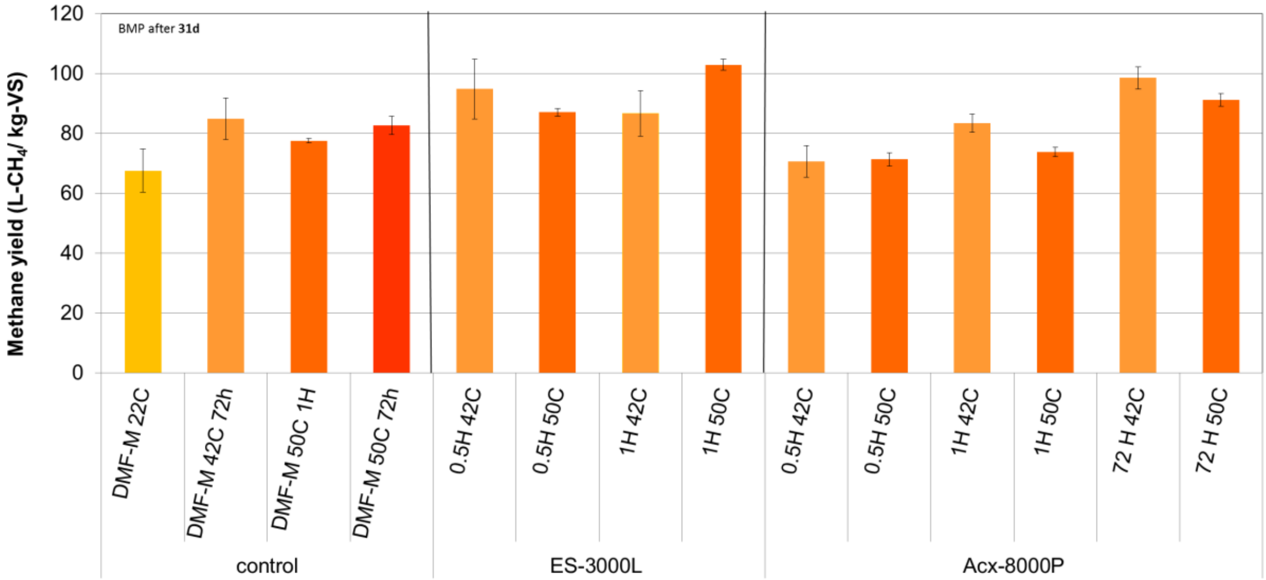

Figure 4. Methane yield after thermal treatment at 42°C and 50°C without enzyme addition (control) and using ES-3000L and ACx-8000P.

<u>Overall conclusions for implantation of enzymatic treatment in the Re-Injection Loop</u>

From the investigations performed, the following overall conclusions can be drawn for implementing enzymatic treatment in the Re-Injection Loop concept:

- The enzyme blend ES-3000L was identified to be the most suitable enzyme blend, as it showed an effect on increasing the methane yield of DMF by 10 m^3-CH_4/t-VS in average at rather low costs.
- Addition of ES-3000L also showed an increasing effect on the methane yield for only 0.5 h and 1.0 h of the enzymatic treatment, indicating that the enzyme blend may still have a hydrolytic effect under anaerobic conditions when added to the BMP test.
- pH adjustment during enzymatic treatment of DMF increases the efficiency of the enzymatic hydrolysis, but counteracts the performance of the subsequent anaerobic digestion process.

- No significant difference of the increasing effect of the enzyme blends was observed for enzymatic treatment at 40ºC and 50ºC and the elevated temperature showed a thermal effect without enzyme addition.

Task 2.3. Evaluation of the ultrasound treatments on the digested fibre fraction output

The objectives of the ultrasound treatment experiments carried out to determine the effect of the ultrasound treatment of lignocellulosic fibres on the organic matter solubilisation and the methane potential. The best operating conditions in terms of specific energy applied were also identified.

The ultrasonic treatment was applied to improve the anaerobic digestion (AD) of biomasses such as sewage sludge or lignin-rich materials. In fibrous materials, ultrasound acts by delignifying the fibres. The final purpose of the treatment is to increase the bioavailability of the organic matter. This is usually controlled by the increase of soluble chemical oxygen demands (CODs) and the CODs/COD ratio. The consequence on the AD is either an increase of the biomethane potential (BMP) or the process kinetics (lower hydraulic retention time necessary). The following tables summarize the results included in the mentioned deliverable. In all cases, the response variable to the pretreatment was the soluble COD and the biochemical methane potential (BMP).

Table 3. Summary of results of US treatment applied to wheat straw (WS1-WS8) (Lab-scale Vibracell VCX 750).

Sample concentration 9 gTS/L	WS1	WS2	WS3	WS4
Specific energy applied (kJ/kg$_{TS}$)	11111	22222	44444	66667
Initial CODs (mg/L)	442.0 ± 19.8	418.0 ± 22.6	537.0 ± 117.4	523.0 ± 89.1
Final CODs (mg/L)	572.0 ± 48.1	680.0 ± 73.5	850.0 ± 56.6	792.5 ± 53.0
CODs increase (%)	29.8 ± 8.4	63.4 ± 10.8	61.0 ± 6.7	52.9 ± 6.7
Sample concentration 18g$_{TS}$/L	**WS5**	**WS6**	**WS7**	**WS8**
Specific energy applied (kJ/kg$_{TS}$)	5556	11111	22222	33333
Initial CODs (mg/L)	764.0 ± 59.4	886.5 ± 101.1	867.5 ± 3.5	1020.0± 21.2
Final CODs (mg/L)	1020.0 ± 0.0	1272.5 ± 95.5	1622.5 ± 53.0	1730.0± 63.6
CODs increase (%)	33.9 ± 0.0	45.1 ± 7.5	87.0 ± 3.3	69.6 ± 3.7

The effect on the solubilisation of organic matter measured as chemical oxygen demand (COD) increased with the applied energy until a maximum at around 22 kJ/gTS in the experiments. The highest increment in the soluble COD (87%) was observed for the sample with 18g$_{TS}$/L after application of a specific energy of 22222 kJ/kgTS. The BMP of the treated sample was around 10% higher than the untreated sample.

Table 4. Summary of results of US treatment applied to digested fibres (Bench-scale Hielscher).

Code	Specific energy (kJ/kgTS)	Amplitude (µm)	Intensity (W/cm^2)	sCOD (mg/L)	Increment	BMP (LCH_4/kgVS)	Increment
MO-0	0	-	-	5200	-	134	-
MO-1	997 ± 2	50	18.0 ± 1.7	6360	22%	246	84%
MO-2	4990 ± 5	50	18.0 ± 0.0	8950	72%	211	58%
MO-3	9966 ± 13	50	18.7 ± 0.7	7975	53%	241	80%
HTN-0	0	-	-	8875	-	126	-
HTN-1	1002 ± 0	50	18.5 ± 0.4	1225	38%	145	15%
HTN-2	5008 ± 1	50	18.0 ± 0.1	17200	94%	229	82%
HTN-3	10039 ± 49	50	18.2 ± 0.8	18450	108%	248	96%
HTN-0'	0	-	-	6865	-	148	-
HTN-4	1037 ± 0.1	100	36.6 ± 0.4	13113	91%	110	-25%
HTN-5	4977 ± 3	100	38.9 ± 1.0	21750	217%	109	-27%
HTN-6	9948 ± 1	100	37.5 ± 0.6	29300	327%	146	-1%

MO: Morso digested fibres. Screw press, diluted to 104.7 gTS/L; HTN: HTN digested fibres. 65.8 gTS/L (obtained by bow screen).

All treatments yielded an increment of the CODs. In the HTN digested fibres, two different amplitudes were tested (50 and 100 microns). At the same levels of specific energy applied, a higher value of the amplitude resulted in a higher intensity of the treatment and higher COD increment. In relation with the BMP tests, all treatments, except for the ones performed at higher amplitude, yielded increments in the methane potential.

The treatment efficiency was evaluated in terms of the energy balance, comparing the energy consumed by the sonicator with the extra energy obtained, thanks to the methane increase due to the ultrasound treatment.

Table 5. Energy balances for the ultrasound treatment applied to the digested fibres with the bench-scale equipment and 50 microns amplitude.

Code	Energy consumption (kWh/kg$_{VS}$)	Extra energy produced (kWh/kg$_{VS}$)
HTN1	0.45	0.18
HTN2	2.72	0.97
HTN3	5.69	1.15
MO1	0.52	1.06
MO2	4.04	0.73
MO3	9.57	1.01

As it appears in the table above, the extra energy produced was in all cases lower than the energy required to apply the treatment on the digested fibres. These calculations were based on treatment with the professional bench-scale equipment from Hielscher.

Task 2.4. Evaluation of combined experiments with enzyme additions and physical treatment on the digested fibre fraction

The combined experiments were evaluated by extensive chemical analyses. The results of the evaluation are available in [D 2.1], an overview of the experiments is presented in the below figure.

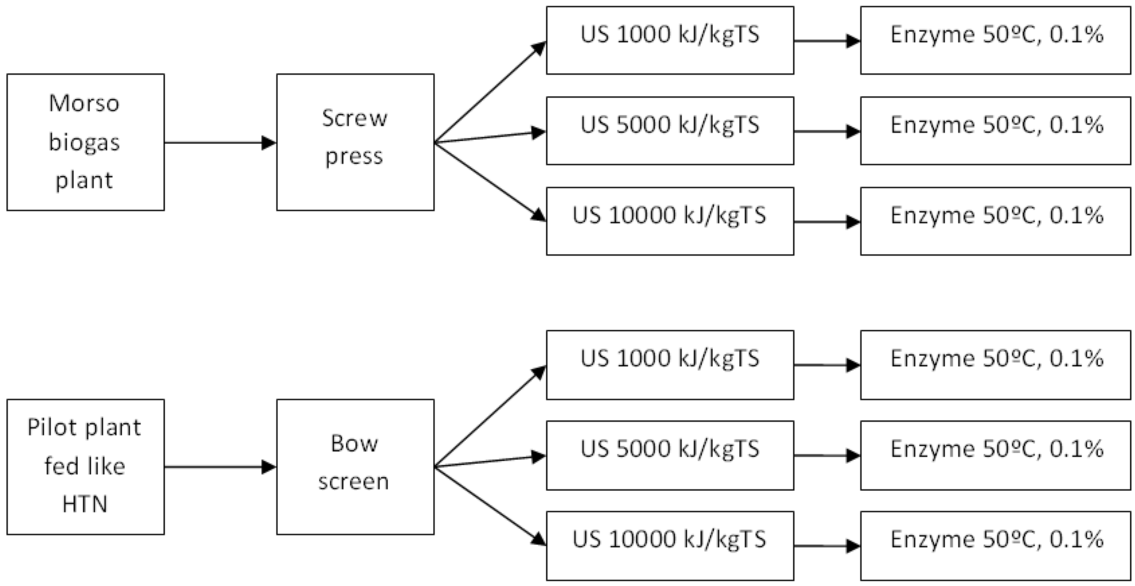

Figure 1. Treatment combinations

The following tables show the results in terms of organic matter solubilization of the treatment combinations analyzed.

Table 6. Overview of ultrasound and enzymatic treatment on Morso and HTN digested fibres.

	Morso		**HTN**	
	CODs, mg/L	***Increment****	***CODs, mg/L***	***Increment****
Crude fibre fraction	5200	-	8875	-
US 1000 kJ/kg_{TS}	6360	22%	1225	38%
US 1000 kJ/kg_{TS} + enzyme	5910	14%	13425	51%
US 5000 kJ/kg_{TS}	8950	72%	17200	94%

US 5000 kJ/kg$_{TS}$ + enzyme	11200	115%	17300	95%
US 10000 kJ/kg$_{TS}$	7975	53%	18450	108%
US 10000 kJ/kg$_{TS}$ + enzyme	10250	97%	20525	131%

*compared to the untreated fibre. Ultrasound treatment (US) with HIELSCHER Bench-scale equipment (UIP2000hd, sonotrode BS2d22L2B), max. temp. 40ºC, A= 50 µm, specific energy of 1000, 5000 and 10000 kJ/kg$_{TS}$. The enzyme treatment enzyme from ENZYME SUPPLIES (cellulase ACx 3000L – liquid natural pH found in the fibres, at 50ºC during 30 min, dosage 0.1 g enzyme per 100 g of TS).

The highest yield in terms of CODs increment was observed at 10000 kJ/kg$_{TS}$ for the HTN fibres (131% CODs increment with the whole treatment sequence), and at 5000 kJ/kg$_{TS}$ for the Morso fibres (115% CODs increment). The effect of the enzyme treatment was more pronounced in the Morso fibres. Oppositely, the ultrasound seemed to be more effective on the HTN fibres. This could be attributed to the different composition in the feed of the plants.

As main conclusion, in order to get similar CODs increments, the HTN fibres needed more energy and the Morso fibres required higher amounts of enzyme – although the fibre content was the same, Morso fibres were more concentrated.

Task 2.5. Study of community structure change, adaptation, kinetics and inhibition of the anaerobic digesting microbial consortia. Task 2.6. Preliminary continuous lab-scale anaerobic digestion (AD) reactor operation

Six 2L lab-scale reactors were operated in continuous mode to investigate a potential effect on the microbial community, when enzymes were added. These reactors were denoted R-reactors, which were harvested after 1 HRT (approx. 20 days) and used as inoculum for the Q-reactors operated on digested fibres from HTN. The methanogenic microbial population was characterized by a qPCR assay directed at the 16S rRNA genes of the methanotrophs. The methanogens present in anaerobic mesophile systems normally consist of two guilds, namely the acetoclastic and hydrogenotrohic methanogens, differentiating from each other by their ability to produce methane from different substrates, either from acetate and methyl compounds, or only H_2, CO_2 and formate.

Most pronounced changes occurred in the lab-scale reactors, where a significant population change within the methanogenic population was observed following the enzymatic pretreatment. This was observed in the R-reactors operated on wheat straw, where two different potential enzyme candidates were investigated (ES-3000L and ES-CX 900T). The reactors were dominated by the hydrogenotrophic methanogens and an increase of the acetoclastic methanogen *Methanosaetaceae* was observed in the enzymatically treated reactor.

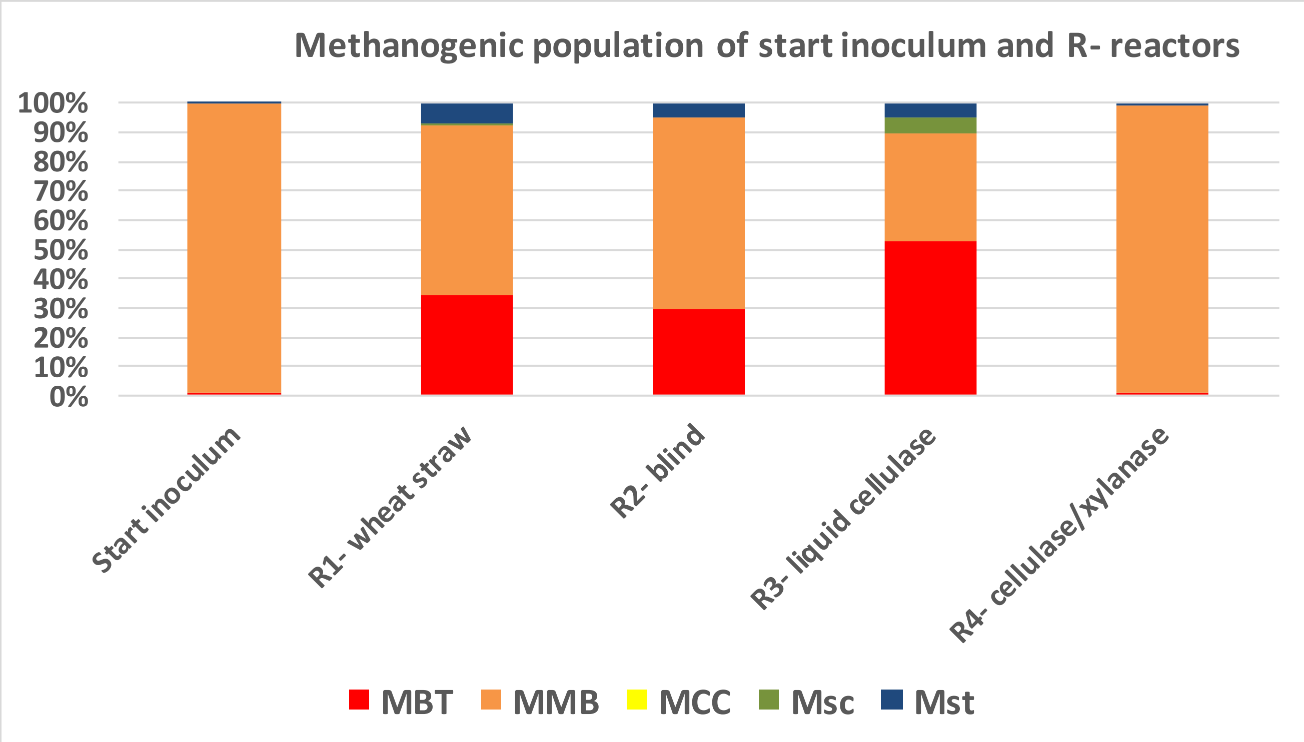

Figure 5: Overview of the initial methanogen population before and after operation of R-reactors depicted. Yellow and red colours correspond to hydrogenotrophic methanogens. Blue and green colours correspond to acetoclastic methanogens.

For the Q-reactors, more pronounced population changes were observed. The population changed from being dominated by a single order of hydrogenotrophs *Methanomicrobiales*, to consist of 30% *Methanobacteriales*. Most pronounced changes were observed, when enzyme addition was conducted and/or removed again. A population increase (or decrease) of the acetoclatic *Methanosaetaceae* was observed from 0-46%, depending on the enzymatic treatment. The reason for this pronounced change is not currently known, but is probably related to the end products produced by the cellulytic bacteria present.

The overall conclusion of this experiment illustrates that the microbial community composition responds to changes in operation as illustrated with the enzymatic treatment. However, it is not possible to conclude whether these changes will always occur in full scale.

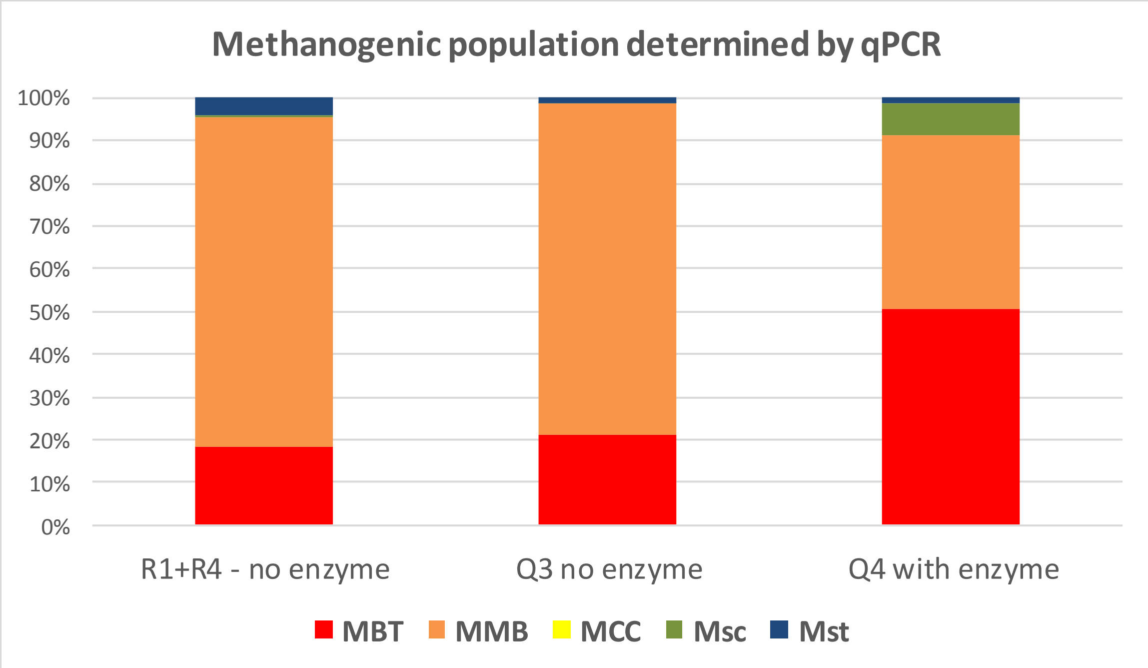

Figure 6: Overview of the methanogenic population determined by qPCR for the q-reactors operated on degassed wheat straw. Yellow and red colours correspond to hydrogenotrophic methanogens. Blue and green colours correspond to acetoclastic methanogens.

The microbial community of the pilot-scale reactors were also investigated using qPCR and here no effect on the community level could be observed, which was most likely related to the reinjection of the microorganisms making it more difficult to observe minor population changes. This was not pursued any further.

4.1.5 WP3: Pilot plants validation of the Re-Injection Loop

Pilot plant operation and validation

Initially three different unitary operations were proposed: solid-liquid separation (bow screen, screw press), ultrasound and enzyme treatment. Finally, three unitary operations were tested in WP3: solid-liquid separation by bow screen, solid-liquid separation by screw press and enzyme treatment. Ultrasound treatment was not included in the pilot-scale experiments, as the outcome of WP2 suggested a negative energy balance. All treatments were applied separately on the fibre fraction or similar substrates and the operating conditions were optimized. The operating conditions of these treatments as well as all the results of WP3 are included in detail in the deliverable D3.1. Below the main results and conclusions of WP3 are included.

Experimental design and operation of the experimental set-up

The anaerobic digestion experiments were carried out in pilot anaerobic digesters with a working volume of 30 L. Eight experimental digesters were in operation. The digesters were mixed continuously by an electric mixer of 25 W. Temperature control was provided by hot water recirculation. The digesters were manually fed once a day. The digester content was analysed weekly to determine the concentration of total and volatile solids (TS and VS), single volatile fatty acids (SVFA), pH, ammonia nitrogen ($N\text{-}NH_4^+$), total alkalinity and alkalinity ratio. Biogas flow and composition (CH_4, CO_2, O_2, H_2, H_2S) was continuously measured by means of Ritter Milligascounters® MGC-10 and Awite® Serie 6 gas analyser, respectively.

Figure 7. Pilot plant

The solid-liquid separation and subsequent pretreatment were performed differently depending on the treatment. The next unitary operations were tested in WP3:

1. **Solid-liquid separation by bow screen:** The bow screen technology was initially selected for this purpose according to the results of WP2, since it provided the correct concentration of total solids for the subsequent enzyme treatment. In order to simulate the process in pilot scale, a specific set-up was prepared.

2. **Solid-liquid separation by screw press or centrifugation:** The separation was made with a press, achieving total solids (TS) concentrations of 30-40%.

3. **Enzyme treatment:** The enzymatic treatment was applied in a Thermomix device where the substrate and the enzyme were mixed slowly in the specified proportions and in the conditions of temperature and time fixed in WP2 by AAU. After some additional tests in pilot scale, the enzyme treatment conditions were adjusted.

The biomass flow is depicted in the next figure. The blank digester was fed with the fresh material (HTN feed or HTN feed supplemented with straw). The feed of the test digesters also included the re-injected fibre, with or without enzymatic treatment.

First of all, the operation of the blank digester included the discharge of digestate and, secondly, the feeding. In the case of the operation of the test digester of the Re-Injection Loop, the operation included discharge of digestate, solid-liquid separation, liquid discarded, and feeding (fresh+digested fibres). Thirdly, the operation included the test digester of the Re-Injection Loop with enzyme treatment, the discharge of digestate, solid-liquid separation, liquid discarded, enzyme treatment of digested fibres (possible dilution with digester content) and feeding (fresh+treated digested fibres).

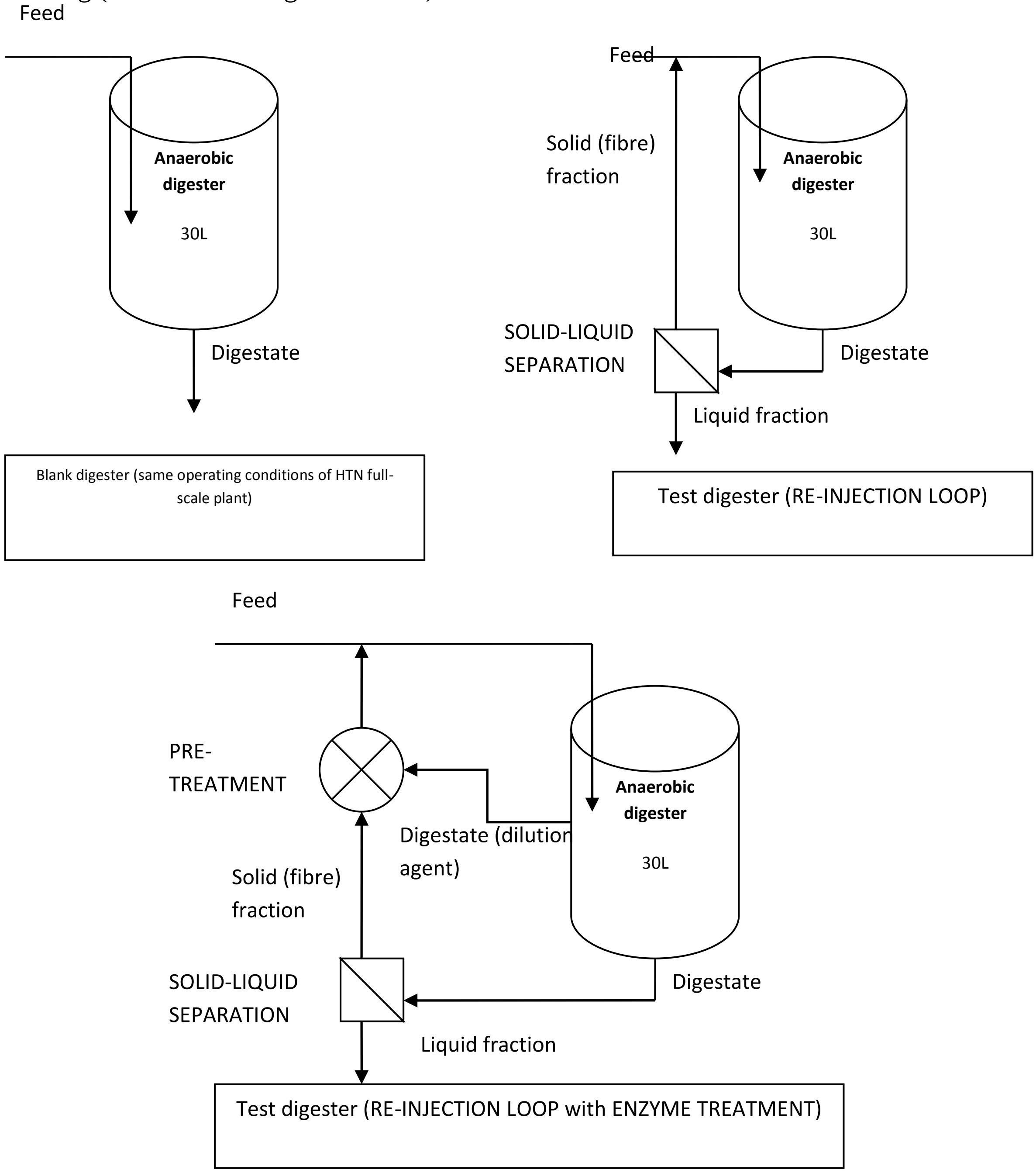

Figure 8. Scheme of the operation of the pilot digesters.

Sampling

The fresh substrate was sampled from the biogas plant of HTN (after the pasteurization step), where full-scale validation was planned to be implemented in WP4. The substrate was composed of the following categories of waste: manure (cattle manure, chicken manure), flotation fats, sludge, wastewater, various food waste, municipal waste, biodiesel by-product waste. Manure contributed to more than 60% of the mixture. Several samples were taken from the HTN biogas plant (deliverable D3.1 includes details of their composition). The HRT of the HTN biogas plant was 32 days. In addition to HTN feed, the experiments carried out from October 2014 until January 2015 included straw. The straw was collected from a farm that uses it for bedding for the animals. The straw was chopped in 1 cm long fibres.

Experimental plan and data acquisition

A summary of the experimental work carried out was included in the next table, including main details of the experiments of WP3.

Table 7. Experimental plan (January to September 2014)

Experiment	**Substrate**	**Conditions**	**Period**
Start-up	HTN feed, HRT 32 d	Sampling, filling, OLR increase to steady state	January-February 2014
HTN-BS	HTN feed, HRT 32 d	Blank Re-injection of fibres after bow screen Re-injection + enzyme* 3000 L at 50°C Re-injection + enzyme* 3000 L at 42°C	March-June 2014
HTN-P	HTN feed, HRT 32 d	Blank Re-injection of fibres after press Re-injection + enzyme* 3000 L at 50°C Re-injection + enzyme* 3000 L at 42°C	July-August 2014
HTN-P-HRT	HTN feed, HRT 17 d	Blank Re-injection of fibre after press + enzyme* 3000 L at 50°C	August-September 2014

*Enzyme treatment conditions: 3000 L, natural pH, 0.5 h, 0.1%, 42 or 50°C

Table 8 Experimental plan (September to December 2014)

Experiment	**Substrate**	**Conditions**
HTN1	HTN feed, HRT 32 d	Blank
HTN2	HTN feed, HRT 32 d	Re-injection of fibres after press (30-40%TS)
HTN3	HTN feed, HRT 32 d	Re-injection + AC-X**
HTN4	HTN feed, HRT 32 d	Re-injection + BG-X**
HTNF1	HTN feed + 5% straw, HRT 32 d	Blank
HTNF2	HTN feed + 5% straw, HRT 32 d	Re-injection of fibres after press (30-40%TS)

HTNF3	HTN feed + 5% straw, HRT 32 d	Re-injection + AC-X**
HTNF4	HTN feed + 5% straw, HRT 32 d	Re-injection + BG-X**

**Enzyme treatment conditions: pH 5.5 (adjustment with HCl), 1%, 1 h, 50°C

Table 9. Experimental plan (December 2014 to January 2015)

Experiment	**Substrate**	**Conditions**
HTN1	HTN feed, HRT 32 d	Blank
HTN2	HTN feed, HRT 32 d	Re-injection of fibres after press (30-40%TS)
HTNF1	HTN feed + 2.5% straw, HRT 32 d	Blank
HTNF4	HTN feed + 2.5% straw, HRT 32 d	Re-injection + BG-X2***

***Enzyme treatment conditions: pH 5.5 (adjustment with HCl), 0.1%, 1 h, 50°C

Data analysis and conclusion

In all digesters, the control process parameters (Alkalinity ratio, VFA, etc.) were stable during operation. The CH_4 and H_2 compositions of the biogas were kept stable. CH_4 was between 60-70% of the biogas and H_2 was below 1000 ppm (data not showed in this summary of results, but included in the deliverable D3.1). Next figures include a summary of main results related to the methane yield (average and standard deviation, SD) of pilot plant experiments from January 2014 - January 2015, both months included.

Effect of re-injection after solid-liquid separation with bow-screen:

With bow-screen (fibre fraction with 8%TS), the re-injection digester showed a slight methane yield increment (9%), statistically significant mean values (t-test, 99% confidence), however, not considering overlapping standard deviation. In case of the re-injection with enzyme pretreatment, no improvement was observed.

A possible loss of volatile compounds due to high temperature of enzyme treatment could be the reason for these results. Pretreatment was applied on the solid fibre fraction and potential volatile compounds as VFA; alcohols and other volatile compounds could be evaporated. Therefore, it seems that a possible loss of volatile compounds means a possible loss of methane yield during the enzymatic treatment at 50°C, or even, at 42°C. The next figure includes the results for the bow-screen and bow-screen with enzyme treatment.

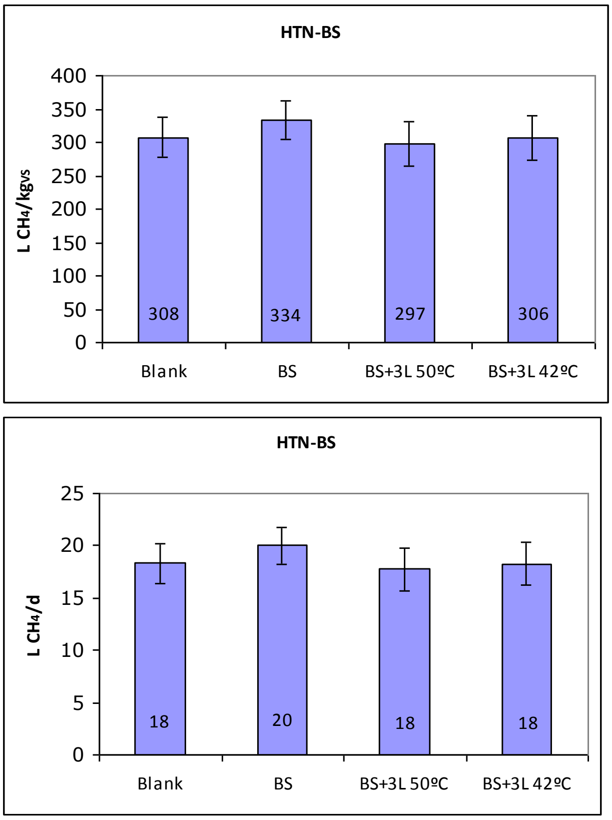

Figure 9. Experiments with bow-screen (data period after 2 HRT only).

Effect of re-injection after solid-liquid separation with screw press:

After the results with bow-screen, it was decided to increase the volatile solids treated in the fibre fraction by changing the solid-liquid separation system to a screw press in order to see if it would increase the effect of the Re-Injection Loop with/without enzyme treatment. The re-injection digester showed a methane increment of 16% (statistics not possible due to temperature drop in August 2014, please see deliverable D3.1 for further details of effect on the data analysis), whereas the re-injection with enzyme pretreatment digesters showed no apparent improvement. Similar to the enzyme treatment, a possible loss of volatile compounds due to high temperature of enzyme treatment could be the cause of this result. The next figure includes the results for the screw press and screw press with enzyme treatment.

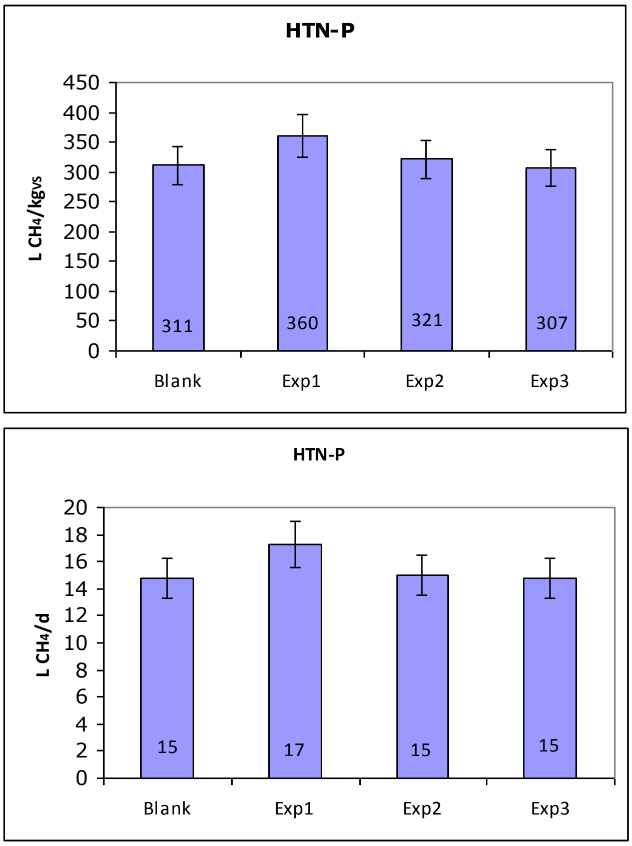

Figure 10. ***Experiments with screw press (total gas of the last 32 days/total volatile solids fed the last 32 days; bars show 10% SD as indication but not real standard deviation)***

Effect of enzyme addition to the fibre fraction with pH adjustment:

After pH adjustment in the enzyme treatment (pH 5.5; addition of HCl 37%), differences were observed with the enzyme treatment (t-test, 95% confidence), while the re-injection increase is not statistically significant (still high SD due to temperature recovery period, this high SD might mask the result, please see deliverable D3.1 for further details). The re-injection with enzyme pretreatment digesters showed increments of 18% and 14% at 50°C and 42°C, respectively, compared with the blank. The next figure includes the results for the screw press and the screw press with enzyme treatment and pH adjustment.

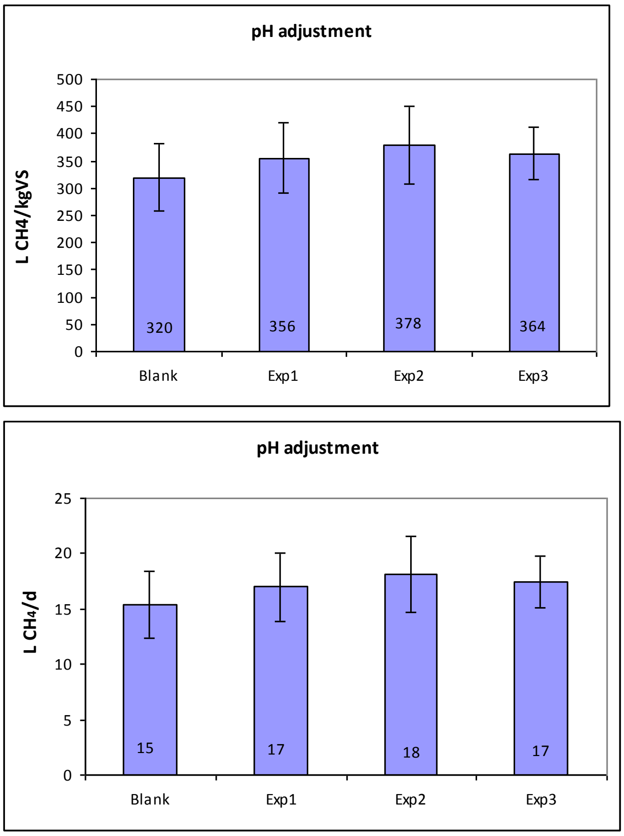

Figure 11. ***Experiments with screw press and pH adjustment in enzyme treatment***

Another focus point was the hydraulic retention time (HRT). If HRT was lowered to 17 (instead of 32) days, the methane yield increased by 33% following re-injection with enzyme. In connection with HRT of 17 days, the amount of fibres partially digested was higher than with HRT of 32 days. For that reason, the effect of the enzyme on the solid fibre fraction was higher than with a higher HRT (32 days). During the test with lower HRT, the pH of enzyme treatment was also adjusted. Although this result is important for other plants, it was not applicable in full scale in BIOMAN. All the details are included in the deliverable D3.1.

Effect of enzyme addition to the fibre fraction with pH adjustment and increase of fibre in the feeding:

As low methane increments were obtained with re-injection, and as the enzyme treatment sometimes did not yield significant results, it was decided to carry out additional experiments with improved conditions. This was achieved through increased efficiency of the solid-liquid separation (total solids in the solid fibre fraction) and enzyme pretreatment (enzyme type, dosage and temperature). The pH conditions of the enzyme pretreatment were already adjusted to optimum values. The re-injection experiment with no enzyme treatment for digester operated with normal HTN feed was carried out together with the new experiments.

The figure below shows that the re-injection of the digester, operated with normal HTN feed, achieved 21% methane yield increment based on mean values, not considering standard deviations (details related to the HTN feed is included in deliverable D3.1). Enzyme treatment added to re-injection did not improve this result (11%). The increase of methane yield was statistically significant (t-test, 95% level of confidence). On the other hand, re-injection of the digester operated with HTN feed and addition of 5% straw yielded a 33-35% methane yield increment, when treating the fibres with BG-X enzyme based on mean values, not considering standard deviations. However, the operating conditions are very extreme (5% straw) and the enzyme dosage used in these experiments was high (1%).

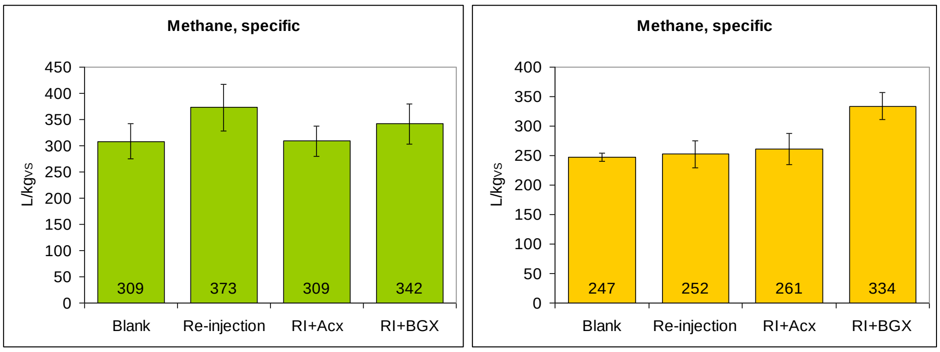

Figure 12. ***Experiments with screw press and pH adjustment in enzyme treatment (HTN, left; HTNF+5%, right).***

These previous operating conditions with a higher dosage of enzyme and higher amount of fibre in the feeding were modified for economical process. The re-injection experiments continued with no enzyme treatment for digesters operated with normal HTN feed, and a new re-injection experiment was initiated with BG-X2 enzyme experiment with less fibre (2.5% straw) and enzyme dosage 0.1%. Nevertheless, in the figure below, no significant difference (t-test, 95% confidence) was observed in the methane yield with re-injection and BG-X2 enzyme with fibre supplemented (2.5% straw) in the HTN feed.

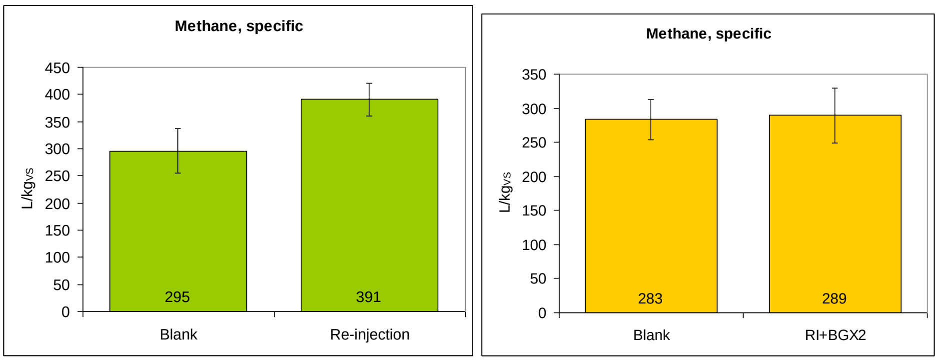

Figure 13. ***Experiments with screw press and pH adjustment (HTN, left; HTNF+2.5%, right).***

Main conclusions

- The effect of re-injection after solid-liquid separation only using bow screen (fibre fraction with 8 % TS) resulted in 9% methane yield increment, whereas with screw pressing, simulating a decanter or screw press (fibre fraction 30-40%TS), 21-33%

methane yield increment was observed. This is based on mean values and not considering standard deviations. Taking the mentioned results into account, re-injection of the digestate fibre fraction is an interesting and fairly simple strategy to increase the methane yield by 9-33% without altering the fresh feed rate.

- The total solid concentration achieved on the solid-liquid separation had a significant impact on the methane yield (differences between bow screen and screw press).

- The enzymatic treatment showed an effect only if the fibre content was high. The effect of enzyme addition to the fibre fraction had visible effects with high fibre contents, pH adjustment and higher dosages. However, in the HTN feed there was only a medium fibre content (manure), representing around 60-70% of the mixture. Therefore, only a small amount of the mixture could be affected by the enzymatic pretreatment meaning that sometimes there was a small increase in the methane yield.

- When the enzyme was a multicomponent fungal cellulase with addition of xylanase, there was a significant increase on the methane yield (33-35%) when the fibre concentration in the substrate was high (feeding 5% straw). That was based on mean values not considering standard deviations.

- Implementation of Re-Injection Loop after the decanter seems feasible for full-scale implementation. Re-Injection Loop without enzyme addition requires a relatively low investment. However, due to economical reasons, the results of the pilot scale were not implemented at full-scale in the HTN biogas plant:

    - Considering the current regulations related to feed-in tariff in Spain, it is not economically profitable for HTN biogas plants to increase their biogas yield. The feed-in tariff for biogas plants with more than 500 kW power installed has decreased from 10 cents to 4 cents per kWh and the HTN biogas plant has a contract to sell a fixed amount of electricity, so there was no benefit in producing more. The HTN biogas plant is running at maximum electricity production of the plant in the BIOMAN project.

    - Additionally, a high income at the HTN biogas plant was related to the current payment of the waste management. A source of income for HTN was related to processing of organic waste (different dairy products). For that reason, it was not interesting to reduce the income from waste management in order to increase the fibre in the feeding to get an increase in the methane yield.

    - In addition, other applications of the biogas such as biomethane were considered. However, it was not economically feasible for the company due to the reduction in income of the biogas plant because of the latest regulations (taxes, etc.).

4.1.6 WP4: Development of full scale Re-Injection Loop operation and additional lab-scale experiments

The objective of this work package was altered as no full-scale implementation was performed. Consequently, only a concept diagram of the entire process was developed as well as specification and requirements for each process step, see below figure.

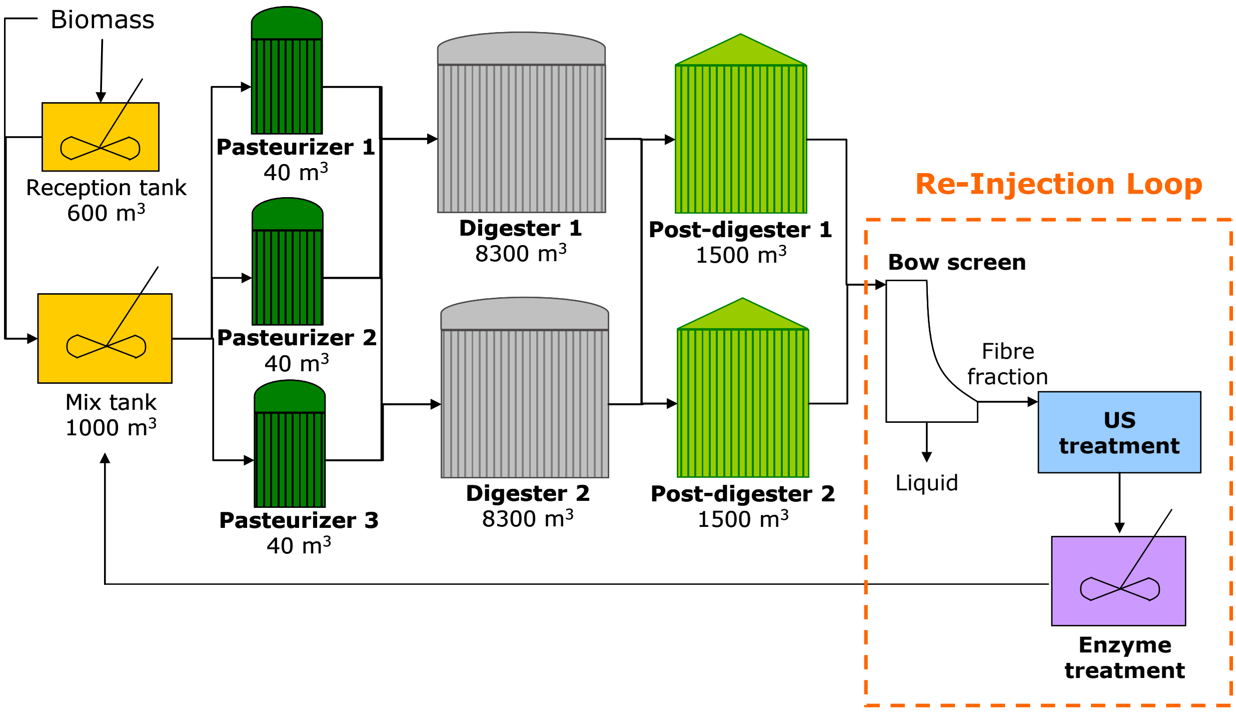

Figure 14. Overview of the HTN biogas plant. The red dotted line show the Re-Injection Loop concept.

Instead of the full-scale implementation, three new tasks were formulated specifically for the need of the involved SMEs, namely Hielscher, Enzyme Supplies and Bigadan. However, the data are considered confidential and are thus not included in the report.

4.1.7 The potential impact and the main dissemination activities and exploitation of results.

Initially, the Re-injection Loop was supposed to be implemented at the HTN biogas plant in Spain. However, the business foundation of biogas production in Spain has changed significantly during the course of the project. Now, the HTN biogas plant is producing the maximum allowed electricity (fixed number); thus, producing more biogas does not provide more income and the heat from the process cannot be sold. Moreover, HTN receives subsidies for receiving various types of organic waste to be used for bio-gasification. Because of this and given the capacity of the plant, HTN has no interest in the Re-Injection concept at present, as the provided subsidies would decrease. This is the main reason for the concept not being implemented at HTN during the project. Lastly, the concept holder Bigadan does not consider investment in equipment to use the Re-Injection Loop concept, as they do not consider the concept economically feasible.

However, the business foundation of biogas production is very different elsewhere in Europe. Below is an outline of a rough business plan for the Re-injection Loop based on higher subsidies for electricity and no subsidies for waste handling.

During the project, three different configurations of the Re-Injection Loop have been studied:

- Re-injection alone (no treatment besides separation)
- Re-injection with enzymatic treatment
- Re-injection with ultrasound treatment.

The ultrasound treatment increased the biogas yield significantly; however, the energy balance (applied energy vs. energy increase) proved to be negative. The enzymatic treatment for most of the experiments proved not to have any significant effect in comparison to the re-injection without enzymatic treatment. Consequently, re-injection alone, without ultrasound treatment nor enzymatic treatment, is included in the below business plan.

The business plan is outlined below on following assumption:

- The Re-Injection Loop increases the methane yield with 10 %, from 300 L/KgVS to 330 L/KgVS. The figures are average figures from the pilot experiments, but they have been varying considerably. Moreover, the biogas yield will be very dependent on the composition of the feed and biogas plant operation. Thus, the potential increase in methane may vary significantly in the different plants.
- A selling price of the electricity of 0.043 €/kWh. This number varies significantly in the different regions/countries and will therefore affect the potential (excess) profit.
- The start-up expenses is set to 250,000 €, depreciation over 5 years, and annual maintenance costs of 10,000 €. The Re-injection has not been operated in full-scale; thus, the figures are rough estimates.

Based on the above, the outlined business plan indicates that the Re-Injection Loop may be feasible; however, the data used for the calculation is associated with uncertainties.

Despite of these uncertainties, there are still manure-based anaerobic digester plants that could benefit from installation of the Re-Injection Loop concept, namely digester plants operating on manure and deep litter with little or no contribution from easily degradable substrates. Digesters that could benefit from the Re-Injection vary in size from small farm-based digesters, up to large digesters operating on mono substrates e.g. chicken manure, cow manure. A survey conducted within BIOMAN on substrate availability in Europe, revealed a large potential for including manure in biogas plants that are not used today. The potential for biogas production based on manure in the EU is 827 PJ; however, today only about 50 PJ is produced in agricultural biogas plants using animal manure as well as energy crops and various types of organic waste. The number of farm scale biogas plants in Europe is estimated to be around 5000 and these plants are found all over Europe. In Germany, more than 6000 biogas plants operate on energy crops (mainly maize) and here with addition of manure or deep litter and other recalcitrant substrates, the Re-Injection Loop concept could boost the economy of the biogas plants.

Even though the Re-injection Loop have not reached the marked, the involved SMEs have all gained valuable knowledge from participating in the BIOMAN project. According to the SMEs, this knowledge will be and have already been used for selecting/ prioritizing areas of potential new business areas:

- Enzyme Supplies has gained important knowledge on the enzymatic potential within the anaerobic digestion sector. This information has already been used to exploit the business potential of EZ to new areas within biogas, and bioethanol. EZ has gained an advantage by developing enzymes directed at specific substrates, and thereby provide the customers with tailor-make enzymes packages for clients in Europe, Africa and South America. Enzymatic pretreatment is extremely important for better use of biogas potential from waste fruits, and vegetables directly at the premises for growth, and for substrates used for bioethanol production.
- Bigadan and DTI worked closely together during definition of the BIOMAN project and consortia composition. Bigadan has the status as concept holder of the Re-Injection loop. The primary business of Bigadan is to design, build and operate biogas plants for different clients. The knowledge gained during BIOMAN will potentially influence future-design solutions of biogas plant e.g. for large mono substrate biogas plants or smaller conventional manure-based biogas plants. Extensive data on the residual biogas potential from manure and other recalcitrant substrates has been obtained during the project, which in turn can be used to evaluate the feasibility of investments either for new plants or plants requiring increased capacity operating on low value substrates.
- HTN biogas plant, could at the time of the project, not see the economically incitement for installing the Re-Injection loop concept as their main source of income originates from waste management and not electricity production. However, if the business foundation for biogas production changes, the Re-Injection loop could potentially be of interest.
- The ultrasound equipment producer, Hielscher, has expanded their knowledge on ultrasound treatment of substrates that could potentially be used for anaerobic digestion. Within BIOMAN, it was demonstrated that at manure-based biogas plants, it is very difficult to obtain a net positive energy balance when applying ultrasound to the fibrous substrates. However, ultrasound is already being applied in some sludge-based biogas plants and here it is anticipated that the economic balance will favour ultrasound treatment. By being a partner of the BIOMAN project, Hielscher has gained access to research that has demonstrated in which directions future business segments could be of interest. Moreover, the ultrasound treatment of a number substrates have been optimized, enhancing Hielscher' general knowledge about their products and process-parameters.

4.2 Use and dissemination of foreground

For several years, there has been a strong focus on developing green energy sources as an alternative to fossil fuels. One area that has received increased great attention is biogas production and many projects have been funded by the EU, e.g. BIOMAN. To develop biogas into a sustainable business, cheap and reliable technologies are needed to aid biogas production from low-value substrates as manure and 2^{nd} generation biomasses. Therefore, it is relevant to identify cheap pretreatment techniques (and combinations of these) that can improve the biogas yield without adding a significant cost to the biogas processing.

This project provides knowledge on a treatment concept termed the Re-Injection Loop, where the fibres from e.g. manure is undergoing a series of treatments to open up the fibres, before they are reintroduced into the anaerobic digester for biogas production.

For the BIOMAN project, a number of dissemination activities were formulated in the application, which are listed in Table 10. These include dissemination activities for the project, for the project partners, and activities specifically directed at the RTD participants (AAU, AINIA and DTI) and SMEs (Hielscher, Enzyme Supplies, HTN and Bigadan).

Table 10. Overview of BIOMAN deliverables during the project.

Work Package Deliverables		**Delivery month**
D6.1	Press release	1
D6.2	Project website - updated every 3^{rd} month	2
D 6.3	Interim Plan for Use and Dissemination of Knowledge	12
D6.4	Final Exploitation and Dissemination Plan	29

A press statement was released at month 1, to media of the different partners' country. The project website was released in month 2, which was hosted and updated by DTI. The Interim plan for use and dissemination was released at month 12. The final use of dissemination was released at month 29. All deliverables have been uploaded.

Below is depicted the dissemination activities of the RDTs. Abstracts concerning the BIOMAN project have been submitted to many different scientific conferences where also companies have participated. In general, interest from both the scientific and the company communities resulted in discussion on the Re-Injection Loop concept. Dissemination started around project initiation and we have just been granted a presentation at the Anaerobic Digestion conference 14, in November 2015.

Table 11. Overview of all dissemination activities during BIOMAN for RTDs

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES FOR RTDs

NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
1	*Publication*	*Caroline Kragelund*	*Engelske enzymer i danskledet EU-biogasprojekt*	*Summer 2012*	*Enterprise Europe network*	*Scientific community*	*10000*	Denmark
2	*Presentation*	*Ruben Rodriquez*	*Participation in a workday*	*September 2012*	*Spanish National Center of Alimentary Technology and Safety. San Adrián, Navarra (Spain)*	*Industry (Plant operators)*	*100*	*Spain*
3	*Poster and 5 min presentation*	*Caroline Kragelund*	*Economically efficient biogas production from manure fibres and straw*	*November 2012*	*Danish society of Microbiology Copenhagen, Denmark*	*Scientific community*	*300*	Denmark
4	*Presentation*	*Hinrich Uellendahl*	*Boosting the economy of manure-based biogas plants*	*April 2013*	*BiogasWorld 2013, Berlin, Germany*	*Scientific community, industry*	*3000*	*Developing countries, i.e. eastern Europe*
5	*Publication*	*Hinrich Uellendahl*	*Boosting the economy of manure-based biogas plants*	*April 2013*	*Dry Fermentation, Substrate Treatment, Digestate Treatment" Proceedings of the international anaerobic digestion symposium within the BiogasWorld 2013*	*Scientific community, civil society, policy makers*	*200000*	
6	*Poster*	*Caroline Kragelund*	*BIOMAN, Economically efficient biogas production from manure fibres and straw*	*June 2013*	*21st European biomass conference and Exhibition – Setting the course for a biobased economy 2013, Copenhagen, Denmark*	*Scientific community, industry*	*10000*	*Mainly Europe but participants from all over the world participate*
7	*Poster*	*Hinrich Uellendahl*	*Bioman Economically efficient biogas production from manure fibres and straw*	*June 2013*	*13th World Congress on Anaerobic Digestion-Recovering (bio) resources for the world. IWA conference, Santiago de Compostela, Spain*	*Scientific community, industry.*	*5000*	*Western countries*
8	*Presentation*	*Beatriz MolinuevoSalces*	*Key factors for achieving profitable biogas production from*	*June 2013*	*13th World Congress on Anaerobic Digestion-Recovering (bio) resources*	*Scientific community,*	*5000*	*Western countries*

			agricultural waste and suistainable biomass		*for the world. IWA conference, Santiago de Compostela, Spain*	*industry*		
9	*Presentation*	*Paz Gomez*	*Agro-industrial biogas: new approaches and technological developments for a viable future in Spain / Biogás agroindustrial: nuevos enfoques y desarrollos tecnológicos para un futuro viable en España*	*July 2013*	*NH Madrid Nacional Hotel (Madrid, Spain)*	*Scientific community, biogas and energy industry*	*75*	*Spain*
10	*Publication*	*RETEMA*	*European project BIOMAN, use of manure fibres to produce agro-industrial biogas / Proyecto europeo BIOMAN, aprovechamiento de la paja de estiércol para generar biogás agroindustrial*	*October 2013*	*Revista Técnica de Medio Ambiente Nr. 169 "Especial Bioenergía"(Spanish journal of environment*	*Scientific community, civil society, policy makers*	*100000*	*Spain*
11	*Poster*	*Bjørn Malmgren-Hansen*	*The Re-Injection Loop concept Economically efficient biogas production from manure fibres and straw*	*June 2014*	*Nordic biogas conference, Reykjavik, Iceland*	*Scientific community, industry*	*500*	*Nordic countries*
12	*Poster*	*Hinrich Uellendahl*	*Testing the effect of different enzyme blends on increasing the biogas yields of straw and digested manure fibres*	*October 2014*	*Biogas science, Vienna, Austria*	*Scientific community, industry*	*288*	*European countries*
13	*Presentation*	*Begoña Ruiz*	*Ultrasound pretreatment to increase the biogas yield of straw and digested manure fibres*	*October 2014*	*Biogas science, Vienna, Austria*	*Scientific community, industry*	*288*	*European countries*
14	*Presentation*	*Hinrich Uellendahl*	*Towards economically efficient pretreatment of lignocellulosic biomass for higher biogas production*	*June, 2015*	*Biogas seminar, Ås, Sweden*	*Scientific community*	*50*	*Norway and other Nordic countries*
15	*Presentation*	*Hinrich Uellendahl*	*Sense and no-sense of pretreatment for increasing biogas yields*	*September 2015*	*IBBA Workshop Malmö*	*Scientific community*	*200*	*Baltic conutries*
16	*Presentation*	*Gracia Silvestre*	*Boosting methane yield of lignocellulosic materials with*	*November 2015*	*14th world congress on anaerobic digestion, Chile*	*Scientific community*	*5000*	*Western countries*

			enzymatic treatment and Re-Injection Loop		*(http://www.ad14chile.com)*	*industry.*		

Table 12. Overview of all dissemination activities during BIOMAN for SMEs

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES FOR SMEs								
NO.	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
18	*Exhibiting*	*HTN*	*Expobioenergia*	*October 2013*	*Valladolid, Spain*	*Scientific community, industry*	*500*	*Spain*
19	*Exhibiting*	*Enzyme Supplies*	*UK Anaerobic digestion & Biogas 2014*	*July 2014*	*ttp://www.adbiogas.co.uk/uk-ad-biogas-2013/uk-ad-biogas-2014/, Birmingham, UK*	*Scientific community, industry*	*10000*	*UK, Europe*
20	*Exhibiting*	*Hielscher*	*ESS 14*	*June 2014*	*Avignon, France*	*Scientific community, and industry*	*150*	*worldwide*
21	*Exhibiting*	*Enzyme Supplies*	*Biogas expo & congress*	*October 2014*	*Offenberg, Germany*	*Scientific community*	*900*	*Europe*
22	*Exhibiting*	*HTN*	*EUROTIER (focus BioEnergy Decentral)*	*November 2014*	*http://www.eurotier.com/ ,Hanover, Germany*	*Industry, farmers.*	*156000*	*Europe*
23	*Exhibiting*	*Bigadan*	*Agromek 2014*	*November 2014*	*http://www.agromek.com/*	*Industry, farmers*	*46619*	*Northern Europe*
24	*Exhibiting*	*Enzyme Supplies*	*Anaerobic digestion & bio-resources conference*	*July 2015*	*http://adbioresources.org/uk-ad-biogas-2015 Birmingham, UK*	*Scientific community, policy makers*	*500*	*Europe*
25	*Exhibiting*	*Enzyme Supplies*	*Biogas expo & congress*	*November 2015*	*http://www.biogas-offenburg.de/en/biogas Offenberg, Germany*	*Scientific community, policy makers*	*1000*	*Europe*

4.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	**FP7-SME-2012.315664**
Title of Project:	**Economically efficient biogas production from manure fibres manure fibres and straw**
Name and Title of Coordinator:	**Dr. Caroline Kragelund Rickers**

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***no*** ***0Yes 0No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	No
• Did the project involve patients?	No
• Did the project involve persons not able to give consent?	No
• Did the project involve adult healthy volunteers?	No
• Did the project involve Human genetic material?	No
• Did the project involve Human biological samples?	No
• Did the project involve Human data collection?	No
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	No
• Did the project involve Human Foetal Tissue / Cells?	No
• Did the project involve Human Embryonic Stem Cells (hESCs)?	No
• Did the project on human Embryonic Stem Cells involve cells in culture?	No
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	No
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	No
• Did the project involve tracking the location or observation of people?	No
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	No
• Were those animals transgenic small laboratory animals?	
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	

• Did the project involve the use of local resources (genetic, animal, plant etc)? Plant material as wheat straw	**Yes**
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	**No**
DUAL USE	
• Research having direct military use	No
• Research having the potential for terrorist abuse	No

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	1	
Work package leaders	2	1
Experienced researchers (i.e. PhD holders)	3	3
PhD Students		
Other- bachelor students		2

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	
Of which, indicate the number of men:	0

Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project?**	X	No

6. Which of the following actions did you carry out and how effective were they?

Not effective at all — **Very effective**

- ❑ Design and implement an equal opportunity policy ○ ○ ○ ○ ○
- ❑ Set targets to achieve a gender balance in the workforce ○ ○ ○ ○ ○
- ❑ Organise conferences and workshops on gender ○ ○ ○ ○ ○
- ❑ Actions to improve work-life balance ○ ○ ○ ○ ○
- ○ Other:

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

- ○ Yes- please specify
- x No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

- X Yes- please specify: Two bachelor students got their degree while working on BIOMAN (D.2.2)
- ○ No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

- ○ Yes- please specify
- X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

- X Main discipline[2]: 1.5 biological sciences
- X Associated discipline[2]:2.3 other engineering sciences
- X Associated discipline[2]:4.1 Agriculture, forestry, fisheries and allied sciences

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	X	No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

- ○ No
- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research

[2] Insert number from list below (Frascati Manual).

○ Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ ○	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

x No
○ Yes- in framing the research agenda
○ Yes - in implementing the research agenda
○ Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
x No

13b If Yes, in which fields?

Agriculture Audiovisual and Media Budget Competition Consumers Culture Customs Development Economic and Monetary Affairs Education, Training, Youth Employment and Social Affairs		Energy Enlargement Enterprise Environment External Relations External Trade Fisheries and Maritime Affairs Food Safety Foreign and Security Policy Fraud Humanitarian aid		Human rights Information Society Institutional affairs Internal Market Justice, freedom and security Public Health Regional Policy Research and Innovation Space Taxation Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**0**
To how many of these is open access[3] provided?	**0**
How many of these are published in open access journals?	**0**
How many of these are published in open repositories?	**0**
To how many of these is open access not provided?	**0**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[4]: ……………	
15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*	**0**

16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**

[3] Open Access is defined as free of charge access for anyone via Internet.
[4] For instance: classification for security project.

17. How many spin-off companies were created / are planned as a direct result of the project?	**0**
Indicate the approximate number of additional jobs in these companies:	

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
x	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*
Difficult to estimate / not possible to quantify	x

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

Yes X No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X	Press Release	X	Coverage in specialist press
❑	Media briefing	X	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
X	Brochures /posters / flyers	X	Website for the general public / internet
❑	DVD /Film /Multimedia	X	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

X	Language of the coordinator	X	English
X	Other language(s)	X	Spanish

Question F-10: Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

5 Final report on the distribution of the EUROPEAN UNION financial contribution

THE TRANSACTION

Please provide a list of the actual cost incurred by the RTD performers during the performance of the work subcontracted to them. These costs refer only to the agreed 'Transaction' for the BIOMAN project.

Table 10. Personnel, subcontracting and other major costs items for RTDs for period 2

Name of RTD Performer	Number of person months	Personnel Costs (€)	Durable equipment	Consumables	Computing	Overhead Costs (€)	Other Costs (€)	Total by RTD performer
DTI	20.49	431.968,10	0	11.321,19	0	0	8.471,28	**451.760,57**
AAU-CPH	6.7	206.749,46	601.12	0	0	0	15.616,42	**222.967,00**
AINIA	44.70	198.822,49	0	34.512,79	0	0	8.224,72	**241.560,00**
TOTAL	**71.89**	**837.540,05**	**601.12**	**45.833,98**	**0.00**	**0.00**	**32.312,42**	916.287,57