Technological solutions for the use of biomass with evaluation of cost effectiveness

Report

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Introduction

Increasing attention in the European Union is paid towards broader use of various renewable energy sources. High amounts of grass biomass being wasted on a field has a potential to be used in many alternative ways, including production of biofuel, biogas or pellets for further energy generation or other purposes.

Within the frame of the LIFE+ project "Alternative use of biomass for maintenance of grassland biodiversity and ecosystem services" research on possible solutions for use of grass biomass has been carried out. The aim of this study has been to test various technical and technological options for processing of biomass for production of biobutanol and biogas. The research included field works – collection of biomass samples from different habitats in Sigulda and Ludza Municipalities (Latvia) and in different times of the year during the vegetation season in 2014, 2015 and 2016. During the laboratory tests the collected biomass has been exposed to various physical and chemical conditions, microorganisms in order to find out the optimum combination of these variables for pre-treatment of grass biomass. The obtained results will serve as the bases for specification of technical parameters for pilot facilities and highlight possible synergies in a process of production of biogas and biobutanol.

Second generation biofuel - biobutanol produced from lignocellulosic biomass is regarded as a good alternative to cellulosic ethanol. However, its fermentation is closely linked to effective pre-treatment and hydrolysis of the biomass. Application of complicated techniques limits the introduction of the technology to a wider scale, thus, options for a simple pre-treatment/hydrolysis must be introduced. The aim of this study was to test various simple biomass conversion techniques for biobutanol production to demonstrate an efficient technology. The report describes the research performed to identify and describe several critical points in technology development. As a result a method of mechanical grinding, followed by boiling and enzymatic hydrolysis at low temperature (30°C) for 24 hours is defined and applied for natural biomass sample analysis collected over 3 months period 2014 –2016 in Sigulda and Ludza Municipalities.

Besides traditional substrates – maize, manure also different grass biomass – green grass, hay, silage and digestate (waste product from anaerobic fermentation) can be used for production of biogas. The report presents laboratory tests and the research carried out for obtaining biogas from grass biomass and digestate. Laboratory tests show that pre-treatment of biomass e.g., by steam explosion ensures essential disintegration of fibrous structures. Results show that it is possible to produce 70 m^3 biogas per 1 ton of grass biomass (methane concentration < 52%). Green grass gives the best indication on maximum biogas production volumes. In parallel laboratory tests of biogas production from digestate have been performed. Based on the research results, it can be calculated that about 60 m^3 of biogas per 1 ton of digestate can be produced (methane concentration ca. 53%). Laboratory tests on optimum conditions and proportions of different grass substances and pre-treatment conditions for digestate are being continued.

Based on research results the cost effectiveness assessment of the proposed technological solutions for production of biogas and biobutanol will be performed. The report includes methodology for cost effectiveness assessment to be followed when the laboratory investigations are finalised. The project envisages testing possibilities of production of pellets from grass biomass obtained during restoration activities of grasslands in Sigulda and Ludza Municipalities. Cost effectiveness of production of grass pellets will be evaluated, too.

1. Collection of biomass

In order to obtain grass biomass for further investigations, field data collection was conducted. The first biomass sample (total weight of ca. 100 kg) was collected from semi-natural grassland in More Parish, Sigulda Municipality and used for initial laboratory investigations in 2014. Other samples (161 in total) were collected from 67 randomly selected semi-natural grassland plots in Sigulda and Ludza Municipalities that correspond to 6 habitat types of Community importance (the most common habitat types within these municipalities) (Table 1.1, Annex 2).

Table 1.1: Number of samples collected in Sigulda and Ludza Municipalities

Habitat type	Number of biomass samples
6120 Xeric sand calcareous grasslands	17
6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates	31
6270 Fennoscandian lowland species-rich dry to mesic grasslands	45
6410 Molinia meadows on calcareous, peaty or clayey-silt-laden soils	18
6450 Northern boreal alluvial meadows	18
6510 Lowland hay meadows	32
Total	**161**

Almost half of the samples were collected in June when grassland biomass has the highest fodder value, but almost one third – in August – in a time that correspond to late mowing (Table 1.2.).

Table 1.2: Number of collected biomass samples per sampling time and habitat type

Habitat type	June	July	August	September	Total
6120 Xeric sand calcareous grasslands	10	5	1	1	15
6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates	15	8	8	0	25
6270 Fennoscandian lowland species-rich dry to mesic grasslands	16	10	18	1	35
6410 Molinia meadows on calcareous, peaty or clayey-silt-laden soils	6	2	8	2	15
6450 Northern boreal alluvial meadows	8	2	5	3	15
6510 Lowland hay meadows	21	7	2	2	23
Total	**76**	**34**	**42**	**9**	**161**

Most of the samples (88) were collected in 2014. During subsequent years there were collected 34 to 39 samples a year (Table 1.3).

Table 1.3: Number of collected biomass samples per sampling year

Habitat type	June	July	August	September	Total
2014	35	23	30	0	88
2015	7	11	12	9	39
2016	34	0	0	0	34
Total	**76**	**34**	**42**	**9**	**161**

Location of the sampling plots within selected semi-natural grassland was purely driven by a visual assessment – in the most representative place for the habitat. In each site, one 1x1 m vegetation plot was sampled before the first cut or the beginning of the grazing period (late June or early July). In sites that were

managed by late mowing, the second sample was collected in late July or August, but in unmanaged sites – the third sample was collected in September 2015. To assess the sugar yield in early June biomass, one sample from each habitat type were collected in early June 2015. In all cases, biomass samples were clipped at 2 cm and 10 cm above the ground level within 1x1 m square using hand shears (Figure 1.1).

(a)   *(b)*

Figure 1.1: 1x1m square frame sampling plots before (a) and after (b) collection of grass samples

The collected material was stored in pre-weighed plastic bags and brought to the laboratory where total fresh biomass was measured. Afterwards, half of each sample were dried in the oven at 105°C for 24h and weighed again to measure the dry matter, but another one – frozen or grinded and then frozen (prepared for detecting the sugar yield later phase). Before clipping vegetation descriptions (the species richness of vascular plants) for each square were prepared to get the data on species composition necessary for result interpretation.

2.   Use of biomass for production of bio-butanol

Worldwide energy consumption is increasing and available fossil fuel resources are decreasing from year to year. The increase in fossil fuel and oil prices and climate change due to greenhouse gases has increased the need for alternative fuel that would be economical. During the latest decades there has been an increasing interest for the production of fuels from renewable resources. First generation biofuels are produced from agricultural cultures, e.g., ethanol from corn. However, their use is controversial and several studies have shown that the impact on the climate[1], biodiversity[2] and availability of land for food production[3] can be negative. As an alternative, second generation biofuel production has been proposed.

The term "second generation biofuels" refers to biofuels produced from lignocellulosic biomass, i.e. from cellulose-hemicellulose-lignin composed feedstock[4]. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO_2 to the atmosphere[5]. Moreover, there is no competition with food production. The most popular biofuel types are liquid biofuels (bioethanol, biobutanol), biodiesel and biogas.

Anaerobic digestion technology for methane production is regarded as the most efficient method for energy generation from biomass than other biological processes, such as cellulosic ethanol[6]. However, there still is a need for liquid fuels that can be blended with conventional petrol or diesel.

Bioethanol is already produced on a fair scale and is easily applicable in present day internal combustion engine vehicles (ICEVs), as mixing with gasoline is possible. Ethanol is already commonly used in a 10% ethanol/90% gasoline blend. Adapted ICEVs can use a blend of 85% ethanol/15% gasoline (E85, in flexible fuels vehicles) or even 95% ethanol (E95). Ethanol addition increases octane and reduce CO, VOC and particulate emissions of gasoline. And, via on board reforming to hydrogen, ethanol is also suitable for use in future fuel cell vehicles (FCVs)[7]. Nevertheless, lately an alternative to bioethanol has been proposed. Biobutanol is considered a superior biofuel due to easy blending with gasoline or diesel at any ratio, low octane values, lower energy density, lower vapour pressure, biodegradability and ease in transportation due to lower explosiveness and corrosiveness[8,9]. Currently butanol is mainly produced via chemical synthesis; however, it can also be obtained from renewable resources (biomass) via acetone-butanol-ethanol (ABE) fermentation naturally performed by genus *Clostridium* bacteria[10].

Irrespective of biofuel type, effective and economically efficient conversion of lignocellulosic biomass to fuel is still a challenge. In biological conversion process biomass pre-treatment prior hydrolysis and fermentation is needed. The pre-treatment phase in general represents at least 20% of total production costs in all available technological approaches and it is regarded as the single most expensive process stem[11]. Thus, the aim of this study was to test various simple biomass conversion techniques for biobutanol production to demonstrate an efficient technology.

Lignocellulose material composition and sources

Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose and lignin (Fig. 2.1).
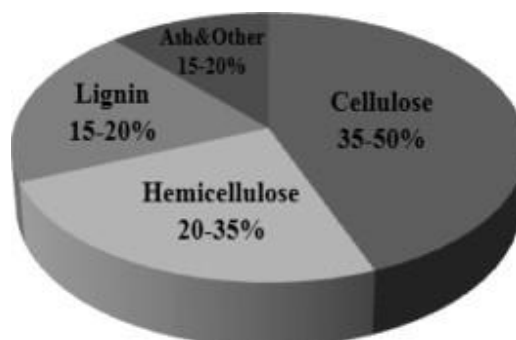

Figure 2.1: General composition of lignocellulosic biomass feedstock[12]

Lignocellulosic biomass with high cellulose and hemicellulose content is required for higher yield of butanol. However, it also consists of lignin, ash, protein and waxes in smaller amounts (Table 2.1.). On one hand where the relative proportions of cellulose: hemicellulose: lignin are the key factors in determining optimum energy conversion route for each type of biomass; the other contents lead to diminution of the theoretical butanol yield (g butanol/g LCB) when compared with sugar and starch crops[13].

Table 2.1: Composition of some lignocellulosic biomass (% of dry matter)[14]

	Cellulose	Hemicellulose	Lignin[a]	Acid detergent lignin	Crude protein	Ash
Crop residues						
Corn stover	38	26	19	4	5	6
Soyabean	33	14	–	14	5	6
Wheat straw	38	29	15	9	4	6
Rye straw	31	25	–	3	3	6
Barley straw	42	28	–	7	7	11
Grasses						
Switch grass	37	29	19	6	3	6
Indian grass	39	29	–	6	3	8
Miscanthus	43	24	19		3	2
Reed canary grass	24	36		2	10	8
Other crops						
Sweet sorghum	23	14	11			5
Pearl millet	25	35		3	10	9

[a] Lignin is total lignin (acid soluble lignin+acid insoluble lignin).

Lignocellulose pre-treatment and hydrolysis

Biomass pre-treatment is fundamental for optimal hydrolysis and downstream operations. During the pre-treatment recalcitrant material of the biomass is disrupted to increase the exposure places of enzymes to cellulose and hemicellulose[15] (Fig. 2.2.). The expected/desired characteristics for pre-treatment method are[16]:

- The more the pre-treatment system is able to process biomass pieces of large dimension, the better the energy balance and the overall process efficiency.
- Pre-treatment is a major energy-consuming step in the lignocellulosic ethanol process. Energy demand should be kept at the lowest possible level, while maintaining high process performances.
- The dimensions of the pre-treatment reactor should be limited to reduce volume and costs, the use of expensive materials should be avoided (this is also dependent on process operating conditions, such as temperature and pressure). Optimum design is a compromise between performances and costs.
- Pre-treatment process conditions should minimize sugar losses.
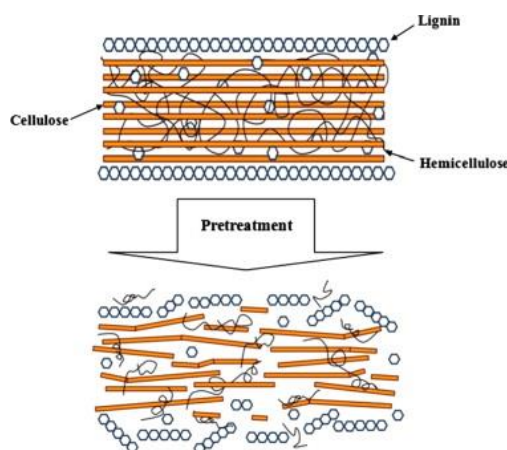

Figure 2.2: Schematic pre-treatment of lignocellulosic material[17]

All pre-treatment methods can be divided into several categories: physical, physicochemical, chemical, and biological or a combination of those (Fig. 2.3).
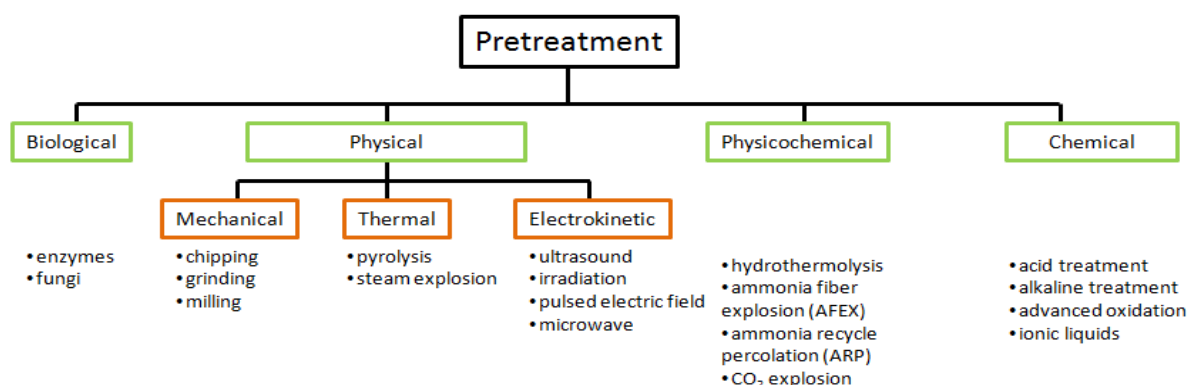
Figure 2.3: Main types of lignocellulosic biomass pre-treatment

2.1. Materials and methods

2.1.1. Mechanical and thermal pre-treatment of biomass

The dried biomass was grounded by grinder (Retsch GM200) and screened with a sieve to obtain various biomass fractions (powder, < 0.5 cm, 0.5 – 1 cm, 1 – 2 cm, > 2 cm). Fresh grass biomass was ground to a size < 0.5 cm. Prior heat treatment the biomass was diluted in 0.05 M sodium citrate buffer (3 % w/v) and either boiled for 5 minutes or heat treated at 121 °C for 15 minutes.

For pre-treatment and hydrolysis studies wheat straw and hay mowed in late June (2014) from lowland hay meadows located in Latvia was used as a reference material.

2.1.2. Enzymatic hydrolysis

For enzymatic hydrolysis, the prepared enzyme (0.2 FPU/mL, 20 FPU/g) was added to the diluted substrates (9% w/v for wet substrates; 3% w/v for dry substrates) and incubated on an orbital shaker for 24 – 48 h at 30 – 50 °C depending on the experiment setup.

Preparation of cellulolytic enzymes

Laboratory scale preparation of cellulolytic enzymes was performed from white rot fungi *Irpex lacteus* IBB 104, grown on agar plates (0.8 g/L KH_2PO_4; 0.2 g/L K_2HPO_4; 0.5 g/L $MgSO_4 \cdot 7H_2O$; 2 g/L NH_4NO_3; 3 g/L yeast extract; 5 g/L glucose; 18 g/L agar; pH = 6.0). The fungi were placed into 250 mL flasks containing 100 mL of liquid medium (10.0 g/L glucose; 2.0 g/L NH_4NO_3; 0.8 g/L KH_2PO_4; 0.4 g/L K_2HPO_4; 0.5 g/L $MgSO_4 \cdot 7H_2O$; 2.0 g/L yeast extract; pH = 5.5–5.8) and incubated on an orbital shaker (150 rpm) at 27°C. After 5–7 days the fungal pellets were homogenized with glass beads. Then 10 mL of homogenized fungal cultures where re-inoculated in the same media with the exception that glucose was substituted with agricultural substrate (hay, raw and dried giant hogweed or Avicel (Sigma)). Enzyme activity assays were performed regularly. After 2 weeks the biomass was removed and $(NH_4)_2SO_4$ (0.5 kg/L) was added to the supernatant, diluted and incubated at 4°C for 24–48 hours. After incubation the enzyme was sedimented by centrifugation (4000 rpm; 10 min) and stored in 0.05 *M* sodium citrate buffer at 4 °C for further use. Enzyme activities were measured according to IUPAC recommendations[18,19] and included the measurements of carboxymethylcellulase (CMCase), filter paper and xylanase assays.

2.1.3. Acid hydrolysis

The grinded biomass samples were diluted in H_2SO_4 and heat treated at various temperatures (Table 2.2). Prior sampling for the released sugars, adjustment to pH 5 with concentrated NaOH was performed. All tests were prepared in triplicates and at least 2 samples from each test were collected for produced sugar measurements.

Table 2.2: Acid hydrolysis treatment conditions

121 °C			Time
0.5%	3%	10%	30 min
0.5%	3%	10%	60 min

2.1.4. Measurements of reducing sugars

Reducing sugar concentration was measured by dinitrosalicylic acid (DNS) method[20]. In brief, to 0.1 mL of 0.05 M sodium citrate buffer in glass tubes 0.6 mL of DNS and 0.1 mL of centrifuged (10 min, 6700 g) sample supernatant was added. For blank control, distilled water was used instead of the sample. Then all samples were boiled for 5 min and transferred to cold water. Then 4 mL of distilled water was added. Absorption was measured with spectrophotometer at 540 nm. To obtain absolute concentrations, a standard curve against glucose was constructed.

2.2. Results and discussions

2.2.1. Evaluation of mechanical pre-treatment effect on sugar yield

To make the handling of the biomass material easier, reduction of the particle size by mechanical treatment is often used. Increased surface/volume ratio increase the effectivity of the biomass hydrolysis[21]. Research related to the effect of particle size has shown high inconsistencies among the reported results[22], indicating on the need for the evaluation of this parameter within each biomass treatment technique. To analyse the effect of hay particle size on enzymatic hydrolysis, reducing sugar concentration in the biomass with the size range of powder, < 0.5 cm, 0.5 – 1 cm, 1 – 2 cm and > 2 cm (Fig. 2.4) was measured directly after grinding and after enzymatic hydrolysis. The results showed that the highest variations among the results of the size groups occurred with the size above 0.5 cm. Significantly different ($p < 0.05$) sugar yields were observed in-between samples of > 2 cm and those below 0.5 cm. No difference ($p > 0.05$) between > 2 cm and 1-2 cm was observed in samples both after grinding and after hydrolysis (Figure 2.5).

Figure 2.4: Various biomass sizes tested for enzymatic hydrolysis
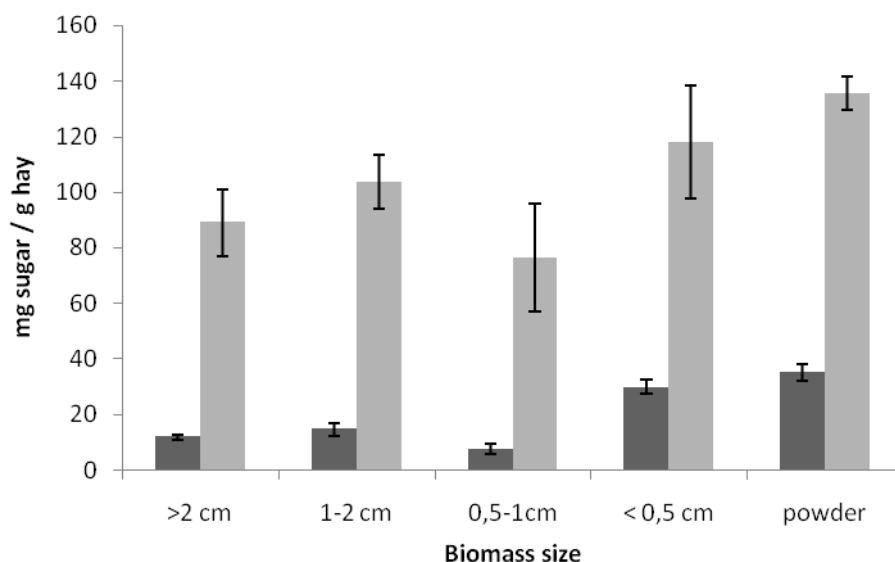
Figure 2.5: The amount of reducing sugars produced from various biomass sizes directly after wetting (dark) and after enzymatic hydrolysis (light). The bars represent average values from 6 replicates

The same observation was also for the samples of powder and < 0.5 cm with only difference that samples of < 0.5 cm gave the highest standard deviation values. This could be explained by variations among the produced grinded biomass size – from powder to 0.5 cm phase in a single sample; creating a real situation – no detailed size check-up during the production process. Other samples showed variable yields in between the experiments or treatments (wetting and hydrolysis), giving raise to the constant inconsistencies of the reported results. Nevertheless, the analysis of the reducing sugar concentrations after the hydrolysis showed 24-35 % higher sugar yields in samples with lower particle size (< 0.5 cm). Due to the observations particle size of < 0.5 cm was used in all further tests. The use of powder type biomass was omitted due to almost double energy consumption when compared to <0.5 cm and the observed yields were only 13 % higher for powder ($p>0.05$). Subsequent fractionation in particle size was omitted due to the fact that commercial availability of such grinding technologies is either limited or highly expensive.

Further the effect of heat treatment was tested. Initially it was observed that samples without any heat treatment were rapidly overgrown with natural biomass bacteria, which effectively consumed sugars produced during enzymatic hydrolysis (Fig. 2.6).

Figure 2.6: Growth of microorganisms in hydrolisates from untreated substrates (right) as opposed to heat treated substrates (left)

As a result tests on effect of thermal treatment on sugar release were performed and showed that even simple 5 minute boiling gave satisfactory results for fermentable sugar release from biomass. Moreover, no significant difference (p > 0.05) was observed for samples treated at 121°C or boiled (Fig. 2.7). Thus, boiling is further recommended as an additional method for sample pre-treatment to minimize natural microbial growth and produced sugar consumption.
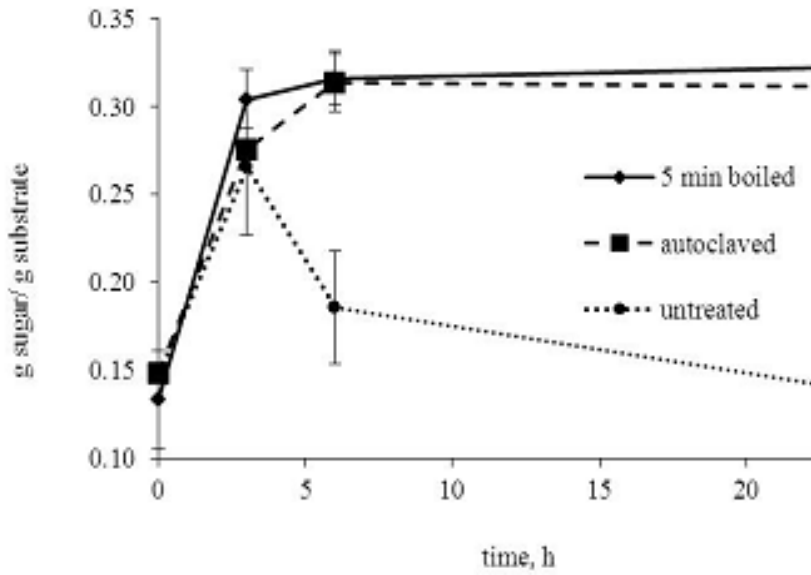
Figure 2.7: Amount of reducing sugars released at various heat treatment regimes

As suggested, dilute acid pre-treatment was evaluated prior enzymatic hydrolysis. The results showed that there is no significant increase in reducing sugar concentration when acid-treated samples are further subjected to enzymatic hydrolysis (Fig. 2.8). Moreover, excess of salt generated due to the necessity for neutralization of acid-treated biomass, limited sample processing.
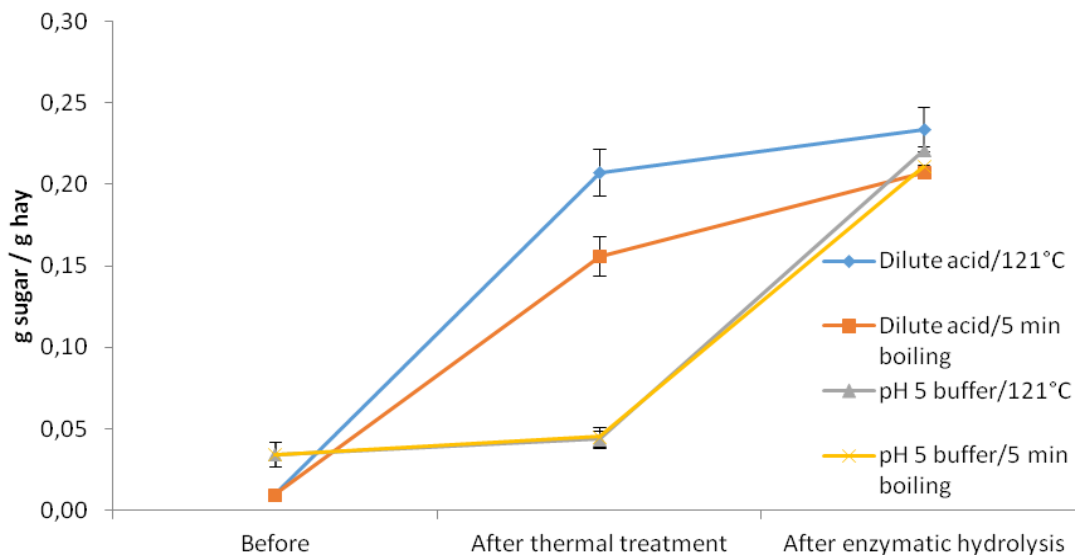
Figure 2.8: Evaluation of dilute acid as biomass pre-treatment technique. Standard deviation represents the dispersion of the results from 3 separate experiments

Further, comparison of acid-treatment with only heat pre-treatment prior enzymatic hydrolysis showed that there is no significant difference in the amount of reducing sugars generated with dilute acid or enzymes, thus, showing the potential of more environmentally friendly and safe method – enzymatic hydrolysis. Pre-treatment used in the research was limited to only mechanical disruption of biomass structures and removal of natural microbiota.

2.2.2. Evaluation of acid hydrolysis effect on sugar yields

Firstly a **standard curve for glucose absorption** was constructed (Fig 2.9.). The obtained formula was further used for all absorption measurements of released sugars.

Figure with y-axis "Glucose concentration, g/l" and x-axis "OD 540nm", showing the equation $y = 2{,}9108x - 0{,}0551$ and $R^2 = 0{,}9981$.
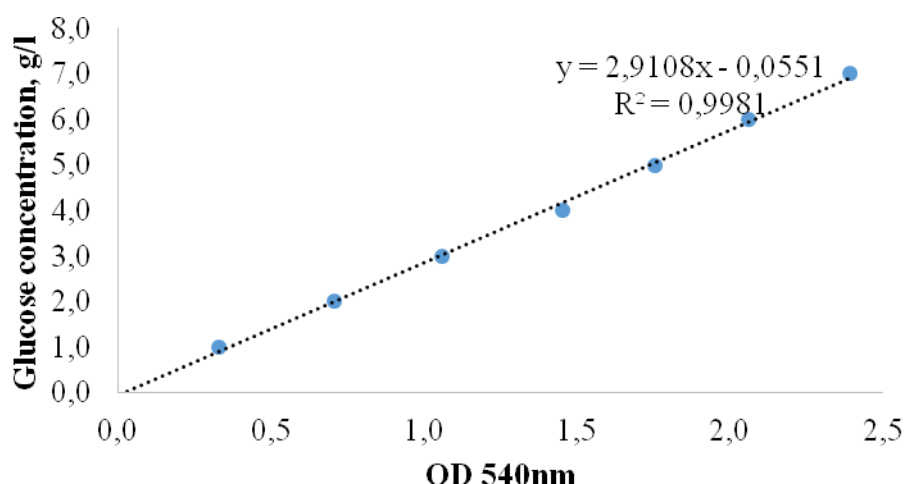
Figure 2.9: Glucose absorption standard at 540 nm. Each point represents an average of 3 separate measurements of identically prepared glucose standard

Further acid hydrolysis was prepared for two substrates – hay and straw, to observe any effect of hydrolysis on material source. Studies with enzymatic hydrolysis have shown that generally higher sugar yields are obtained with hay than with straw[23]. The results with acid hydrolysis (Fig. 2.10) showed the same trend of higher released sugar yields for hay. This was observed irrespective of treatment conditions. The highest released sugar yields with both substrates were obtained when final acid concentration was 3%. For both substrates 3% and 0.5% acid concentrations showed significantly different results ($p < 0.05$) at all temperature/time treatment regimes.

The highest released sugar concentration was observed for samples treated at 170°C for 60 minutes, however, this accounted for only 4.3% at 3% H_2SO_4 and 10.9% for 0.5% H_2SO_4 (hay) and for 4.0% at 3% H_2SO_4 and 21.0% for 0.5% H_2SO_4 (straw) when compared to 30 minutes treatment at 170°C. Thus, released sugar yields and energy consumption needed for hydrolysis must be evaluated. Moreover, the lowest temperature/time conditions for hay treated with 3% H_2SO_4 the observed difference reached only 32%.

To evaluate the effect of acid treatment as a pre-treatment technique which is followed by enzymatic treatment, solid substrates after acid treatment were washed with sterile water, air-dried and subjected to enzymatic hydrolysis. The results showed very low amounts of additional sugars released. Moreover, high amounts of water were required to wash the substrate, thus, giving rise for increase in production costs.
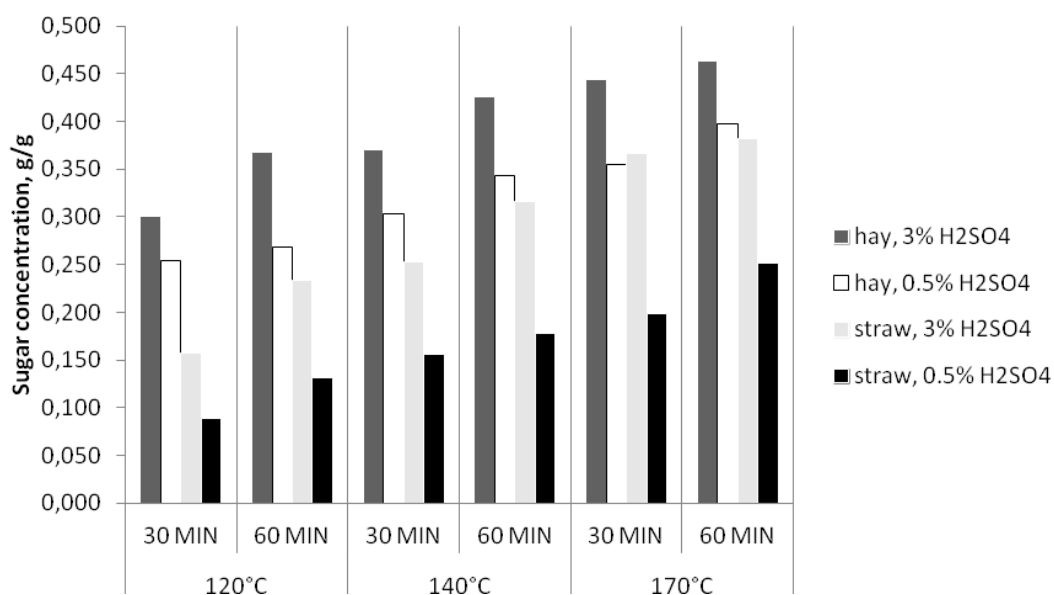
Figure 2.10: Amount of sugars released (g) from 1 gram of hay or straw at various temperature/time treatment regimes of acid hydrolysis. Each bar represents an average of 3 separate experiments

Experiments with 10% H_2SO_4 (reported as highest concentration for diluted acid treatment[24]) resulted in liquids impossible to neutralize for sugar measurements (the amount of concentrated alkali increased the volume of the liquid). Thus, no results were obtained for liquid fractions treated with 10% H_2SO_4. Enzymatic hydrolysis of solid substrates again did not account for significant enzyme production.

2.2.3. Evaluation of enzymatic hydrolysis on sugar yields

Apart from enzyme application in pre-treatment[25], enzyme use in hydrolysis has become more popular than application of any chemicals. Irrespective of longer incubation times enzymatic hydrolysis has been offered as one of most suitable tools for fermentable sugar production. Generally enzyme producing fungi are incubated with the lignocellulosic biomass for 2 to 23 days[26]. Shorter incubations are achieved with commercially available or pre-prepared enzymes at temperatures around 48-50°C when fungal cellulases are regarded as the most effective[27]. At the same time white-rot fungus *Irpex lacteus* is cultured at 28-30°C[28]. Thus, to estimate the necessity of increased temperature (50°C) hydrolysis, a comparative test was performed. The results showed that higher reduced sugar yields from hay biomass were obtained at the temperatures closer to the natural growth and enzyme production temperature of *I. lacteus*. 30% higher reducing sugar yields were obtained in samples incubated at 30°C than at 50°C. A reduction in 9% of sugar yield was observed when the temperature was increased from 30°C to 37°C ($p < 0.05$). It is regarded that mesophilic bacteria found in the biomass are not effective at 50°C temperatures, thus, non-sterile saccharification of lignocellulose has been proposed[29]. The results of this study showed that there is a significant difference ($p < 0.05$) in between the sugar yields of heat pre-treated and untreated samples.

To increase the sugar yields longer hydrolysis was introduced. No significant ($p > 0.05$) improvement in the sugar yields was observed when the samples are incubated at 30°C for 24 or 48 hours. Additionally there was no difference in sugar yields hydrolysed at 30°C or 37°C after 48 hours.

Thus, hydrolysis at 30°C for 24 hours was accepted as a suitable method for further biomass sample analysis.

Summary of optimal conditions

According to the obtained results, the following methodology is suggested for biomass hydrolysis:
- Mechanical pre-treatment by grinding to fractions below 0.5 cm.
- Thermal pre-treatment by 5 minute boiling to remove indigenous microorganisms.
- Enzymatic hydrolysis for 24 hours at 30°C if enzymes from *Irpex lacteus* are used.

2.2.4. Evaluation of biomass resources

To evaluate the amount of sugars released from various grassland biomass sources, enzymatic hydrolysis at optimal conditions as described before was performed. All samples were collected from June to September 2014, 2015 and 2016 (Table 1.1– 1.2). After collection the samples were either frozen or grinded and then frozen to avoid any microbiological activity. Hydrolysis was performed according to optimal conditions defined before.

The first year's results showed significantly different ($p < 0.05$) sugar yields in June than in July and August (Table 2.3). The highest sugar yields were obtained from samples from Semi-natural dry grasslands and scrubland facies on calcareous substrates (6210), but the lowest – in the samples of 6210 collected in August.

Table 2.3: Amount of reducing sugars generated from various biomass resources collected from June till August 2014

Sample No.	Date of collection	Average sugar yield, mg/g sample	Standard deviation*	Volatile Solids, %	Habitat No	Sugar, mg/g dry matter
22	27.06.2014	42.10	4.61	33.34	6120	126.29
23	27.06.2014	52.08	6.80	23.55	6510	221.15
24	27.06.2014	32.26	14.02	28.31	6510	113.95
25	27.06.2014	49.48	10.91	23.52	6450	210.40
26	27.06.2014	31.50	4.35	25.54	6450	123.35
27	27.06.2014	41.85	7.91	30.43	6450	137.51
29	27.06.2014	75.27	21.81	31.96	6210	235.50
30	27.06.2014	41.18	5.05	31.14	6120	132.23
31	28.06.2014	60.52	7.39	32.00	6120	189.13
32	28.06.2014	57.24	10.97	47.01	6120	121.76
33	28.06.2014	51.30	6.06	30.42	6120	168.65
34	28.06.2014	36.46	4.90	31.81	planted grass	114.63
35	28.06.2014	39.80	7.72	25.01	6510	159.12
60	17.07.2014	34.24	4.38	37.50	6450	91.32
61	17.07.2014	29.47	7.32	30.42	6450	96.90
62	17.07.2014	28.34	4.60	32.04	6510	88.45
63	17.07.2014	43.46	6.67	31.71	6210	137.04
64	17.07.2014	50.15	14.35	35.61	6120	140.85
65	17.07.2014	40.51	3.17	38.73	6120	104.60
66	17.07.2014	29.71	5.34	32.31	6120	91.95
68	23.07.2014	31.97	2.77	36.63	6510	87.27
69	23.07.2014	42.23	6.54	33.89	6510	124.61
70	23.07.2014	23.72	8.14	37.29	6270	63.60
71	23.07.2014	34.30	2.09	34.72	6270	98.80
72	23.07.2014	30.21	4.55	34.14	6410	88.50
73	23.07.2014	42.91	6.42	36.17	6120	118.65
74	24.07.2014	29.29	2.01	36.00	6120	81.38
75	24.07.2014	30.06	5.78	34.11	6210	88.14
76	24.07.2014	20.48	12.11	33.09	6510	61.90
77	24.07.2014	40.64	6.55	34.08	6210	119.24
78	11.08.2014	48.39	14.62	33.64	6270	143.83
79	11.08.2014	31.82	5.67	30.13	6270	105.63
80	11.08.2014	25.65	9.81	35.05	6270	73.19
81	11.08.2014	30.92	2.24	24.61	6270	125.61

Sample No.	Date of collection	Average sugar yield, mg/g sample	Standard deviation*	Volatile Solids, %	Habitat No	Sugar, mg/g dry matter
82	11.08.2014	39.73	11.29	35.46	6410	112.05
83	11.08.2014	32.54	3.61	33.57	6410	96.92
84	11.08.2014	33.58	3.98	31.87	6410	105.36
85	11.08.2014	35.51	3.68	35.53	6410	99.95
90	15.08.2014	34.27	7.04	32.23	6270	106.34
93	15.08.2014	31.91	5.12	30.93	6270	103.18
94	15.08.2014	27.49	6.22	33.73	6270	81.50
95	15.08.2014	27.04	6.32	32.49	6270	83.23
96	15.08.2014	23.38	5.41	31.90	6270	73.29
98	15.08.2014	34.71	6.50	32.29	6270	107.51
99	15.08.2014	33.01	3.20	29.59	6270	111.54
104	22.08.2014	29.19	3.88	39.99	6210	73.00
105	22.08.2014	45.14	11.41	38.31	6210	117.83
106	22.08.2014	25.98	8.21	43.79	6210	59.32
107	22.08.2014	33.49	8.77	43.67	6210	76.70

* Standard deviation represents the average value from 6 separate measurements of randomly selected sample fractions from the specified biomass sample.

To further evaluate the available biomass resources, samples from the same habitats were collected also in June – September 2015 and the same methodology was used to determine the amount of sugar produced (Table 2.4).

Table 2.4: Amount of reducing sugars generated from various biomass resources collected from June till September 2015

Sample No.	Date of collection	Average sugar yield, mg/g sample	Standard deviation*	Volatile Solids, %	Habitat No	Sugar, mg/g dry matter
112	08.06.2015	54.00	4.76	23.95	6120	225.46
113	08.06.2015	45.58	5.89	25.83	6210	176.44
114	08.06.2015	44.78	4.65	30.74	6450	145.69
115	08.06.2015	41.66	6.20	26.21	6450	158.95
116	08.06.2015	43.83	4.84	26.92	6510	162.81
117	08.06.2015	46.20	2.12	33.08	6410	139.66
118	08.06.2015	53.06	8.89	31.12	6510	170.50
119	01.07.2015	51.71	8.30	35.64	6270	145.10
120	01.07.2015	42.10	5.35	27.43	6270	153.48
121	01.07.2015	42.63	9.90	31.37	6270	135.89
122	01.07.2015	32.26	6.79	27.22	6510	118.53
124	01.07.2015	32.59	1.87	37.32	6270	87.31
125	02.07.2015	46.21	7.20	27	6270	171.15
126	02.07.2015	49.60	6.00	31.39	6210	158.00
127	02.07.2015	46.65	14.59	32.74	6410	142.48
128	02.07.2015	30.67	10.00	34.53	6510	88.83
129	02.07.2015	36.35	7.37	33.33	6510	109.07
139	13.08.2015	38.19	2.87	35.96	6410	106.19
140	13.08.2015	32.01	4.10	34.48	6450	92.84
142	13.08.2015	33.35	3.65	37.24	6410	89.55
143	10.09.2015	31.70	2.81	43.35	6510	73.12

Sample No.	Date of collection	Average sugar yield, mg/g sample	Standard deviation*	Volatile Solids, %	Habitat No	Sugar, mg/g dry matter
144	10.09.2015	19.21	1.44	44.44	6450	43.22
145	10.09.2015	26.52	3.58	40.01	6510	66.30
146	10.09.2015	33.27	4.05	36.39	6410	91.42
147	10.09.2015	38.72	4.75	43.49	6410	89.04
148	10.09.2015	30.75	5.53	44.5	6450	69.10
150	10.09.2015	39.91	6.40	47.19	6120	84.58
151	10.09.2015	39.05	3.56	35.54	6270	109.87

* Standard deviation represents the average value from 6 separate measurements of randomly selected sample fractions from the specified biomass sample.

In 2015 vegetation period again significantly higher ($p < 0.05$) sugar yields were obtained from samples collected in June than in August or September. No significant sugar yield difference ($p > 0.05$) was observed in-between samples from August and September. The overall tendency of decrease in extractable sugar quantity was observed for all Habitats by the end of the vegetation period.

The highest sugar yields in 2015 attributed to Xeric sand calcareous grasslands (6120), but the lowest ones – as in previous year – in the samples of 6210 collected in August and September (Table 2.4). This contradicts to the results obtained in 2014 where 6210 were the most productive. However, it could be explained by the greater proportion of dicotyledon plants or dicots in samples from 6210 tested in 2014.

In 2016 analyses were performed only in June (Table 2.5) to determine if there is any trend in-between Habitats over the years.

Table 2.5: Amount of reducing sugars generated from various biomass resources collected in June 2016

Sample No.	Date of collection	Average sugar yield, mg/g sample	Standard deviation*	Volatile Solids, %	Habitat No	Sugar, mg/g dry matter
152	02.06.2016	42.57	8.91	21.53	6510	197.72
153	02.06.2016	55.86	10.51	26.82	6510	208.28
154	02.06.2016	53.26	4.84	26.74	6510	199.16
155	02.06.2016	46.88	2.76	28.76	6210	163.01
156	02.06.2016	60.06	14.01	20.72	6410	289.85
157	02.06.2016	51.47	6.24	25.84	6270	199.19
158	02.06.2016	56.56	10.19	27.89	6410	202.81
159	02.06.2016	36.98	7.84	30.71	6450	120.41
160	09.06.2016	54.03	6.19	26.79	6410	201.67
161	09.06.2016	60.49	11.19	33.25	6450	181.92
163	09.06.2016	52.72	8.36	28.71	6450	183.63
164	09.06.2016	100.17	8.39	32.26	6120	310.50
165	09.06.2016	72.00	11.72	27.18	6510	264.91
166	09.06.2016	63.05	7.96	29.14	6210	216.38
167	09.06.2016	74.44	11.21	27.34	6510	272.29
168	09.06.2016	44.53	13.80	28.51	6120	156.19
169	09.06.2016	64.81	6.98	27.71	6510	233.89
170	29.06.2016	42.89	9.67	21.14	6210	202.89
172	29.06.2016	42.24	11.32	27.41	6210	154.09
173	29.06.2016	47.16	9.31	27.75	6210	169.93
174	29.06.2016	46.32	8.13	28.34	6270	163.43
175	29.06.2016	45.82	6.22	26.33	6270	174.02

Sample No.	Date of collection	Average sugar yield, mg/g sample	Standard deviation*	Volatile Solids, %	Habitat No	Sugar, mg/g dry matter
176	29.06.2016	36.77	4.98	27.2	6270	135.19
177	29.06.2016	40.62	10.35	32.84	6270	123.68
178	29.06.2016	39.70	9.52	32.23	6270	123.18
179	29.06.2016	23.72	2.77	31.2	6270	76.04
180	29.06.2016	44.28	11.36	24.94	6270	177.53
181	29.06.2016	43.42	8.25	28.7	6270	151.28
182	29.06.2016	52.02	8.96	28.81	6510	180.55
183	29.06.2016	44.76	6.66	39.81	6510	112.42
184	29.06.2016	45.11	5.18	24.02	6510	187.79
185	29.06.2016	43.48	10.42	27.04	6510	160.79

In 2016 the highest sugar yields were also obtained from the habitats No. 6120. Moreover, it was observed that product yields decrease ($p < 0.05$) even within one month of vegetation period (samples collected between 02.06 to 09.06 and 29.06.2016).

The average amount of the reducing sugars highly varies not only seasonally, but also by years. For example the reducing sugars from the biomass harvested in June 2016 (a month with the most comprehensive data set) were 3% to 58% more than in those collected in June 2014 and June 2015 for all habitats except 6210 (Table 2.6).

Table 2.6: Amount of average reducing sugars generated from biomass resources according sampling times and habitats

EU habitat code	6120	6210	6270	6410	6450	6510
June, 2014	147.61	235.49	-	-	157.08	164.74
July, 2014	107.48	114.80	81.20	88.49	94.10	90.55
August, 2014	-	81.71	101.34	103.56	-	-
June, 2015	225.46	176.44	-	139.66	152.32	166.66
July, 2015	-	158.00	115.49	142.48	-	105.48
August, 2015	-	-	-	97.87	92.84	-
September, 2015	84.58	-	109.87	90.23	56.16	69.71
June, 2016	233.35	181.26	147.06	203.67	161.98	201.88

One of the reasons for such large differences within one habitat is its heterogeneity. The habitats listed in the annexes of EU Habitats Directive are not classified in a single hierarchical system. It includes habitats separated by the phytosociological classification of plant communities as well as habitat groups that include several similar habitats divided by specific environmental conditions. For example, the habitat *Molinia* meadows on calcareous, peaty or clayey-silt-laden soils 6410 includes *Molinion* grasslands, grasslands where low height sedge species like *Carex flacca*, *Carex hartmanii*, *Carex hostiana*, *Carex panicea*, *Carex buxbaumii* dominate as well as grasslands without pronouncedly dominant species with many dicotyledonous plant species if all of them are with high species diversity and located in periodically drying soils. That explains the fact that sugar yields in samples from 6410 collected in August 2014 and July 2015 were higher than in samples collected in July 2014 and June 2015 as at July 2014 and June 2015 sampling were done in sedge grasslands while in August 2014 and July 2015 – in *Molinia* grasslands and 6410 grasslands without pronouncedly dominant species.

Besides, Due to diverse environmental conditions, species composition and vegetation structure can be very diverse within one grassland plot, and even in small areas several plant communities that still belongs to one habitat subtype can be found. It could explain the fact that the sugar yields in samples from 6270 collected in August 2014 are higher than in samples from the same habitat collected in July 2014, but to give the precise explanations of these variations, a more sophisticated analysis of species compositions in tested samples is needed.

2.3. Conclusions

- To effectively destroy lignin structures and provide accessibility to cellulose and hemicelluloses, biomass mechanical grinding must be performed. Moreover, higher sugar release yields were obtained in samples with lower mesh size.

- 5 minutes of sample boiling is enough to neutralize natural microorganisms prior enzymatic hydrolysis where the highest sugar yields were obtained after 24 hour incubation at 30°C.

- Enzymatic hydrolysis showed to be as effective as acid hydrolysis, which was closely related to acid concentration and treatment conditions used. The highest sugar yields were obtained in samples treated with 3% H_2SO_4 for 60 minutes at 170°C.

- Analyses of biomass samples showed that generally higher sugar yields are obtained for samples collected in June and originating from Xeric sand calcareous grasslands (6120), Semi-natural dry grasslands and scrubland facies on calcareous substrates (6210) and Lowland Hay meadows (6510) – the habitats with a greater proportion of dicotyledon plants or dicots.

3. Use of biomass for production of biogas

Technologies for production of biogas and the main biochemical parameters of substrate

Technological solutions for production of biogas depend on available substrates. Main parameters for substrates are biomethane (biogas) potential, total solids (TS), and the biodegradation time of substrate in reactor which is being reflected by the hydraulic retention time (HRT).

Silage obtained from maize is the most widely used substrate for production of biogas. Its biogas potential is up to 200 Nm^3/t of natural sample at TS 35-38%. The biogas potential of liquid cow manure is up to 24 Nm^3/t of natural sample at TS 8-9%. The biogas potential of silage obtained from perennial grasslands is up to 128 Nm^3/t of natural sample at TS 25%. The biogas potential of natural grasslands (including grasslands habitats) is up to 98 Nm^3/t of natural sample at TS 35%[30].

In typical biogas stations located at rural area, manure and energy plants are the main substrates for biogas production, TS in reactor is within the range between 9-15%. HRT is within the range from 60 to 90 days. Furthermore, maize silage and silage obtained from natural grasslands have approximately the same period of biodegradability. This means that using the same amount of biomass and keeping it for the same time in bioreactor, totally different results in amount of biogas will be produced. Or, in order to obtain the same amount of biogas from maize silage and from silage obtained from natural grasslands, with the same rate of biodegradation of both substrates, reactors of different volume are needed. Indeed, when grass substrate from natural grasslands is used, two times larger reactor is needed compared to one for maize silage. Here the question of payback raises and even more the question of profitability of the whole biogas production process. Currently grass biomass is rarely used as the only substrate for biogas production (some plants in Germany, none in Latvia). For fermentation of grass biomass either liquid fermentation in continuous-flow stirred-tank reactors (CSTR type reactors), with total solids of 9-15%, or dry fermentation, where TS 30-35%. Interesting technological solutions are used for reutilisation of digestate[i] for production of biogas. Digestate comprises the unused potential of biogas production, biomass of micro-organisms, as well as macro and micro elements necessary for anaerobic fermentation (AF) processes. There is a technological solution of separation of digestate: the liquid phase is directed into a lagoon for sedimentation and further disperses on fields, while the solid phase (TS ~28%) is partly reverted directly into the fermentation process. The major part is dried and used for litter in cow-sheds and further together with liquid manure again directed to biogas reactor.

Other technologies intend the reversion of the liquid phase to the fermentation process thus keeping the required level of TS in reactor (up to 9%). There are other technologies requiring pre-treatment of substrate i.e. aerobic hydrolysis. Here the fresh substrate is mixed with digestate, aerated and exposed to hydrolytic, acidogenic micro-organisms. As the result, hydrolyses and acidogenesis are ensured to a large extent already before directing the substrate into the biogas reactor. As a whole this method ensures a quicker biological processing of substrate.

3.1. Materials and methods, results and discussion

3.1.1. Justification of the choice of technological solution for biogas production

The objectives of the GRASSSERVICE project envisage development of a new bioreactor. Development of technological solutions comprises the following tasks and goals to be achieved:
- A competitive technological solution in terms of energy consumption for self-consumption needs;
- High concentration of methane in biogas;
- Synergy with technology of biobutanol production;
- Balancing the amount of digestate with the required amount of organic fertilizer for habitat maintenance needs.

In the current market of biogas technologies, self-consumption of electricity in a range from 4-5% of produced electricity is regarded as a good indication. Substrate mixers and substrate pumps are the main consumers of electricity in a biogas plant. Besides, there is a coherent ratio between the optimal size of reactor and the size of installed mixer when the electricity consumption of these processes is the lowest.

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[i]Digestate - material remaining after the anaerobic digestion of a biodegradable feedstock

Increasing or decreasing the size of the bioreactor, the electricity consumption increases. In small biogas stations (electric capacity Q_{el} up to 50 KW) electricity consumption for own operational needs is high. It is between 6 to 10 % of produced electricity. When developing a bioreactor of a new type, the goal is preferably not to exceed 4% level of electricity self-consumption at installed Q_{el} up to 50 KW.

The framework scheme of a biogas reactor (Figure 3.1.) shows that the substrate mixing is ensured by movement of the same substrate within the subsections of a reactor (2, 3) caused by an increase in biogas pressure above the substrate when valves (4, 5) are closed. In addition, blending with low capacity gas pump takes place in subsections (according to AIR LIFT principle). At a certain biogas pressure, a valve No. 4 or 5 opens, biogas is emitted from the bioreactor and the level of substrate (according to the principle of communicating vessels) restores in subsections. In the main section of bioreactor (1) mixing takes place only due to substrate flows generated by the movement of substrate to and from subsections.
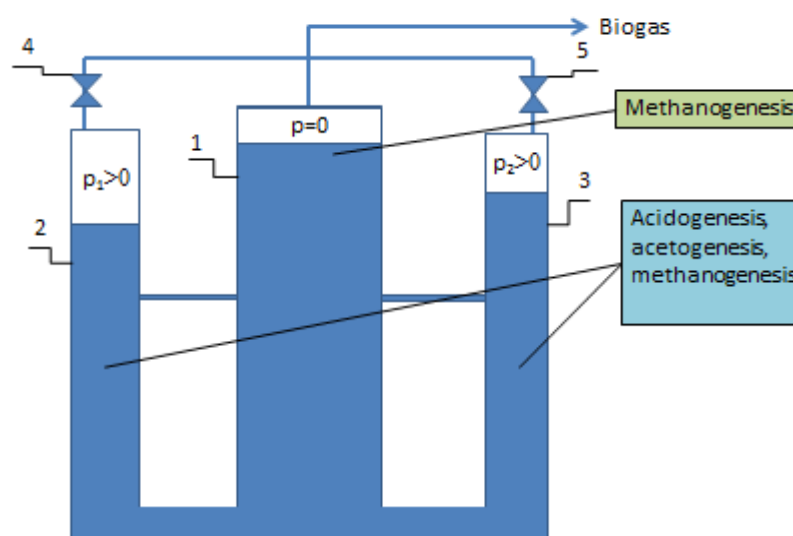

Figure 3.1: Framework scheme of pilot installation of a biogas reactor. 1 – main section of bioreactor; 2, 3 – subsections of bioreactor, 4, 5 – valves

Pressure developed by released biogas above the substrate in combination with mixing of gas (*AIR LIFT*) ensures that part of carbon dioxide (CO_2) - an undesired component of biogas dilutes in the substrate (water). At the same time in a presence of hydrogen methanogenic bacteria utilise CO_2 for synthesis of methane (CH_4). The process can be described by the following formula of chemical reaction:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O.$$
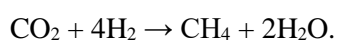
Acidogenesis, acetogenesis and to a lesser extent methanogenesis take place in sub-sections of bioreactor. The volume ratio in the main section and in subsections is essential. Methanogenic phase is a limiting factor for synthesis of biomethane in AF processes. Methanogenic phase can be about 4 times longer than acidogenic phase. This aspect is taken into account when determining the size of main section and subsections. Also hydrogen (H_2) is one of the final products of microbiological process (acidogenesis) taking place in subsections. This means that there are preconditions for the synthesis of methane from CO_2. Theoretically for methane formation of about 70% of acetates and only around 30% of H_2 and CO_2 are consumed, but microbiological process in reactor can adapt depending on the substrates available in a long term. Basically, each biogas reactor will develop its own bacterial consortium, which ensures the formation of methane. Proposed design of bioreactor stimulates the consumption of CO_2 for the formation of methane, thus increasing the total concentration of methane in biogas.

The process of hydrolysis is common for technologies of production of biogas and biobutanol. Simple sugars (carbohydrates) obtained at the beginning of hydrolyses are required for production of biobutanol. Next sequential step of hydrolysis is acidogenesis. Both simple carbohydrates and organic acids can serve as raw materials for biogas production. This circumstance serves as a basis for the potential technological synergies for production of biobutanol and biogas.

In addition, biomass of bacteria is generated during the production process of biobutanol. Bacteria has to be periodically removed from the processes. After certain treatment these bacteria can potentially serve as a substrate for production of biogas.

The assessment of the above mentioned synergies is one of the goals of the GRASSSERVICE project Activity 2.

3.1.2. Substantiation of selected substrates for production of biogas from grass biomass obtained in grassland habitats

The main prerequisite for selection of substrate for biogas production is its availability all the year around. The framework scheme presented in Figure 3.2 justifies the necessity to prepare both hay and silage from the grass obtained in grassland habitats in order to ensure supplies of substrate all year round for biogas production.
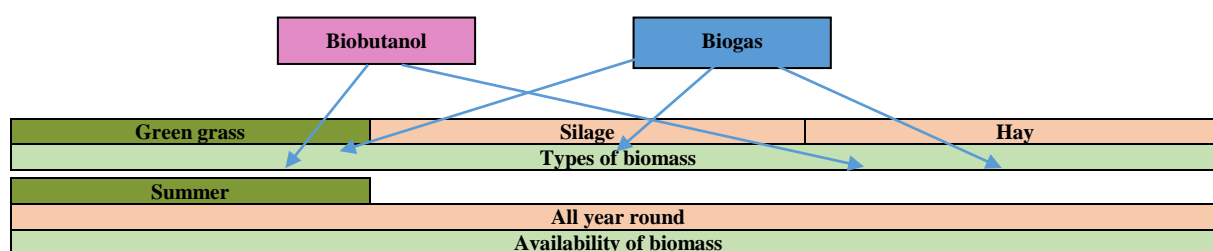

Figure 3.2: Possible utilisation of different types of grass biomass for production of biogas and biobutanol

As presented in the scheme, the competition on raw material for production of biogas and biobutanol arises mainly in summer. Fresh green grass biomass is the best substrate for production of biobutanol.

By the end of the A2 action, the optimum share of different types of grass biomass will be defined to ensure the most efficient solution for biogas production from grass biomass all the year round, taking into account the C:N:P:S ratio, biogas potential and biodegradation period.

3.1.3. Laboratory testing of biochemical parameters of grass biomass

Assessment of biochemical parameters of a substrate is the first step towards choosing the most appropriate technological solution. Biochemical parameters of grass biomass collected from grassland habitats include total solid (TS), volatile solid (VS), biogas potential, C:N:P:S ratio, content of macro elements (N;P; K;S).

Another important step is preparation of the average sample of grass biomass for further investigation. When the necessary parameters of the average sample are measured, possible deviation caused by differences of grass collected in different habitats is estimated. Possible deviation within the range of ±10% is considered as negligible. Data on total solids in grass biomass samples collected in grassland habitats in 2014 are obtained. Hay and silage samples from grass biomass samples collected in 2014 are prepared in the laboratory.

Currently a laboratory investigation is being prepared to evaluate the biogas potential of all 3 types of grass biomass – green grass (collected in 2015), silage (prepared in 2014), and hay (prepared in 2014) in anaerobic fermentation system simultaneously. Beforehand, it is planned to analyse the C:N:P:S ratio and the content of macro elements (N;P; K;S) in all the above mentioned substrates.

3.1.4. Laboratory testing of physical parameters of grass biomass

Conditions of mechanical grinding (milling)

Acceleration of the biodegradation process of biomass by mechanical grinding is possible by increasing the biomass surface area. For example, if cutting a piece of straw (10 cm long, 4 mm in diameter and wall thickness 0.7 mm) into 5 pieces (2 cm each), the surface accessible to microorganisms is increased by cross-sectional area of eight cuts being comparatively small increase in comparison to the size of straw surface. It is important to achieve the division of the smallest parameter of straw (in this case - wall thickness of straw). This means that in order to accelerate the biodegradation process by mechanical grinding, the particle size of chopped straw should be below 0.7mm. Of course, a positive effect is also achieved by shredding of a piece of straw in a length of 10 cm to 2-3cm. Accordingly the mechanical mixing power is reduced, load on substrate pumps is also decreased. This project gives an opportunity to assess different samples of chopped grass obtained in various grassland habitats, as grinding of hay in hammer mills for production of grass pellets is planned. Usage of the finest grinded fraction of hay (< 0.5 mm) for production of biogas could be tested. Depending on sieve of hammer mill, this fraction can comprise 10-20% of the total amount of grinded hay. It has to be assessed:
- whether separation of the finest fraction does not impair the mechanical properties of grass pellets (lignin content);
- what will be the increase of milling costs to obtain bigger share of fine fraction of hay.

Minimizing adverse effects of floating layer in reactors

Formation of a floating layer inside the reactor (digester) excludes from the cycle part of nutrients necessary for microorganisms, hinders evaluation of microbiological processes and can lead to emergency situation in the reactor. Floating layer typically is composed by substrates with higher fibber content. Freshly cut green grass has the lowest potential of floating layer formation. In general, grass silage has a medium potential, but hay has the highest potential of formation of floating layer.

Typically to prevent formation of floating layer in biogas plants, the mixing intensity and the position of mixers (in some technological solutions) is changed, often leading to increased electricity consumption during the mixing process.

Pre-treatment of biomass e.g., grinding of hay, is another preventive action to be considered. So far sedimentation test of various biomass samples has been carried out. The methodology is as follows: the biomass sample (5-10g) is placed in the cylinder, 150 ml saline (0.9% sodium chloride solution in water) is added. At the beginning of the experiment almost all amount (~ 97%) of the sample has emerged. Every day, the content of cylinder is shaked and after the settling the floating and sediment layer is recorded. Figure 3.3 presents images reflecting the sedimentation test with samples of grinded fraction of hay (0.05-1.0 mm) being prepared from grass obtained in grassland habitats in July, 2014. Test results are reflected in Table 3.1.
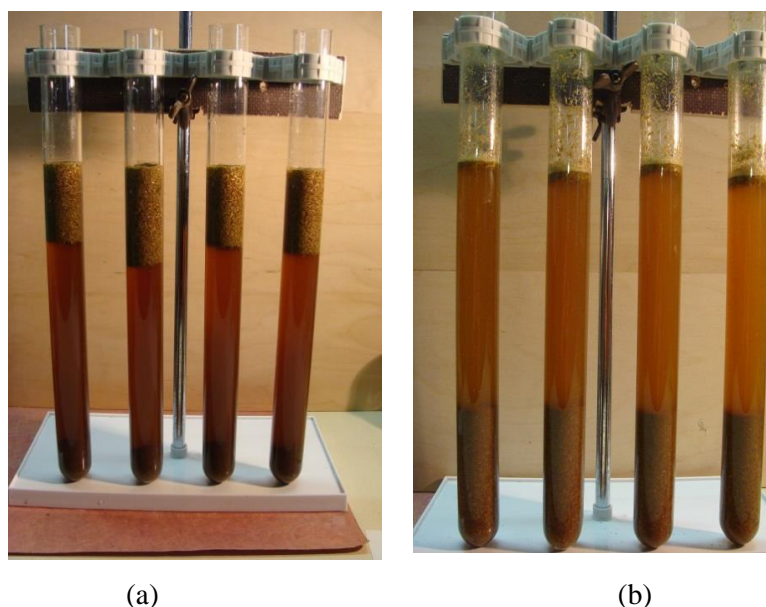

(a)   (b)

Figure 3.3: Sedimentation test of grinded hay: (a) at first day (beginning of experiment), (b) at the 5th day (end of experiment)

Table 3.1: Results of sedimentation test with samples of grinded hay

Grinded hay 0.05- 1.0 mm	Days				
	1	2	3	4	5
Floating layer [%]	97	72	68	20	8
Sedimented layer [%]	3	28	32	80	92

Similar tests have been conducted with different fractions of hay prepared from grass obtained in grassland habitats: 3-5 mm, 2- 3 mm, 1-2 mm. Fractionation was carried out with the help of laboratory sieves. Sedimentation rate of biomass is proportional to the particle size of the ground hay. The finer is the grind, the faster is the sedimentation process. Numerical values of sedimentation speed are important.

90% of the ground hay with the particle size of 3-5 mm has settled in 15-17 days, while 90% of the ground hay with the particle size of 0.05-1.0 mm has settled in four days. Thus it can be concluded that the floating layer in the reactor can be reduced by grinding of biomass. The particle size should be less than 1 mm. This refers in case of hay. It is more complicated to grind fresh grass and silage to a size below 1 mm, although this is not as crucial as fresh grass and silage biomass form the floating layer in reactor to a lesser extent. Knowledge about the structure of floating layer allows selecting the optimum solutions for substrate mix.

A positive aspect of a floating layer is its capacity of immobilization of bacteria thus being used in some technological processes.

Assessment of impacts of thermal treatment of biomass (steam explosion effect)

Steam explosion technology is well known and advanced technology. It is used for degradation of biomass fibre structures. The technology comprises placing a sample of biomass (straw, hay) into the reactor followed by injection of saturated water vapour. The temperature is raised up to 145-190°C, the pressure increases to 4.5-12 bar, the sample is kept at this temperature for 5-10minutes. Sample structure becomes saturated with water molecules. Then reactor is opened rapidly and the pressure explosively decreases; water vapour dilates with the structure of a biomass sample and thus the sample is destroyed principally from inside. Cellular structure of biomass is disrupted thus increasing the degree of biodegradability. The limits of the treatment are set by energy consumption and appearance of undesirable by-products. During the degradation process of lignin phenols are generated which thus possibly inhibiting production of biogas in anaerobic fermentation process. Within the GRASSSERVICE project the BI-H$_2$ installation has been used. It has been supplied with a tailor-made equipment ensuring collection of biomass fibre structures and also partial collection of volatile substances originated during the explosion process.

Experiments are carried out in order to assess the benefits of application of steam explosion method within reducing (CO$_2$) environment. The methodology is as follows: a sample of grass biomass (hay 10-40g) is placed in the reactor, water (200-450 g) is added. Before heating starts, a gas (CO$_2$) is blown into the reactor for 10 minutes. The reactor is closed and temperature rise till 145-165°C is started. Simultaneous vacuuming of expansion column to p = -0.8 bar is started. Biomass sample is kept at certain temperature for up to 10 min. Then the reactor is opened rapidly and biomass together with water vapour fills the expansion column. After cooling of the reactor, biomass sample is collected from the reactor. Currently, chemical oxygen demand (COD) is measured in the liquid phase to characterise the degree of destruction of biomass structure. Results of a few series of experiments are shown in Table 3.2.

Table 3.2: Results of experimental series of steam explosion tests

Sample No	t [ºC]	p [bar]	time [min]	COD [mg/l]
TvSp12	145	4.0	10	11450
TvSp23	150	4.7	10	12100
TvSp26	150	4.7	10	12050
TvSp32	155	5.5	10	13460
TvSp34	155	5.5	10	13790
TvSp43	160	6	10	15910
TvSp44	160	6	10	16130
TvSp51	165	6.8	10	17800

Based on the experiments carried out so far final conclusions may not be yet drawn on optimal technological regimes. Technological boundaries for application of this method in small-scale biogas plant are related to the availability of steam corresponding to certain parameters. Steam can be produced in a heat exchanger of a gas operating cogeneration plant if installed in the exhaust stream of the engine. Small capacity engines can produce only a small amount of steam. If biogas is used for reaching the required parameters of steam, then cost-effectiveness of this technology can certainly be low. Therefore it would be necessary to apply the steam at the lowest possible temperature and accordingly the lowest pressure.

Benefits of steam explosion will be fully evaluated after performing the laboratory tests with treated and untreated biomass in batch anaerobic fermentation reactors.

Analysis of experimental data makes it possible to evaluate whether this technology can be incorporated into technological process of large-scale biogas production facilities. Full range of calculations, including the economic cost effectiveness estimations will be carried out. Based on calculation results it will be possible to define the size of the biogas production facilities when this technology would be profitable. Technology manufacturers will be interviewed in order to get basic information of feasibility of technical solutions and the respective costs. Already now there have been identified some limiting factors, for example, for a biogas pilot plant with a cogeneration unit(CHP) of size of 2-4 KW$_{el}$, steam explosion technology cannot be applied due to shortage of steam corresponding to certain parameters.

3.1.5. Analysis of organic loading rate (OLR), hydraulic retention time (HRT) and biogas yield of grass biomass using bioreactor BR 100

The aim of using continuous flow stirred-tank reactors is to gain necessary information about basic biogas production parameters necessary for development a biogas pilot plant for utilisation of grass biomass (fresh cut grass, silage, hay) obtained in grassland habitats (Activity C2).

Substrate for laboratory tests in CSTR mode was made as an average sample from the biomass samples collected in grassland habitats located in project pilot areas.

During experiments biogas production of about 70 m^3 per 1 ton of green grass was achieved with concentration of methane <52% at the organic loading rate 2.8-3.0 kgVS/m^{3}V*day. At higher organic loading rates the concentration of methane decreased below 50% and concentration of volatile organic acids in the substrate increased above 5000 mg/l. HRT has been in the range from 45 to 55 days. These results were obtained without supplementary micro elements for optimisation of AF process and continuously (over 20 months) feeding reactors with grass biomass in different regiments.

Several technical solutions have been evaluated for pre-treatment of biomass prior to feeding to the reactor. Laboratory testing of optimal proportions of substrate (fresh grass: hay: silage) for production of biogas during the continuous operation of the biogas plant all-year-round is still ongoing. Results show that presence of green grass has a very high influence on stability of biogas production in anaerobic fermentation process. Accordingly green grass gives the best indication on maximum biogas yield and admissible organic loading rates. When green grass is used in full scale biogas plants in the first half of summer, higher sensitivity of AF processes is also observed.

These results provide base for elaboration of technological solutions for biogas pilot facility. According to experimental results it seems that anaerobic fermentation process will have to be optimised by applying supplements of micro elements, preferably in AF reactor having low level of VS (up to 4%). This can be achieved by physically separating phases of the AF process and creating a separate technology for hydrolysis. Separation of hydrolytic phase would be optimal, because it would allow simultaneous production of biogas and biobutanol as both processes utilise products derived from hydrolysis. In such way hydrolysis phase of anaerobic fermentation will serve as synergy for biogas and biobutanol production.

3.1.6. Assessment of potential of biogas production from digestate

Digestate is often considered as waste product of anaerobic fermentation and consists mainly of bacteria and biomass residue. Biomass residues contain mainly fibrous substances - cellulose, hemicellulose, and lignin. Depending on the technological regime of biogas production - organic loading rate (OLR), the hydraulic retention time (HRT), structure of substrate and movement of the substrate in the reactor, digestate may also contain fraction of easily degradable structures (polysaccharides, fats etc.).

Laboratory tests were performed with the aim to determine the biogas potential of solid fraction of digestate. Separation of digestate was performed. Another task was to evaluate the effect of low-temperature heat treatment on the biogas potential and its production dynamics. Organic loading rate was 3.2-3.5 kgVS/m^{3}V*day. For laboratory testing to 1kg of digestate 4 kg of water was added. One part of obtained sample 24 hours was exposed to temperature of 70°C. Each batch reactor was filled in with 500 g of treated or not treated digestate and 300 g of seed material. Laboratory tests of anaerobic fermentation were performed under mesophilic regime (t$_m$=+37.5+0.5°C). The duration of AF test was 2246 hours (94 days). The graph of cumulative biogas production is presented in Figure 3.4.
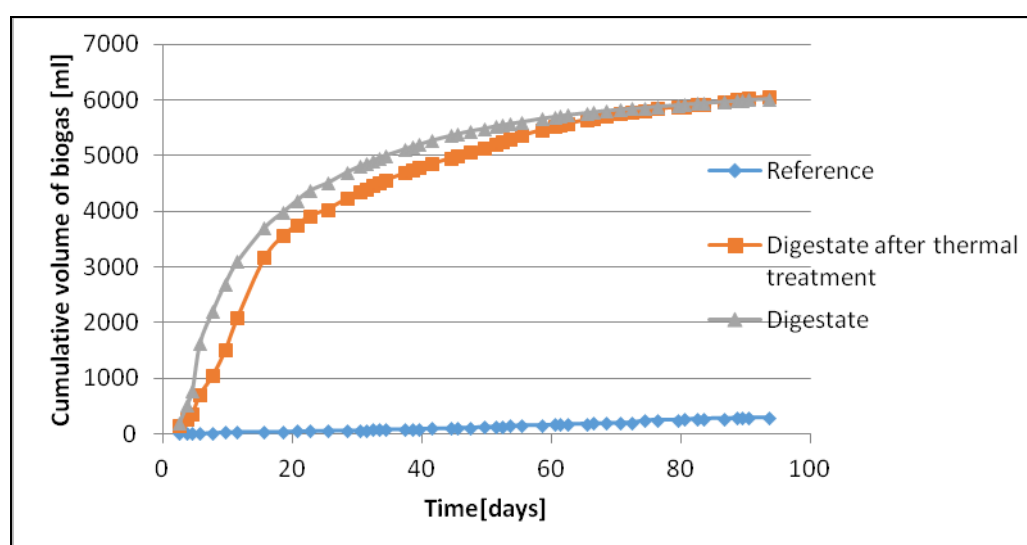
Figure 3.4: Production of biogas from thermally treated and untreated digestate

Thermally not treated digestate had the highest speed of biogas production - 724 ml/day achieved at the sixth day of anaerobic fermentation. Highest biogas production speed of thermally treated digestate was 300 ml/day (2 peaks were observed at around day 6 and 12 of anaerobic fermentation). The biogas production speed is presented in Figure 3.5 (scaled).
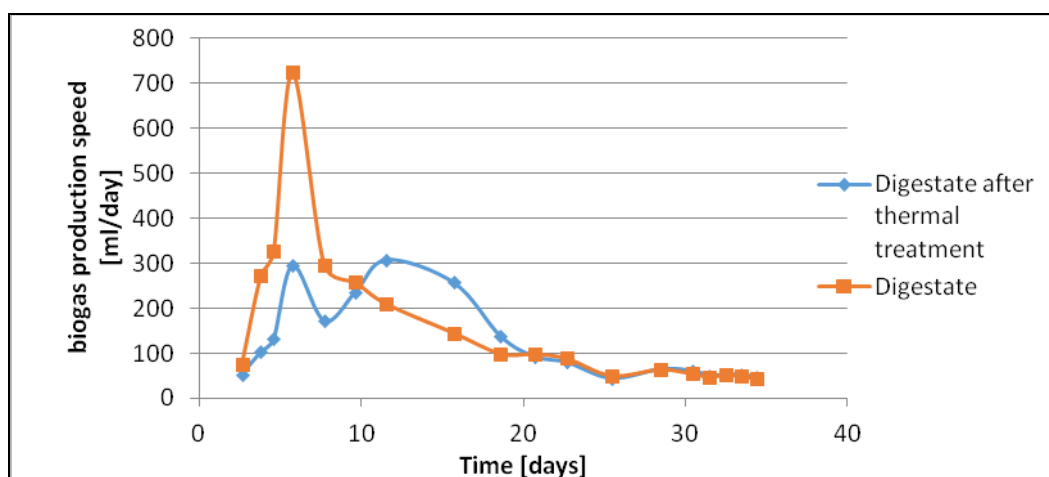
Figure 3.5: Speed of biogas production during 40 days of anaerobic fermentation

Test results and calculation of biogas production is presented in Table 3.3.

Table 3.3: Test results and calculation of biogas production

Reactor	Substrate (g)	Biogas (ml)	Biogas (m^3/t)	Degrad. of solids (%)	Methane conc. (%)	Biogas 80% (ml)	Time period for 80% of biogas (days)**
Thermally treated digestate	800	6065.33	60.7	35.6	55.3	4852	42
Digestate without thermal treatment	800	6023.33	60.2	36.4	53.3	4819	30
Reference	700	298	0.4	20.0	51.8		

*** For optimisation of AF processes, time period when 80% of total biogas volume has been obtained is often evaluated*

3.1.7. Assessment of structural changes in lignocellulose of digestate after ozone treatment

Cellulosic and lignocellulosic structures degrade slowly in anaerobic fermentation (AF) process. Degradation process can be accelerated by either pre-treatment of biomass or by activating hydrolysis. Lignin (the third main component of fibrous materials) degrades very slowly in AF process. Moreover, phenolic compounds, inhibiting the AF process are formed. Concentration of phenol derivatives (phenols) in digestate increases in continuously operating biogas reactors at long-lasting AF process (4-6 months) when substrates with high fibre proportion (straw, hay) are used as raw materials. Phenols in water solutions react very well with ozone. When low concentration of phenols in water is treated with ozone multiple organic acids (formic acid, etc.) are formed. The acids do not participate in further reaction with ozone but in the same time they decrease the pH level thus limiting the activity of ozone. Already after one cycle of ozone treatment phenolic rings can be broken and toxicity of compounds decreases.

Ozone treatment of digestate has several goals:
- To disrupt phenol compounds in the liquid phase of digestate, thus increasing the potential of biogas production at the recycling of AF processes;
- To disrupt the structure of lignin in solid phase of digestate, thus increasing the potential of biogas production at the recycling of AF processes;
- To disrupt cell structure of microorganisms present in digestate, thus increasing the potential of biogas production at the recycling of AF processes.

The methodology for ozone treatment of digestate is the following: As a testing sample a liquid part of filtered digestate (particle size < 0.5mm) obtained from biogas reactors BR 100 of continuous operation (4-6 month, utilising fibre rich substances – straw, hay) has been used. Sample was placed in the flow ozonation unit OZ 3000 (Figure 3.6), where treatment with ozone was performed.

Figure 3.6: Researchers of AB2 BioRE working with the flow ozonation unit OZ 3000

Technological parameters (variables) are: duration of treatment, intensity of the ozone flow, concentration of alkali metal ions in sample substance. Parameters analysed are: speed of increase of oxidation / reduction potential (ORP) expressed in mV/min, that characterises the dilution speed of ozone into the model substance and the respective dynamics of chemical reaction; the absolute value of ORP expressed in mV, being achieved within the duration of the experiment and characterising the overall balance of oxidation / reduction after treatment with ozone; COD in test substance filtered through filter (0.2 µm) that characterises the level of chemical degradation. Figures 3.7 and 3.8 show the dynamics of ORP speed and the absolute value of ORP during the experiment ($O_zD4/1$).
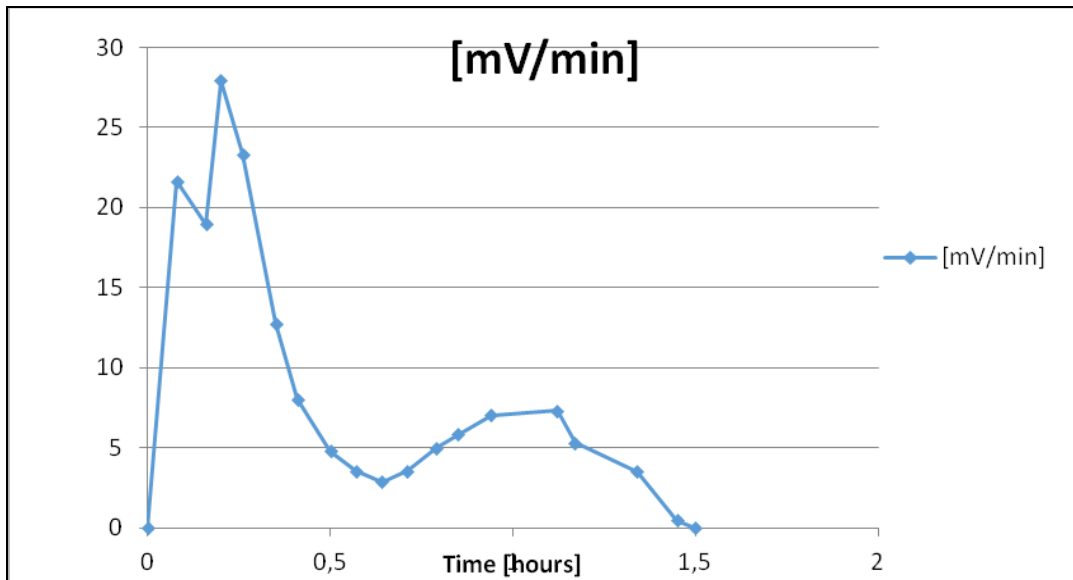

Figure 3.7: Dynamics of ORP within the laboratory experiment No. OzD4/1
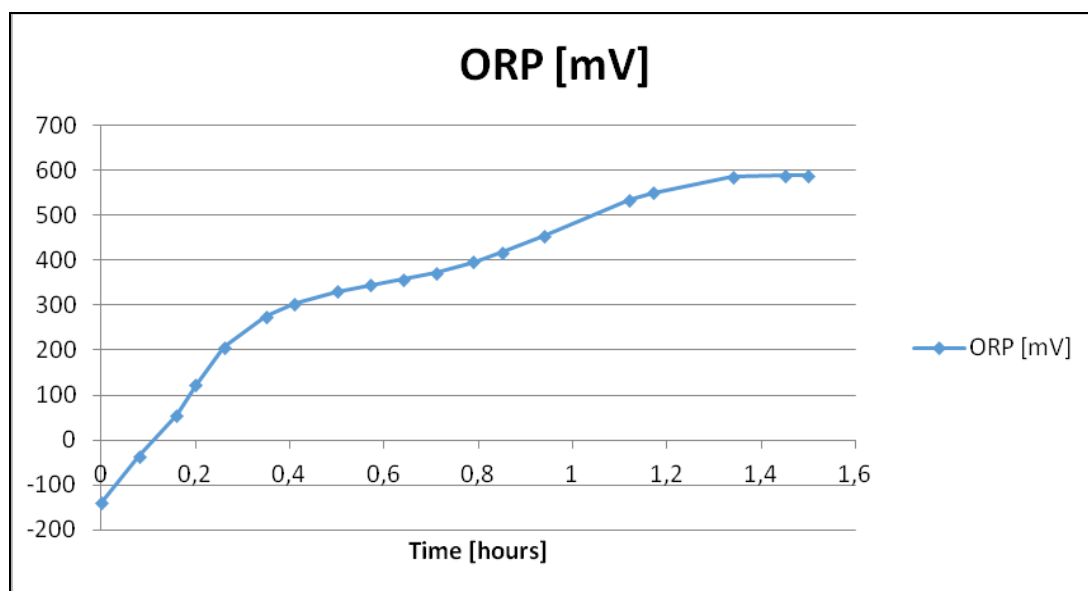
Figure 3.8: Dynamics of total ORP within the experiment No. OzD4/1

So far the highest result achieved in the dynamics of ORP (without adding an external source of hydroxyl group (-OH) is 800 mV, the COD difference in the filtered test substance is in a range of 25-35%. ORP is valuable process indicator allowing to follow the process treatment. COD is a parameter allowing to compare obtained results with the results of other investigations. It can also be used for extrapolation of the potential biogas yield.

The results obtained so far serves as base data for further development of technological solutions. In opposite to steam explosion technology, where availability of steam is a limiting factors in small scale biogas plant, ozone treatment has several advantages e.g., low energy consumption.

After these tests, the digestate treated with ozone is planned to be fed into the AF processes in single load reactors to assess the biogas potential and the dynamics of production of biogas. The results will be compared with the results of untreated digestate. Based on the results obtained and evaluation of cost effectiveness, final conclusions will be drawn on application of this technology for treatment of digestate with ozone.

3.2. Conclusions

- Based on laboratory tests a framework scheme of pilot installation of a biogas reactor has been developed.

- Hydrolyses of biomass is a synergy stage of production of biogas and biobutanol.

- Treatment of biomass with steam explosion ensures essential disintegration of fibrous structures. COD in the liquid phase of a test substance increased up to 17800 mg/l.

- Ozone treatment of digestate showed increase of COD in the liquid phase for 25-35%.

- During the analyses of the reactor of continuous operation, when habitat grass was used as the feeding substrate, it was found out that micronutrient sufficiency play a much more significant role in efficient biogas production compared to the C: N: P: S ratio. Therefore, additives of microelements are advised to be used during biogas production.

- When feeding the reactor with fresh grass mown at the beginning of the vegetation season, there is a risk of increase in $NH4^+$ and following inhibition of AF processes. Accordingly, special technological adjustments must be applied to control the processes.

4. Use of the digestate in grassland management

Considering the low productivity of many semi-natural types of grassland, there is an interest if an application of digestate supports productivity without compromising biodiversity. To answer the question, a study was conducted, which aimed to investigate the effect of grassland management and applications of digestate on above ground biomass yield and biodiversity.

4.1. Material and methods

To analyse the digestate impact on diversity and productivity of semi-natural grasslands, six study sites were chosen in Sigulda and Ludza Municipality (4 sites in Sigulda and 2 sites in Ludza). The sites were selected to represent the typical vegetation of the most common semi-natural grassland types in the project sites – Sigulda and Ludza Municipality – that correspond to Community Importance habitats 6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates, 6270 Fennoscandian lowland species-rich dry to mesic grasslands and 6510 Lowland hay meadows. Skujas, Griezes and Aizupes represent *Cynosurion* grasslands where *Festuca rubra* and *Agrostos tenuis* or *Festuca rubra* and *Briza media* dominate; Ludza and Vecslabada – *Festuco-Brometea* grasslands where *Poa angustifolia*, *Centa scabiosa* and *Agrimonia eupatoria* dominate; but Laurenči – *Arrhenatherion* grasslands with *Festuca pratensis, Phleum pratensis* and *Dactylorhiza glomerata* (Table 4.1).

Most of the sites (4) were mulched up to 5 years before 2014. Others – unmanaged or irregularly grazed (Table 4.1).

Before the start of the study, the sites were mowed and harvested once per season at August 2014. The mean dry matter yield of forage ranged from 2.5 to 6.3 t/ha (Table 4.1).

Table 4.1: Description of experimental sites

Experimental site	EU habitat code	Dominant species	Previous management	Dry matter, t/ha	Yield assessment time
Aizupes	6270*-1	*Festuca rubra, Agrostis tenuis, Briza medi*	Mulching	3.1	10.08.2014
Skujas	6270*-1	*Festuca rubra, Agrostis tenuis, Briza media*	Mulching	3.1	14.08.2014
Griezes	6270*-2	*Festuca rubra, Agrostis tenuis*	Mulching	3.0	15.08.2014
Laurenči	6510-1	*Festuca pratensis, Phleum pratsense, Dactylorhiza glomerata*	Mulching	4.5 6.3	27.06.2014 08.08.2014
Ludza	6210-2	*Poa angustifolia, Centa scabiosa, Agrimonia eupatoria*	Irregularly extensively grazed	2.5	21.08.2014
Vecslabada	6210-2	*Poa angustifolia, Centa scabiosa, Agrimonia eupatoria*	Unmanaged	2.5	25.08.2014

In each site, 10 soil samples were taken at the beginning of April 2015 at a depth of 0–20 cm. All samples from each site were combined to get one representative sample for basic soil chemical analysis. The analysis was performed by the methods approved by the rules for agrochemical soil research in Latvia (Ministry of Agriculture Arrangement No.21 from 29 August 2014 "Procedure for assessment of agrochemical research of soils"). The data on soil parameters are described in Table 4.2.

Table 4.2: Soil descriptions of experimental sites

Experimental site	Soil type	pH KCL	Organic matter, %	K_2O	P_2O_5
Aizupes	Sandy loam	5.6	3.2	49	3
Skujas	Loamy soil	5.7	2.7	64	2
Griezes	Loamy soil	4.5	2.5	41	2
Laurenči	Sandy loam	6.1	2.2	71	12
Ludza	Sandy loam	7.4	2.6	178	47
Vecslabada	Loamy soil	6.9	3.6	130	83

In each site, four permanently marked management plots of 250-400 m² were established. Two of them were mowed and harvested, but two – mowed, harvested and fertilized. Within the plots that were selected for fertilisation, 10x10 m squares were marked for digestate application. These squares were placed in the middle of the marked management plot to avoid the other influences.

Fertilization took place in May 2015 and April 2016 (Table 4.3) with a dry fraction of digestate from a biogas plant (supplied with cattle slurry and maize silage) with 35% DM content and a content of N, P_2O_5 and K_2O of 7.6 kg/t, 5.9 kg/t and 14.0 kg/t DM, respectively. The amount of N applied on the plots ranged between 10 and 33 kg/ha/y and was calculated to match 50% of the N exported from each plot by harvested biomass in 2014. These amounts were applied, as mass flows for N of about 50% into the digestate have been reported by Wachendorf et al.[31] (2009) during biogas processing of semi-natural grasslands. The amount of P_2O_5 and K_2O applied ranged between 8 and 26 kg/ha/y for P_2O_5 and 18 and 62 kg/ha/y for K_2O (Table 4.4).

Nutrient removal through harvesting of the biomass was calculated from the data on biomass yield in each management plot and the average concentration of constituents in the biomass of semi-natural grasslands indicated in the Report for Forage Analysis in Latvia prepared by Latvian Rural Advisory and Training Centre[32] (Siliņa et. al. 2013).

Determination of the (above ground) biomass yield was carried out in August 2014 by cutting one 1x1 m square in the most characteristic place of each management plot. The methodology for determination of the above ground biomass is described in Chapter 5. The data for yield assessment repeatedly were collected during 1st or 2nd July 2015 and on 29th June 2016 (Table 4.7). The number of samples per sampling time ranges from one to three from each management plot depending on habitat heterogeneity.

Fertilization was carried out by hand, applying digestate evenly across the plot. All plots were mowed once per season in July and August (Table 4.3). Harvested material was collected and removed from the site with hands.

Table 4.3: Digestate application and harvesting time

Experimental site	Application of digestate		Mowing and harvesting		
	2015	2016	2014	2015	2016
Aizupes	06.05	18.04	12.08, 26.08	08.08, 21.08	09.08, 11.08
Skujas	06.05	18.04	21.08, 29.08	01.07, 05.07	02.07, 06.07
Griezes	13.05	15.04	18.08, 29.08	12.08, 26. 08	10.07, 14.07
Laurenči	13.05	15.04	08.08, 26.08	18.08, 29.08	29.07, 02.08
Ludza	12.05	08.04	25.08, 25.08	22.08, 29.08	20.08, 23.08
Vecslabada	12.05	08.04	27.08, 27.08	25.08, 30.08	05.09, 06.09

Table 4.4: Descriptions of applied digestate

	The amount of scattered digestate (35%DM), kg/100m²	The amount of scattered N kg/ha	The amount of scattered P_2O_5 kg/ha	The amount of scattered K_2O kg/ha
Aizupes	68.6	18	14	34
Skujas	68.6	18	14	34
Griezes	65.7	17	14	32
Laurenči	125.7	33	26	62
Ludza	37.1	10	8	18
Vecslabada	37.1	10	8	18

For the botanical inventory, data on species numbers and vegetation coverage in 30 random 1x1 m squares were collected in each management plot before mowing and harvesting. The cover of all vascular plant species rooting in the 1x1 m squares was visually estimated directly in percentages. As permissions of the land owners were gained in mid-summer 2014, the first botanical inventory were made in July and August 2014, but in later years (2015, 2016) – in June and July.

Initial assessment of biodiversity showed a high number of vascular plant species on two of six sites (more than 30 species per 1m²), but a high number indicator species for semi-natural grasslands were found on five to six sites (more than 10 indicator species per study site) (Table 4.5).

Table 4.5: Results of botanical census at the beginning of the experiment

Experimental site	Number of plant species per study site	Number of indicator species per study site	Average number of plant species per 1 m² (n-30)	The range of plant species number per 1 m² (n-30)
Aizupes	91	20	24	9–35
Skujas	74	14	25	13–32
Griezes	83	11	17	10–28
Laurenči	64	8	17	9–25
Ludza	47	12	12	7–20
Vecslabada	48	12	11	6–17

For the invertebrate monitoring, the data on diversity and density of terrestrial beetles were collected by pitfall traps in June 2015 and 2016. The traps (10 in each monitoring plot) were installed using transect method and exhibited for a four week period.

In total, 77 species were indicated in 2015 and 73 species in 2016. In the both years, the largest diversity and density were found in Laurenči, but the smallest – in Ludza site (Table 4.6). In all management plots, except Ludza, a large dominance of one species (*Poecile versicolor)* were indicated (the dominance ranges between 33% in Skujas site and 55% in Vecslabada), while In Ludza site 45% of all individuals were *P. versicolor*, *Pterostichus vernalis* or *Bembidion properans* (17%, 17% and 10% respectively).

Table 4.6: Results of invertebrate census at 2015 and 2016

Experimental site	Number of terrestrial beetle species per study site		Average number of terrestrial beetles per trap (n-10)
	2015	2016	
Aizupes	36	30	7
Skujas	25	29	20
Griezes	37	32	15
Laurenči	50	43	36
Ludza	21	6	2
Vecslabada	31	25	12

4.2. Results and discussion

The impact of digestate application will be analysed in the last year of the project. The initial results show that the application of digestate has influenced the composition of terrestrial beetles and the distribution of particular plant species, but only in some cases the changes can be considered as statistically significant. In most of the cases, the differences in the diversity index do not exceed the range of standard error within the analysed years. According grassland productivity, the initial assessment shows that the yields are significantly affected by sampling time and climatic condition of the particular year. It means that it is hard to compare the data collected in June or during first days of July with the data collected in August, and it is hard to distinguish the influence of digestate application from the effect of climatic conditions in the given year. Still, as the differences in the plots with digestate application in most of the cases is smaller than in plots without application (Table 4.7), it can be concluded that the application of digestate increased the yield. To get a more detailed conclusion, the monitoring activity will be continued in 2017 and detailed data analysis will be included in the Monitoring Report that will be prepared by the end of September 2017.

Table 4.7: Results of above-ground biomass census, dry matter t/ha

Experimental sites	In management plots with digestate application				In management plots without digestate application			
	27 June 2014	10-24 August 2014	1-2 July 2015	29 June 2016	27 June 2014	10-24 August 2014	1-2 July 2015	29 June 2016
Aizupes	-	3.1	2.7	3.0	-	3.1	2.7	2.4
Skujas	-	3.3	4.1	3.7	-	3.1	3.3	2.0
Griezes	-	2.9	3.7	2.8	-	3.1	4.3	2.3
Laurenči	3.8	6.6	7.4	4.0	5.2	6.0	8.7	5.2

5. Total amount of the biomass per habitat type

The data on dry matter was additionally used to determine the total amount of biomass from Community Importance habitats. The data shows that the dry matter ranges from 1.0 to 4.6 t/ha (Table 5.1).

Table 5.1: The amount of grassland biomass per habitat type

Habitat type	Total dry matter, t/ha
6120 Xeric sand calcareous grasslands	1.0
6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates	2.2
6270 Fennoscandian lowland species-rich dry to mesic grasslands	2.9
6410 Molinia meadows on calcareous, peaty or clayey-silt-laden soils	2.8
6450 Northern boreal alluvial meadows	4.5
6510 Lowland hay meadows	4.6

The data shows that total amount of the biomass are significantly impacted by the harvesting time. For example the total amounts of the grassland biomass harvested in June 2014 (in a year with the most comprehensive data set) were 18% to 30% less than those harvested few months later – in August (Table 5.2).

Table 5.2: The amount of grassland biomass collected in 2014, according the habitat type and sampling time

Habitat type	Total dry matter, t/ha		
	June	July	August
6120 Xeric sand calcareous grasslands	0.9	1.1	-
6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates	1.9	2.0	2.5
6270 Fennoscandian lowland species-rich dry to mesic grasslands	2.4	2.8	3.1
6410 Molinia meadows on calcareous, peaty or clayey-silt-laden soils	2.2	3.0	3.1
6450 Northern boreal alluvial meadows	4.2	5.2	5.3
6510 Lowland hay meadows	4.4	5.5	6.3

Comparing data obtained from 2014 to 2016 it were concluded that amount of the biomass also varies greatly from a year to year. The amount of the biomass in 2016 was 33% to 19% less than in 2015 or up to 27% less than in 2014 (Table 5.3).

Table 5.3: The amount of grassland biomass according to the habitat type and sampling year

Habitat type	Total dry matter, t/ha		
	2014	2015	2016
6120 Xeric sand calcareous grasslands	1.0	1.2	0.8
6210 Semi-natural dry grasslands and scrubland facies on calcareous substrates	2.1	3.0	2.1
6270 Fennoscandian lowland species-rich dry to mesic grasslands	2.8	3.2	2.6
6410 Molinia meadows on calcareous, peaty or clayey-silt-laden soils	3.0	2.9	2.2
6450 Northern boreal alluvial meadows	4.5	5.1	3.5
6510 Lowland hay meadows	4.4	5.7	3.9

6. Cost effectiveness assessment of proposed technological solutions

6.1. Methodology for cost effectiveness analysis

6.1.1. General principles

The cost-effectiveness assessment of technological solutions for biomass use is based on the comparison of benefits originating from the respective technological solution with costs of technological solution. The greater is the difference, the more effective the technological solution can be considered.

In order to be correct, the cost-effectiveness is made, taking into account all the benefits and costs arising from the technological solution during its life cycle (lifetime). The discounted cash flow or the present value method is used in order to allow the comparison of benefits and costs arising during different periods of time. With this method net present value is calculated, which characterizes the present value of the difference between the costs and benefits of the technological solution at the beginning of the life cycle. The overall net present value (NPV) is calculated as follows:

$$NPV = \sum_{i=0}^{n} \frac{B_i - C_i}{r},$$
(1)

where i –   Yearly index (within the range from 1 to n);
n –   Life time of the respective technological solution;
B_i –   Benefits from the respective technological solution (in the year i);
C_i –   Costs of the respective technological solution (in the year i);
r –   Discount rate used in the calculation.

The benefits of the technological solution (B) consist of two types:
   1) benefits derived from the final product (e.g., biogas, biobutanol, grass pellets);
   2) benefits from the by-products (e.g., digestate from biogas production process).

Benefits of final products are determined by assessing their economic value derived from the respective technological solution. The economic value of the final product is measured using reference products - equivalent products for which it is possible to determine the market value or provide a reasonable economic value otherwise. Broader explanation of the benefits of the final products is set out in chapters 6.1.2., 6.1.3., and 6.1.4.

Benefits of by-products are taken into account if positive economic value of these products is sufficiently clearly identifiable. In such cases, economic value of these by-products will be determined similar as for final products - using reference products. In cases where by-products do not have significant positive economic value, benefits caused by them are not taken into account in the cost-effectiveness assessment. Detailed explanation on the benefits of by-products is provided in chapter 6.1.2., and 6.1.4.

In addition to these two groups of benefits, there are also benefits related to the residual value at the end of the life cycle of a particular technological solution which consist mainly of various equipment scrap value. However, given that this residual value is relatively small compared to the initial investment, the potential residual value is not taken into account for the cost-effectiveness assessment.

The costs of the respective technological solution consist of two groups:
   1) investment costs;
   2) operational costs.

Investment costs include the cost of the design, creation/acquisition of equipment and machinery, construction works, intangible assets (e.g., Software acquisition) and other investments. Mostly investment costs are created at the very beginning of the technological life cycle. In some cases a technological solution may require additional investments (e.g., joints / equipment with a shorter lifetime needs replacement) during the life cycle of technological solution. More detailed information on the investment costs is given in chapter 6.1.2., 6.1.3., and 6.1.4.

Operational costs are formed by routine costs associated with the use of a technological solution (performance, operation). They mostly include:

1) preparation costs of grass biomass for the needs of the respective the technological solution;
2) the cost of consumables;
3) labour costs;
4) costs for external service;
5) disposal costs of by-products.

For each technological solution specific complementary costs for preparation of grass biomass are assessed. It should be noted that for the largest part of the grassland public co-financing is available to finance costs of grassland management e.g., the EU support for agriculture under direct aid schemes (the so-called single payment scheme). Under the terms of this support grass should be cut and removed from the area being applied for support. Consequently, there is reason to believe that part of the full grass biomass preparation costs are covered by co-financing available for the public. Therefore, for cost-effectiveness assessment of specific complementary preparation costs of grass biomass is used. These costs include the costs that originate after fulfilment of terms of public co-financing. It does not include costs for mowing of grass and collection of hay from the grasslands, but includes all other costs of biomass preparation, including grass grinding costs, further transportation costs to place of storage and so on.

Costs of consumables account for consumables necessary for functioning of the technological solution e.g., lubricants, additives, filter materials, etc. Detailed description of these costs is provided in the chapter 6.1.2., 6.1.3., and 6.1.4.

Labour costs comprise wages and social security contributions for the staff (operators, maintenance staff) necessary for operation of the technological solution. These costs are estimated on the basis of labor load required for operation (use) of the technological solution, required skill level of staff and the gross wage rates (in accordance with the necessary qualification). More detailed description of labour cost evaluation is presented in the chapter 6.1.2., 6.1.3., and 6.1.4.

External service costs consist mainly of maintenance costs for the technological solution. However, taking into account the specifics of the technological solution, these costs may also include costs of other external services. More detailed description on evaluation of these costs is given in the chapter 6.1.2., 6.1.3., and 6.1.4.

By-product disposal costs represent the costs associated with the utilization of by-products that do not have positive economic value, but are created by the respective technological solution. These costs consist mainly of different waste disposal costs. These costs also include a natural resources tax on hazardous / polluting discharges in nature. Detailed description of costs for recycling of the by-products is provided in the chapter 6.1.4.

It should be noted that there are two approaches to use the discounted cash flow method – the approach of nominal size[ii] and the approach of real value[iii]. The real value approach is used in cost-effectiveness calculations, since it is very difficult to predict future costs and benefits in nominal terms accurately enough. The use of real values means that all benefits and costs are projected non-inflationary, i.e. in 2016 prices (purchasing power). For this reason, the real discount rate is also used in calculation. The real discount rate assumed is 5% per annum. This discount rate is an indicative rate, which ensures that the risks associated with the uncertainty of future benefits is taken into account at the same time and avoiding a situation where the discount rate excessively reduce the future benefits of the present value.

Cost-effectiveness assessment of all benefits and costs are assessed without value added tax. In addition it has to be noted that the calculations do not take into account corporate income tax (tax on income from business activities). Taxes related to labour (personal income tax, statutory social insurance contributions) are taken into account.

6.1.2. Assessment of biogas production

Assessment of production of biogas from grass biomass in anaerobic digestion reactors is generally made on the basis of general principles described in the chapter 6.1.1. Biogas production has been assessed in three directions:

[ii] Nominal gains and nominal costs are discounted, by applying nominal discount rate
[iii] Real gains and real costs are discounted, by applying real discount rate

1) only for biogas production: the end product - methane;
2) only heat production: the end product – heat;
3) co-generation (CHP): the end product - electricity and heat.

The first option allows the assessment of overall economic efficiency of production of biogas from grass biomass in anaerobic digestion reactors (without going into deeper details on the subsequent use of biogas). In this case, economically useful component of biogas - biomethane and not biogas (which is a mixture of different gases - mostly methane and carbon dioxide) is considered as the final product. Natural gas is considered as the reference product of biomethane, being widely used product with an identifiable market value.

The economic value of biomethane is calculated by the following formula:

$$B_{ch4} = (V_{bruto} - V_{pp}) \cdot P_{dg},$$
(2)

where B_{ch4} –   economic value of biomethane;
V_{bruto} Gross value of methane, m^3/year;
V_{pp} –   Self-consumption of methane in technological processes, m^3/year;
P_{dg} –   Market price of natural gas, EUR/m^3.

Methane gross value (V_{bruto}) is calculated on the basis of organic solids (OS) and the amount of methane in the outcome of the OS. Organic solids volume is estimated based on a percentage of dry matter in grass biomass and OS proportion of dry matter. Methane yield of OS is determined in accordance with the technical characteristics of the technological solution. When calculating V_{bruto}, it has been assumed that the biogas production occurs in an average of 11 months (approximately 330 days) in a year, and one month is needed for maintenance and similar needs.

Self-consumption of methane for ensuring technological process (V_{pp}) has been evaluated in accordance with the technical characteristics of the technological solution, taking into account the different need for heating of the anaerobic fermentation process during the year (in winter the consumption is higher than in summer).For calculation purposes it assumed that the calorific value of methane is about 10 kWh/m^3 and the heat efficiency is about 90%.

Market price of natural gas (P_{dg}) has been evaluated under the Public Utilities Commission (PUC) set tariffs. For calculation purposes natural gas tariffs for consumer class are used, being close to the amount of biomethane, which can be obtained within a year by using the technological solution[iv]. For calculation purposes it is assumed that the price of natural gas is considered to be the price of methane (without corrections).It should be noted that the methane content of natural gas is not 100%, but is slightly smaller. However, this assumption provides a more conservative assessment of the benefit.

Investment costs account for the basic investments needed for technological process to produce biogas from grass biomass. Investment costs do not include investments in further purification of biogas to obtain high methane content in the gas.

Basic operational costs include the costs related to:
1) preparation and transportation of grass to the place of production of biogas;
2) consumables for grass biomass preparation and biogas production process;
3) labour costs for processing of grass biomass and biogas production process;
4) maintenance costs of the biogas reactor and the associated equipment.

By-product disposal costs within the frame of this option are not taken into account, as assumed that such costs are negligible or can be offset by potential gains from the sale of digestate[v].

The second assessment option provides a deeper assessment of economic efficiency. In this case, the end product is heat. Benefits of the final product are evaluated similarly to the first option:

$$B_{th} = (Q_{bruto} - Q_{pp}) \cdot P_{th},$$
(3)

where B_{th} –   economic value of heat;

[iv]In order to ensure a conservative assessment of benefits, the most similar class of consumption has been used having lower tariff for consumption of natural gas
[v] Benefits originating from selling of digestate are not included in the assessment of this option.

Q_{bruto} gross volume of heat, MWh/year;
Q_{pp} –   self-consumption of heat for technological processes, MWh/year;
P_{th} –   reference value of heat, EUR/MWh.

Gross volume of heat (Q_{bruto}) is calculated in accordance with the estimated V_{bruto} and calorific value of methane (10 kWh/m³). Self-consumption of heat for technological process (Q_{pp}) is estimated as in the first option. Reference value of heat (P_{th}) is determined on the basis of market price of natural gas, calorific value of natural gas and defined efficiency factor of natural gas boiler, as well as on the estimated specific investment cost in natural gas boiler:

$$P_{th} = (\frac{P_{dg}}{q_{dg} \cdot \eta_{dg}} + i_{dg}) \cdot 1000 , \tag{4}$$

where q_{dg} –   Defined calorific value of natural gas, kWh/m³;
    η_{dg} –   Defined efficiency factor of natural gas boiler;
    i_{dg} -   Specific investment costs for natural gas boiler, EUR/kWh.

Defined calorific value of natural gas (q_{dg}) is assumed as 9.35 kWh/m³. Such a level is usually referred indifferent sources of literature, as well as used in practical calculations of heat energy. Defined efficiency factor of natural gas boiler is assumed 0.9. This level is used in the various laws and regulations(e.g., Cabinet of Ministers Regulation559, of the year 2012) governing the regulatory calculation of the amount of energy when replacing fossil fuels with renewable energy sources.

Specific investment costs for natural gas boiler (i_{dg}) are calculated on the basis of defined investment costs and assuming that the average service life of natural gas boiler is 5 years:

$$i_{dg} = \frac{I_{dg}}{24 \cdot 330} \cdot \frac{r}{1 - (1+r)^{-5}} , \tag{5}$$

where I_{dg} –   Defined investment costs for natural gas boiler, EUR/kWh.

In formula (5) the assumption is used that natural gas boiler is operated around the clock 330 days a year on average (the same assumption as used in relation to biogas production).Defined investment cost for natural gas boiler (I_{dg}) is assumed as 116.68 EUR/kWh[vi]. In order to take into account the fact that the investment costs incur at the moment of the purchase of natural gas boiler, but the heat production occurs evenly throughout the service period, the discount rate r is included in formula (5) (the same rate that is used in other cost-effectiveness calculations).

Given that today gas boilers are intelligent (very minimal labour consumption is needed during the operation of the boiler) and have low maintenance costs, in calculation of reference value of heat (see. the formula (4)), other costs are not taken into account, assuming that they do not pose significant impact on P_{th}.

In the second assessment option investment costs compose investment costs as of first and the additional investment costs for biogas boiler and its associated equipment. Operational costs consist mainly of the same costs as in the first evaluation option. Additional operating costs represent the cost of consumables for biogas boiler, labour costs related to operation of biogas boiler, and maintenance costs of a biogas boiler.

The third option provides evaluation of economic efficiency for functionally broader technological solutions resulting in two final products-electricity and heat energy. Benefits of these final products are valued as follows:

$$B_{CHP} = (E_{bruto} - E_{pp}) \cdot P_{el} + (Q_{bruto*} - Q_{pp*}) \cdot P_{th} , \tag{6}$$

where B_{CHP} –   Economic value of final products of cogeneration;
    E_{bruto} –   Electricity gross volume, MWh/year;
    E_{pp} –   Self-consumption of electricity for technological processes, MWh/year;
    P_{el} –   Reference value of electricity, EUR/MWh;
    Q_{bruto*} –   Gross amount of heatin cogeneration, MWh/year;
    Q_{pp*} –   Self-consumption of heat for technological processes in cogeneration, MWh/year.

[vi] Reference value of investment costs for natural gas boiler as given in Cabinet of Ministers Regulations No 559 (2012)

Electricity gross volume (E_{bruto}) is calculated in accordance with the estimated V_{bruto}, methane calorific value (10 kWh/m^3) and the electrical efficiency of co-generation. Electricity self-consumption for technological process has been evaluated in accordance with the technical characteristics of cogeneration. Gross amount of heating cogeneration (Q_{bruto*}) is calculated in accordance with the estimated V_{bruto}, methane calorific value(10 kWh/m^3) and the heat efficiency of cogeneration. Given that the self-consumption of heat for technological process are mostly related to the biogas production process, it is assumed that $Q_{pp*} \approx Q_{pp}$.

Reference value of electricity (P_{el}) has been assessed based on the market price for electricity at the consumption rate which can be produced by the technological solution within one year. It should be noted that there are two prices on the electricity market:
1) The DDP price or the price for the supply, which includes the distribution costs and the mandatory procurement component (OIK);
2) EXW price or price by the manufacturer, which is the price at which electricity can be sold on the network. This price is significantly lower than the DDP price.

For calculation purposes DDP electricity price is used as the technological solution of CHP is targeted at users who use the electricity for their needs, thus replacing part of the electricity purchased from outside. In this case, the reference electricity value is estimated as follows:

$$P_{el} = P_{NP} + \pi_T + \pi_S + \pi_{OIK} \,,\qquad\qquad(7)$$

where P_{NP} –   The weighted average price of electricity in Nord Pool stock exchange, EUR/MWh;
        π_T –   sellers' premium, EUR/MWh;
        π_S –   Distribution fee, EUR/MWh;
        π_{OIK} –   OIK, EUR/MWh.

Sellers' premium, fee for distribution and OIK has been assessed based on the existing charges in the electricity market as being applied for electricity consumers with relatively low level of electricity consumption.

In the third option (option of CHP) investment costs comprise investment costs of the first option and cogeneration equipment investment costs. Operational costs in this case as in the second option consist mainly of the first option operational costs. Additional operating costs represent the cost of consumables for cogeneration plants, the cost of labour operating cogeneration plants and maintenance costs of cogeneration plants.

6.1.3. Assessment of production of biobutanol from grass biomass

The assessment of production of biobutanol from grass biomass is generally carried out based on the general principles described in the chapter 6.1.1.

Since biobutanol is a second-generation biofuel having physico - chemical characteristics and applications very close to petrol[vii], petrol is selected as the reference product to biobutanol. The economic value of biobutanol is calculated by the following formula:

$$B_{bu} = V_{bu} \cdot q_{bu} \cdot \frac{P_{be}}{q_{be}} \,,\qquad\qquad(8)$$

where B_{bu} –   Economic value of biobutanol;
        V_{bu} Outcome of biobutanol, l/year;
        q_{bu} –   Calorific value of biobutanol, kWh/l;
        P_{be} –   Price of petrol, EUR/l;
        q_{be} –   Calorific value of petrol, kWh/l.

Outcome of biobutanol (V_{bu}) is calculated solely on the basis of sugar content in the grass biomass and the estimated outcome of biobutanol from sugars. With respect to the calorific value of biobutanol (q_{bu}) and calorific value of petrol (q_{be}) information provided in technical literature has been used.

[vii] Biobutanol is even closer to petrol than bioethanol

Price of petrol (P_{be}) is determined in accordance with the petrol price in the retail market as technological solutions envisages production of biobutanol in relatively small volumes and are targeted to substitution of already purchased fuel and not to fuel sales,.

The assessment of the costs is done on the basis of the investment costs and the basic operational costs. Investment costs comprise investments in various machinery and equipment necessary for production of biobutanol from grass biomass. Investment costs do not include the cost of processing of residue for further utilisation.

Basic operational costs include the costs for:
1) preparation and transportation of grass to the biobutanol production site;
2) consumables necessary for preparation of grass biomass and production of biobutanol;
3) heat necessary for biobutanol production process;
4) electricity necessary for biobutanol production process;
5) labour for preparation of grass biomass and for production of biobutanol;
6) biobutanol production installations and equipment maintenance.

The costs of heat energy are assessed in accordance with the planned heat energy consumption during the production process using the estimated P_{th} (see chapter 6.1.2.). Electricity costs are estimated in accordance with the planned electricity consumption during the biobutanol production process. Calculations are done based on the DDP electricity prices (see chapter 6.1.2).

The appearance of by-products with a negative value is not planned. It is assumed that the grass biomass residues have a zero value. Therefore, neither the disposal costs of by-products nor the related benefits are taken into account.

6.1.4. Assessment of production of pellets from grass biomass

Cost efficiency assessment of production of pellets from grass biomass is generally based on general principles presented already in the chapter 4.1. The evaluation includes cost efficiency assessment of production of pellets for combustion. Taking into consideration that currently there is no market for grass pellets for combustion, the assessment comprises combustion of pellets for production of thermal energy.

Thus, the final product of this technological solution is heat energy. The economic value of heat energy is estimated as follows:

$$B_{zg\_th} = M_{zg} \cdot q_{zg} \cdot \eta_{zg} \cdot P_{th},$$
(9)

where
B_{zg_th} – Economic value of heat produced from grass pellets;
M_{zg} – Amount of grass pellets, t/year;
q_{zg} – Calorific value of grass pellets, MWh/t;
η_{zg} – Defined efficiency factor of grass pellet boiler.

The amount of grass pellets (M_{zg}) is estimated according to the amount of total solids of grass biomass and the amount of production of grass pellets. Calorific value of grass pellets (q_{zg}) has been assessed on the basis of technological trials.

Assessment of the costs of this technological solution comprises investment costs and operational costs. Investment costs include investments in grass pellet production installations and in the related equipment and investments in grass pellet boiler. It should be noted that the characteristics of grass pellets are different from wood pellets - grass pellets have a higher proportion of ash and relatively low ash melting point. For this reason, the investment costs for grass pellet boiler are higher than for wood pellet boiler of the same capacity.

Operational costs include the following costs:
1) grass preparation and transportation to grass pellet manufacturing site[viii];
2) consumables for grass biomass preparation and grass pellet production process, as well as during the combustion process;

[viii] For the calculation purposes, it will be presumed that combustion of pellets takes place besides the grass pellet production site, thus there are no significant transportation costs from the production site of grass pellets to the site of combustion

3) electricity costs for grass pellet production process and the combustion process;
4) labour costs for preparation of grass biomass and for production of grass pellets, as well as for combustion process;
5) costs for grass pellet production installation and maintenance costs for equipment costs, as well as maintenance costs of grass pellet boiler;
6) disposal costs of harmful by-products.

When making the assessment of costs for consumables, electricity, labour and maintenance for grass pellet combustion process, it has been acknowledged that operation of grass pellet boiler is more complex and labour-intensive than exploitation of wood pellet boiler.

Disposal costs of harmful by-products are related to the fact that combustion of grass pellets is potentially causing large emissions of nitrogen oxides. It is planned to explore this factor more thoroughly. Then it will be also evaluated if additional costs are to be expected (e.g., Natural resource tax) for nitrogen oxide emissions.

It has to be noted that the grass pellet combustion process results also in a by-product with a potentially positive value - grass pellet ash. These ashes have high concentration of potassium (K) and have a high potential of reducing soil acidity (pH-increasing) effect. Thus, it is possible to use the ash as organic potassium fertilizer, which simultaneously reduces the acidity of the soil (as opposed to potassium fertilizers that acidify the soil).

6.2. Results of cost effectiveness analysis

6.2.1. Biogas

The following three options of technological solutions for biogas production have been assessed:
1) a pilot facility with a processing capacity of 50 kg a day of green biomass;
2) a potential biogas production facility with a processing capacity of 1000 kg a day of green biomass;
3) a potential biogas production facility with a processing capacity of 3000 kg a day of green biomass.

Based on the results of the study by the Latvian Fund for Nature, it was assumed that the content of dry matter in green biomass is 33.3% on average and the content of organic dry matter (ODM) in dry matter is 93.0%. In accordance with the research outputs of company "Bio RE" Ltd., the yield of methane is assumed as 174 normal m^3 per 1 t ODM. Given that grassland productivity differs in Sigulda and Ludza Municipality, cost-effectiveness analysis was carried out separately for the both municipalities.

According to Chapter 6.1.2, three sub-options have been analysed for each option:
1) only biogas production;
2) only heat production;
3) co-generation or combined heat and power (CHP).

The main parameters of technological solutions are presented in Table 6.1.

Table 6.1: The main parameters of technological solutions for biogas production

Parameters	Technological solutions		
	I	II	III
Biogas production:			
Processing capacity of green biomass, kg/day	50	1 000	3 000
Total processed green biomass, t/year	16.5	330.0	990.0
Total dry matter, t/year	5.5	109.9	329.7
Total organic dry matter, t/year	5.1	102.2	306.6
Methane, m^3/year	889	17 782	53 347
Methane, MWh/year	8.9	177.8	533.5
Only heat production:			
Total heat energy, MWh/year	8.0	160.0	480.1
CHP:			
Electricity, MWh/year	2.7	62.2	192.0
Heat energy, MWh/year	5.3	97.8	288.1

Source: Calculations by "Edo Consult" Ltd. from data by "Bio RE" Ltd.

Based on the actual costs of pilot plant development and estimates, investment costs for biogas technological solutions have been calculated (see Table 6.2).

Table 6.2: Investment costs for biogas technological solutions

Parameters	Technological solutions		
	I	II	III
Biogas module, EUR	90 000	39 292	103 057
Heat generation module (boiler), EUR	200	3 031	9 093
CHP module, EUR	6 500	3 929	12 124

Source: Data by „Bio RE" Ltd.

Investment costs for the pilot facility include also research costs. Although capacity of option II is several times higher than form option I, the investment costs of option I for biogas and CHP module is higher due to research cost included. The investment costs for option II and III have been estimated by taking into account the potential scale economies from increasing capacity.

The analysis assumes that the average life cycle of a biogas module is 15 years, of a heat production module - 5 years (typical indicator for gas boilers), of a CHP module - 3 years (accelerated depreciation in the conditions of intensive operation).

NPV calculations for the technological solutions of biogas production have been calculated according to the methodology described in Chapter 6.1 (see Table 6.3). It should be noted that in the case of option I, heat energy for ensuring biogas production exceeds the calorific value of the produced biogas due to a low production capacity. Therefore, for calculating benefits from this solution, the gross volumes (without deducting consumption), not the total net methane and heat, have been applied. Instead, consumption of heat energy is assessed as heat energy expenditures using a heat energy reference value (P_{th}).

Table 6.3: Net present value calculations for the technological solutions of biogas production (EUR)

Parameters	Technological solutions		
	I	II	III
Biogas production:			
Sigulda Municipality	-149 873	-89 078	-164 105
Ludza Municipality	-149 876	-89 288	-165 197
Only heat energy production:			
Sigulda Municipality	-150 372	-89 288	-163 410
Ludza Municipality	-150 374	-89 498	-164 501
CHP:			
Sigulda Municipality	-180 732	-70 574	-89 679
Ludza Municipality	-180 735	-70 784	-90 771

Source: Calculations by "Edo Consult" Ltd.

The differences in NPV between Sigulda and Ludza Municipality are related to the differences in green biomass transportation costs. Since Ludza Municipality possesses a lower than average grass yield (about 35.5% lower), a greater area is needed to ensure the needed substrate, which means also a longer mean weighted transportation distance. The influence of transportation costs increases along with the increase of the capacity of a technological solution.

The obtained results show that all technological solutions for biogas production have a negative NPV, which means that they are not economically effective under the existing conditions. In addition, operational costs for options I and II outweigh the benefits from the technological solution. Thus, these solutions are economically ineffective, regardless of the size of the investment. At the same time, it should be noted that a very significant part of the operational costs consists of personnel costs. If personnel costs are excluded from operational costs, options II and III will become economically efficient (NPV>0) even at the level of the existing investment costs. In contrast, benefits from the technological solution for option III (the economic value of electricity and heat) is larger than operational costs. Therefore, this option would be economically efficient, if there were less investment costs.

Summarising the results of the cost-effectiveness analysis for biogas technological solutions, it can be concluded that, although the considered options are not economically efficient, this solution can be considered as a perspective in the future. Further development of this solution by increasing the processing capacity of biomass, reducing personnel costs (man-hours), reducing specific investment costs etc. would help to achieve cost-effective options (NPV>0).

6.2.2. Biobutanol

Two options have been assessed for biobutanol production technology:
1) a pilot facility with a processing capacity of 1 t/year of dry biomass;
2) a potential facility with a processing capacity of 10 t /year of dry biomass.

The analysis of cost effectiveness has been carried out based on the assumption that grass biomass is in the form of hay, because there is more information available on biobutanol production from hay. For the purposes of the calculation, it is assumed that content of dry matter in hay is 85%, of organic dry matter in the total dry matter - 93.0%. According to the study carried out by Riga Technical University, 1 t of organic dry matter yields 269 kg of sugars; 1 kg of sugars yields 0.332 l of butanol. Given that grassland productivity differs in Sigulda and Ludza Municipality, cost-effectiveness analysis was carried out separately for the both municipalities.

The main parameters of technological solutions are presented in Table 6.4.

Table 6.4: The main parameters of technological solutions for biobutanol production

Parameters	Technological solutions	
	I	II
Processing capacity of dry matter, t/year	1.0	10.0
Needed hay amount, t/year	1.2	11.8
Total organic dry matter, t/year	0.9	9.3
Sugars, kg/year	250	2 502
Biobutanol, l/year	83.1	830.6

Source: Calculations by "Edo Consult" Ltd. from data by Riga Technical University

The investment costs for the pilot facility (option I) are estimated at 100 000 EUR including research costs. The investment costs for option II are estimated theoretically at 50 000 EUR. The estimate is based on the assumption that it is possible to significantly reduce investment costs, as well as that there is no need for such large research costs as for the pilot facility.

For the need of the analyses, it is assumed that the average life cycle of equipment is 5 years (typical for equipment). The net present value for technological solutions of biobutanol production have been calculated in accordance with the methodology described in Chapter 6.1 (see Table 6.5).

Table 6.5: Net present value calculations for the technological solutions of biobutanol production (EUR)

Parameters	Technological solutions	
	I	II
Sigulda Municipality	-149 873	-89 078
Ludza Municipality	-149 876	-89 288

Source: Calculations by "Edo Consult" Ltd.

The differences in NPV between Sigulda and Ludza Municipality are associated with differences in the costs of hay transportation. As the hay consumption is relatively low, the differences in the net present value are accordingly small.

The obtained results show that the both options of technological solutions have a negative net present value, therefore, they are not economically effective. It should be noted that the operational costs for the both options exceed the benefits (biobutanol economic value) several times. Thus, these options are economically ineffective, regardless of the size of the investment.

In parallel to biobutanol, acetone is being formed (about 50% of butanol volume), and big amounts of dry matter residues remain that can be used for biogas production. Thus, there are synergies for combining this option with biogas production.

Summing up the results of the analysis, it can be concluded that the production of biobutanol from grass biomass itself is not economically effective. Given the very high investments and operational costs at a relatively small amount of processed dry matter, it is unlikely to make the technological solutions economically effective by increasing processing power and the savings of the operational costs. However, there might be cost-effective solutions where biobutanol production from grass biomass is combined with biogas production.

6.2.3. Grass pellets

In the study, the production of grass pellets as fuel has been analysed. The following three options of technological solutions have been assessed:

1) a facility with a pellet production capacity of 45 kg/h;
2) a facility with a pellet production capacity of 300 kg/h;
3) a facility with a pellet production capacity of 1000 kg/h.

For the purpose of the calculations, it is assumed that the content of dry matter is 85% in hay and 90% in hay pellets, and losses during the production process reach 3%. It is assumed that the lower heating value of grass pellets is 4.43 MWh/t, and the efficiency of grass pellet boilers is 85% (lower than for wood pellet boilers). Given that the grassland productivity differs between Sigulda and Ludza Municipality, cost-effectiveness analysis was carried out separately for the both municipalities. The main parameters of the technological solutions are presented in Table 6.6.

Table 6.6: The main parameters of technological solutions for grass pellet production

Parameters	Technological solutions		
	I	II	III
Produced pellet amount, t/y	87.1	580.8	1 936.0
Energy value of produced pellets			
Gross production, MWh/y	385.9	2 572.9	8 576.5
Net production, MWh/y	328.1	2 187.0	7 290.0
Needed amount of raw material			
Dry matter of grass, t/y	80.8	538.9	1 796.3
Hey, t/y	95.1	634.0	2 113.3
Needed capacity of grass pellet boiler, kW	41.4	276.1	920.5

Source: Calculations by "Edo Consult" Ltd. from data by "Baltic Unique Solutions" Ltd.

Based on the data provided by company "Baltic Unique Solutions" on the market prices of equipment for the production of grass pellets, investment costs for facilities have been estimated (see Table 6.6). The investment costs, which are related to grass pellet boilers, are estimated in accordance with the boiler capacity required for the corresponding option. On average, specific investment costs for grass pellet boilers have been accepted as 200 EUR/kW (higher than for wood pellet boilers).

Table 6.7: Investment costs for the technological solutions of grass pellet production

Parameters	Technological solutions		
	I	II	III
Equipment for production of grass pellets, EUR	1 455	18 182	126 446
Grass pellet boilers, EUR	8 284	55 227	184 091

Source: Calculations by "Edo Consult" Ltd. from data by "Baltic Unique Solutions" Ltd.

The analysis assumed that the average life cycle of a grass pellet production facility and a grass pellet boiler is 5 years. NPV calculations for technological solutions of grass pellet production have been carried out in accordance with the methodology described in Chapter 6.1 (see Table 6.8).

Table 6.8: Net present value calculations for the technological solutions of grass pellet production (EUR)

Municipality	Technological solutions		
	I	II	III
Sigulda Municipality	-57 244	130 773	603 681
Ludza Municipality	-57 289	129 990	598 918

Source: Calculations by "Edo Consult" Ltd.

The differences in NPV between Sigulda and Ludza Municipality are associated with differences in the costs of hay transportation. If production capacity increases, hay transportation costs and the differences in NVP between Sigulda and Ludza Municipality increase, accordingly.

The obtained net present value suggests that, at the given conditions, option I is not economically effective. In turn, options II and III are economically effective even including considering all investment costs. It should be noted that the net present value of these options is higher than the investment costs, which supports a relatively convincing economic effectiveness.

Summarising the analysis, it can be concluded that, in general, grass pellet production is an economically effective technological solution (with the exception of option I with a low capacity). However, it should be noted that cost effectiveness assessment of the technological solutions for grass pellet production was carried out on the basis of relatively incomplete information. For example, the specific investment costs for grass pellet boilers, the efficiency of grass pellet boilers, personnel costs, etc. have been assessed with a high degree of approximation. In particular, potential costs from increased nitrogen oxide (NO_X) emissions from burning pellets have not been analysed due to lack of information. In future, significant additional costs may arise, if legal regulation on NO_X emissions changes, which can significantly reduce the economic effectiveness of the technological solution.

The ash from grass pellet burning could theoretically be used as potassium (K) fertiliser in agriculture. Moreover, ashes from hay pellets made from biologically valuable grasslands, can be used as a valuable fertiliser in organic agriculture. However, given that there is no sufficient market for this type of fertiliser, the potential economic benefits of grass pellet ash has not been taken into account in the assessment of economic efficiency.

6.3. Conclusions

The comparison of all three technological solutions indicates that under the existing conditions (technological parameters, prices of energy resources, operational costs, investment cost) the production of pellets from grass biomass (hay) and using them as fuel is the only solution, which is probably economically effective. This solution has a substantially positive net present value for options II and III.

Under the existing conditions, biogas production is not economically effective as all options have a negative net present value. These results are derived from the experience from the pilot facility being constructed within the GRASSSERVICE project. However, a detailed analysis shows that this solution is perspective because benefits increasingly exceed operational expenditures at higher production capacity. It is possible to achieve economically effective options by further development of this solution (increasing the processing capacity of biomass, reducing personnel costs (man-hours), reducing specific investment costs etc.).

The cost effectiveness assessment based on the experience from the pilot facility within the GRASSSERVICE project reveals that the production of biobutanol from grass biomass is not economically effective under the existing conditions. The yield of butanol is quite low but the operational and investment costs to process dry matter are very high. So this solution might not be economically effective as a stand-alone solution. Nevertheless, this solution can become economically effective if it is combined with biogas production. As only sugars from biomass are used in production of butanol, the remaining dry organic matter can be used to produce biogas. Therefore, there is a potential of production synergy.

Annex 1. Components and sources of lignocellulose materials

Cellulose

Cellulose is a polysaccharide that consists of a linear chain of D-glucose linked by β-(1,4)-glycosidic bonds to each other. It is the main constituent of lignocellulosic biomass[33].

Hemicelluloses

Hemicelluloses are heterogeneous branched biopolymers that contain pentoses, hexoses and/or urgonic acids. Their key role is to provide a link between lignin and cellulose. Since they have amorphous and branched structures they are relatively easy to hydrolyse, however, a wide range of various enzymes are required for complete hydrolysis of hemicellulose into free monomers[34].

Lignin

Lignin is an aromatic and rigid biopolymer which is composed of three phenolic monomers – coumaryl, coniferyl and sinapyl alcohol[35]. Previously, little interest has been given to lignin chemistry potential on hydrolysis. However, lignin components are gaining importance since the adsorption of lignin to cellulases requires a higher enzyme loading because this binding generates a non-productive enzyme attachment and limits the accessibility of cellulose to cellulose http://www.sciencedirect.com.resursi.rtu.lv/science/article/pii/S0360128512000172 - bib76. Furthermore, phenolic groups are formed from the degradation of lignin. These components substantially deactivate cellulolytic enzymes and hence influence enzymatic hydrolysis and subsequent fermentation process[36].

Raw materials

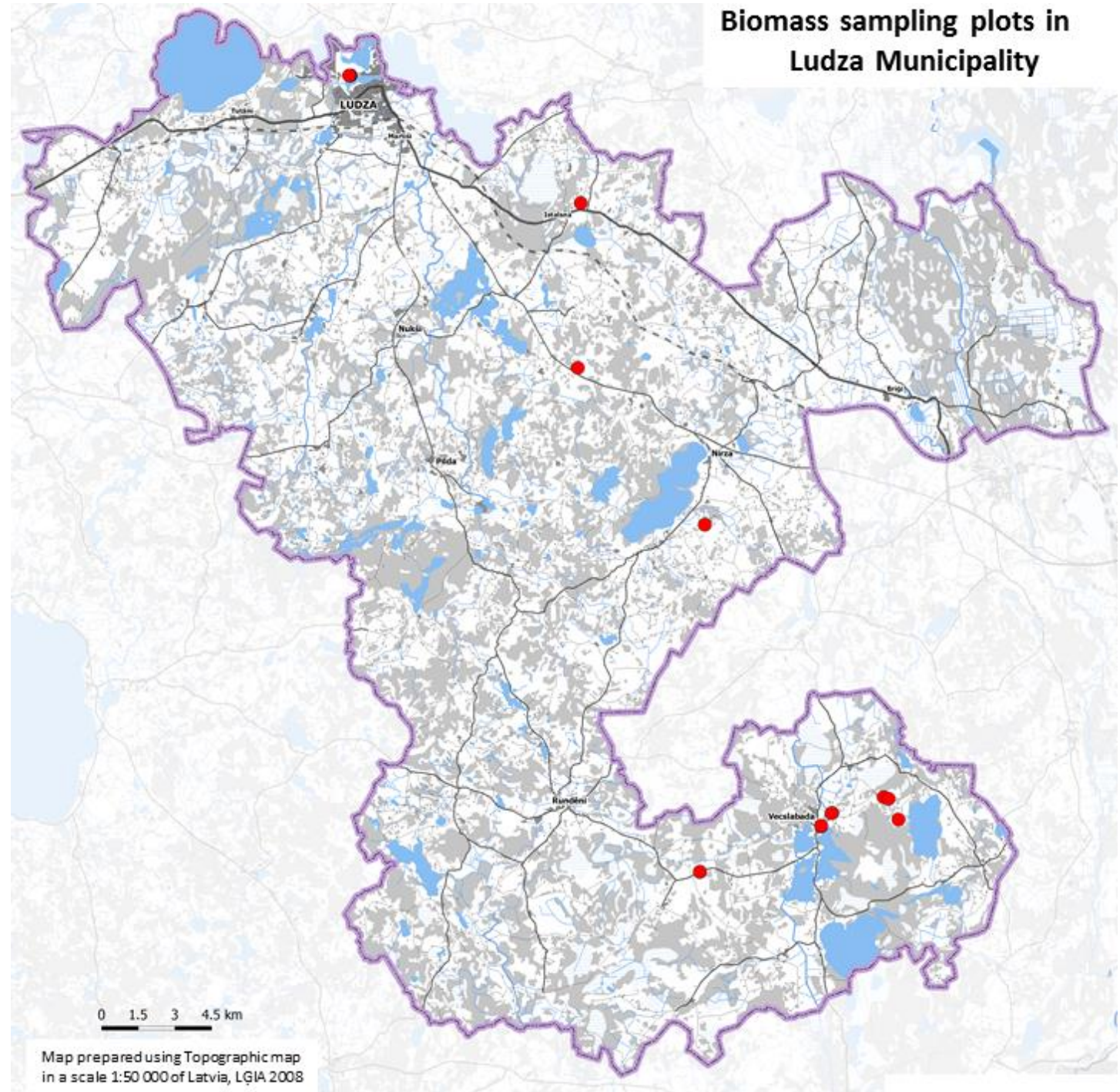Various types of biomass can be defined into 4 main groups:
- woody plants,
- herbaceous plants/grasses,
- aquatic plants,
- manure.

Within this categorisation, herbaceous plants can be further subdivided into those with high- and low-moisture contents. Apart from specific applications or needs, most commercial activity has been directed towards the lower moisture-content types, woody plants and herbaceous species. Aquatic plants and manures are intrinsically high-moisture materials and as such, are more suited to 'wet' processing techniques[37].

With respect to its composition and physiology woody plants can be divided into hardwood and softwood. Generally softwood contains more lignin, making it more difficult to degrade and more resistant to enzymatic hydrolysis than hardwood[38].

Plants/grasses consist of an extensive variety of types. They comprise also agricultural wastes such as corn stover, corn stalks, rice and wheat straws as well as sugarcane bagasse. Crop residues contain more hemicellulosic material than woody biomass (approximately 25–35%). Aside from being an environmentally friendly process, agricultural residues help to avoid reliance on forest-woody biomass and thus reduce deforestation (non-sustainable-cutting plants). Unlike trees, crop residues are characterized by a short-harvest rotation that renders them more consistently available to biofuel production[39].

Annex 2. Location of biomass sampling plots

Biomass sampling plots in Ludza Municipality

Map prepared using Topographic map in a scale 1:50 000 of Latvia, LGIA 2008

Biomass sampling plots in Sigulda Municipality
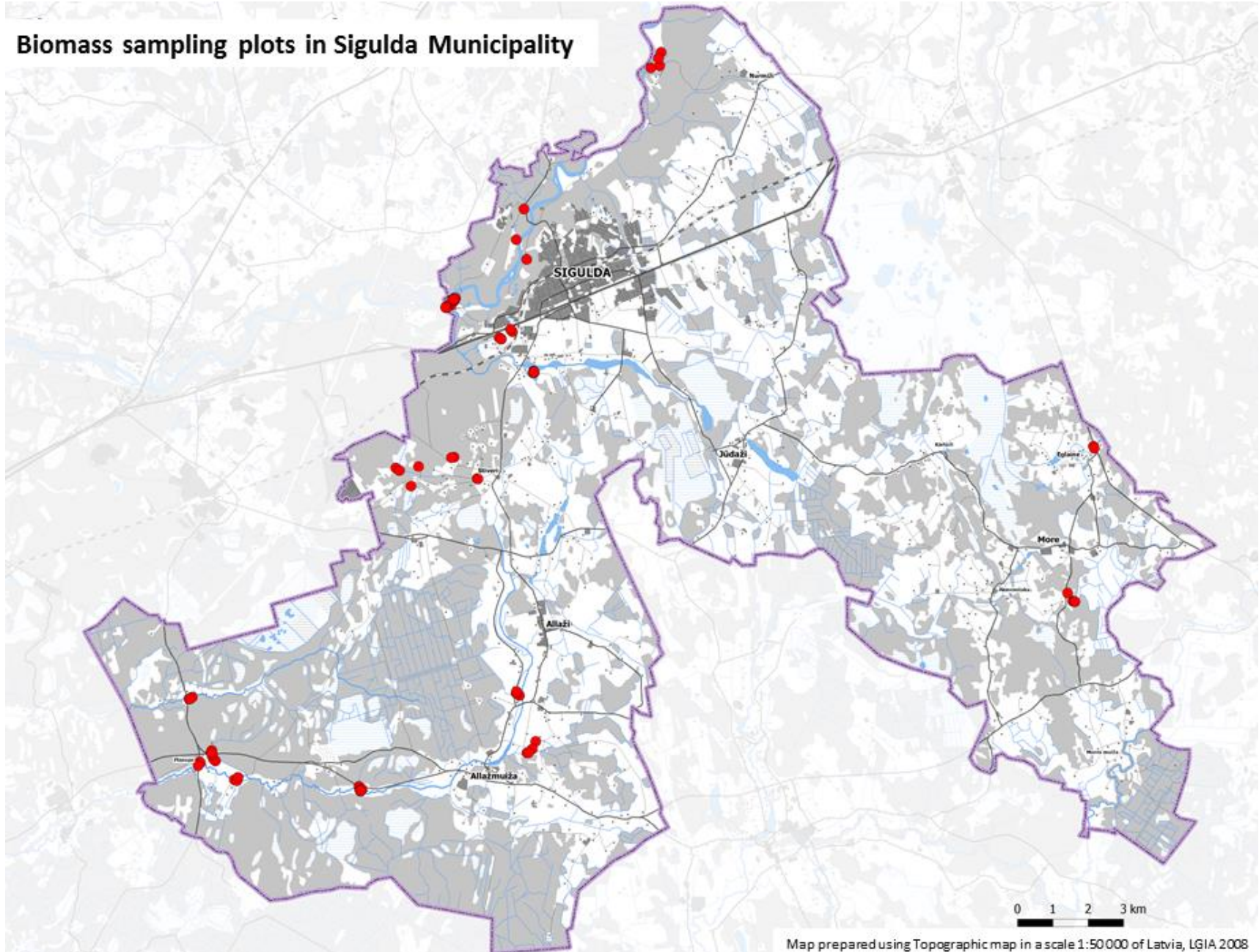
Map prepared using Topographic map in a scale 1:50 000 of Latvia, LĢIA 2008

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