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Methane yield of different energy crops grown in Estonian conditions

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Abstract. To investigate the suitability of different alternative crops in Estonian conditions for methane production, a plant field collection was established in 2008 at the Institute of Agricultural and Environmental Sciences of Estonian University of Life Sciences on Haplic Luvisol (Hypereutric) soil near Tartu (58°23´N, 26°44´E) in Estonia. The species grown in this collection field were: Jerusalem artichoke (*Helianthus tuberosus* L.), fibre hemp (*Cannabis sativa* L.) cv USO–31, energy sunflower (*Helianthus annuus* L.) cv Wielkopolski, Amur silver–grass (*Miscanthus sacchariflorus*), energy grass cv Szarvasi–1, and foxtail millet (*Setaria italic* L.).

The correlation between methane yield and lignin content was significantly negative. The expected average decrease in methane yield is 7.49 LCH_4 $kgTS^{-1}$ with an increase of 1% lignin content in biomass.

The highest methane yield was obtained from *Miscanthus sacchariflorus*, *Helianthus tuberosus* L., and *Setaria italica* L. samples. The alternative non–food crops from southern areas gave higher methane yield in Estonian conditions, because their development rate was slower, lignin content smaller and development stage more suitable for methane production. The variety of plant biomass improves the operational management of biogas plants and favours agro–ecosystem biodiversity.

Key words: Alternative energy crops, lignin content, methane yield.

INTRODUCTION

Biogas with reference to methane yield is a promising renewable energy source. Currently new alternative crops for energy production are studied in Estonian climatic conditions. Recently, fibre hemp and sunflower field experiments have been performed in Estonia. The cultivation of fibre hemp has a very long tradition here, but nowadays the biggest difficulty for comprehensive cultivation of hemp in Estonia is the lack of modern harvest technology. Preliminary results have shown that energy sunflower cultivation is quite promising (Noormets et al., 2010). Furthermore, field experiments with maize, the most popular energy crop in Europe, have also been performed in Estonia. The cultivation of maize requires deep–laid, balanced management between food and non–food crop production. Additionally, it has been proposed that some alternative southern perennial and annual energy crops may have bio–energy potential in Nordic conditions. The current study investigated the suitability of Jerusalem artichoke (*Helianthus tuberosus* L.), fibre hemp (*Cannabis sativa* L.), energy sunflower (*Helianthus annuus* L.), Amur silver–grass (*Miscanthus sacchariflorus*), energy grass cv Szarvasi–1, and foxtail millet (*Setaria italica* L.) in Estonian conditions, although these cultures are originally grown in southern climatic conditions. These potential energy crops produce high above–ground biomass in native habitants (Gunnarson et al., 1985; Basavarajappa et al., 2003; Zabaniotou et al., 2007; Bengtsson, 2009). For example, highly efficient carbon–fixing plants, such as *Miscanthus* sp, can convert approximately 2% of incident solar radiation into biomass (Beale & Long, 1995). The use of cell walls as a renewable form of energy is highly attractive because plants have adapted to proliferate in nearly every biome on the planet (Wright et al., 2004). High above–ground biomass benefits to high energy yield per hectare which is declared to be an alternative measure to compare overall yields than analysing biogas production per tonne of volatile solids (Braun et al., 2009).

An abandoned agricultural or nutritionally depleted land can be used for the production of lignocellulosic (non–food) bio–energy crops to produce energy from cellulose and other cell wall polysaccharides (Campbell et al, 2008; DeBolt et al, 2009). Biogas production from biomass residues has been widely studied (Nallathambi Gunaseelan, 1997; Seppälä et al., 2009). These studies revealed that each group of substrates has a specific methanogenic potential that is linked to their chemical composition characteristics (Klimiuk et al., 2010).

Hemicellulose, cellulose and lignin are the three main components of biomass and generally they cover respectively 20–40, 40–60, and 10–25 wt.% of lignocellulosic biomass (McKendry, 2002).

Cellulose, the most common organic compound on Earth, is the primary structural component of cell walls in biomass. Its amount varies from 90% (by weight) in cotton to 33% for most other plants. Represented by the generic formula $(C_6H_{10}O_5)_n$, cellulose is a long chain polymer with a high degree of polymerization (~10,000) and a large molecular weight (~500,000). It has a crystalline structure of thousands of units, which are made up of many glucose molecules. This structure gives it high strength, permitting it to provide the skeletal structure of most terrestrial biomass (Klass, 1998). Cellulose is primarily composed of d–glucose, which is made of six carbons or hexose sugars (Basu, 2010).

Hemicellulose is another constituent of the cell walls of a plant. While cellulose is of a crystalline, strong structure that is resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It is a group of carbohydrates with a branched chain structure and a lower degree of polymerization (~100–200), and may be represented by the generic formula $(C_5H_8O_4)_n$ (Klass, 1998). There are significant variation in the composition and structure of hemicellulose among different biomass. Most hemicelluloses, however, contain some simple sugar residues like d–xylose (the most common), d–glucose, d–galactose, l–ababinose, d–glucurnoic acid, and d–mannose. These typically contain 50 to 200 units in their branched structures. Hemicellulose tends to yield more gases and less tar than cellulose (Milne, 2002).

Lignin is a complex highly branched polymer of phenylpropane and is an integral part of the secondary cell walls of plants. It is primarily three–dimensional polymer of 4–propenyl phenol, 4–propenyl–2–methoxy phenol, and 4–propenyl–2.5–dimethoxyl phenol (Diebold & Bridgewater, 1997). It is one of the third important constituents of the cell walls of woody biomass. Lignin is the cementing agent for cellulose fibers holding adjacent cells together. The dominant monomeric units in the polymers are benzene rings. Lignin would generally have lower oxygen and higher carbon compared to cellulose or hemicelluloses (Basu, 2010).

The knowledge of chemical characteristics enables to perform analysis of biomass bio–energy potential. For example, Jones et al. (2006) indicated that due to the knowledge of biomass components, it is possible to predict the behaviour of biomass during pyrolysis.

The aim of this research was (i) to evaluate the adaptability of alternative non–food crops from southern areas in Estonian conditions, (ii) to study the total methane yield of selected energy crops, and (iii) to analyse the influence of biomass chemical composition on methane production.

MATERIALS AND METHODS

To investigate the suitability of different alternative crops in Estonian conditions for methane production, a plant field collection was established in 2008 at the Institute of Agricultural and Environmental Sciences of Estonian University of Life Sciences on Haplic Luvisol (Hypereutric) soil near Tartu (58° 23´N, 26° 44´E) in Estonia. The species grown in this collection field were: Jerusalem artichoke (*Helianthus tuberosus* L.), fibre hemp (*Cannabis sativa* L.) cv USO–31, energy sunflower (*Helianthus annuus* L.) cv Wielkopolski, Amur silver–grass (*Miscanthus sacchariflorus*), energy grass cv Szarvasi–1, and foxtail millet (*Setaria italica* L.). For *Helianthus tuberosus* L., *Miscanthus sacchariflorus* and energy grass cv Szarvasi–1 this was the third year of vegetation, the seeds of *Cannabis sativa* L., *Helianthus annuus* L. and *Setaria italica* L. were sown by hand on 23 May 2010. The collection plot size was $5m^2$ without replications. Mineral N fertilizer (NH_4NO_3) was applied by hand on 9 June 2010 (100kg N ha^{-1}). During the vegetation period no pesticides were applied, manual weeding was done only on the plot of *Setaria italica* L. The temperature and precipitation data of 2010 differed from the long term average. The year 2010 was a little more difficult for crop growth, with an average temperature higher than usual (in July the temperature was 5.1^oC higher than long–term average), the total precipitation in the growth period (May–August) being 277mm, i.e. 34mm lower than normal.

The height of the plants were determined before the harvest, samples for chemical analyses and methane amount determination were taken on 12 October 2010; in addition, samples of *Cannabis sativa* L. and *Helianthus annuus* L. were taken also on 18 August. The vegetation period in Estonian climatic conditions has finished by mid–October 2010; the average diurnal temperature was then below 5°C. By this time *Miscanthus sacchariflorus* was in flowering stage, *Helianthus tuberosus* L. did not flower yet, *Setaria italica* L. was in later waxy stage, the other crops were in matured stage.

The percentage of lignin, NDF and ADF in the DM of all plant samples was determined at the Plant Biochemical Laboratory of Estonian University of Life Sciences (Tecator ASN 3430; AOAC, 1990; Van Soest et al., 1991). All samples were ground with Cutting Mill SM 100 comfort (Retsch GmbH) and then with Cutting Mill ZM 200 (Retsch GmbH).

The BMP test performed in this study was based on a modified version of the guidelines described by Owen et al., 1979. The experiment was carried out in triplicate in plasma bottles with an effective volume of 575 ml. Each replica consisted of 150ml of inoculum and 0.3gTS^{-1} of substrate and 50ml of distilled water. Total volume was 200ml.

The inoculum used was collected from the anaerobic reactor of a wastewater treatment plant in Tallinn, Estonia. The inoculum was stored at room temperature, sieved through 2mm mesh and preincubated at mesophilic range (36°C) for 5 days before test setup to ensure activation and degasification of the sludge.

Energy crop samples were conditioned by drying and milling to achieve particle size of less than 1mm for homogenization of sample.

Oxygen from the headspace of the test bottles was flushed out by inducing a flow of N_2/CO_2 during 8 minutes before closing the bottles. Bottles were closed with butyl rubber stoppers and incubated during 77 days at mesophilic temperature (36°C). Gas samples were taken by connecting the test bottles to the gas chromatographer through a plastic tube attached to a needle. Gas production was analyzed by measuring the increase in pressure in the gas phase of test bottles using an absolute pressure transmitter (0–4 bar, SIEMENS). Gas composition of biogas samples were analyzed chromatographically using a gas chromatograph (Varian Inc., Model CP–4900) equipped with 2 columns: a Molsieve 5A Backflush heated column (20m x 0.53mm), and a PoraPLOT U heated column (10m x 0.53mm). Helium and argon were used as carrier gases in columns 1 and 2, respectively. Total solids (TS) and volatile solids (VS) were analyzed according to method 1684 (U.S. Environmental Protection Agency – EPA). TS were determined after drying the sample at 105°C over night. VS in organic wastes were measured as total solids minus the ash content after ignition at 550°C. pH was measured by a Sentron pH–meter 1001pH.

The data was processed using Pearson’s correlation and descriptive statistics. The linear regression model of total methane yield (LCH_4 kgTS^{-1}) was performed using R version 2.12.2 (R Development Core Team, 2011). Predicted methane yields were accompanied by 95% confidence intervals (given in parentheses).

RESULTS AND DISCUSSION

In our field collection the perennial energy crops tolerated the winter cold in Estonia. The height of different crops was 29–71% smaller in Estonian conditions than grown in southern climatic conditions (Table 1).

Table 1. The height of different energy crops in field collection and their comparison with literature.

Energy crop	In field collection	In literature	Source
Setaria italica L.	50–70	120–200	www.fao.org/
Helianthus tuberosus L.	160–170	240	www.aussiegardening.com
Energy grass cv Szarvasi–1	70–110	180–220	Kocsis et al., 2008
Miscanthus sacchariflorus	190–220	240	www.perennials.com/

Presumably the above ground biomass of these crops would be smaller in the same range, such as height in Estonian climatic conditions. The mean above ground biomass in dry matter of *Cannabis sativa* L. and *Helianthus annuus* L. in Estonian conditions depending on N fertilisation has been 3.1–10.9 and 7.7–13.5t ha^{-1}, respectively (Lauk et al., 2009).

The chemical composition of different energy crops above ground biomass is presented in Table 2. Biomass can be classified on the basis of its relative proportion of cellulose, hemicellulose, and lignin. For example, it is possible to predict the behaviour of biomass during pyrolysis by knowledge of these components (Jones et al., 2006). The lignin content of above ground biomass was influenced by plants development stage during harvest time. Lower lignin content was determined in plant samples with lower development rate. Plants derived from southern area developed much slower in northern conditions, because of smaller sum of effective temperatures by harvest time. The lignin content of later harvested *Cannabis sativa* L. and *Helianthus annuus* L. was higher than the lignin contents of plants harvested earlier. The content of hemicellulose and cellulose did not increase steadily.

In our experiment the methane yield was not significantly influenced by hemicellulose–lignin and cellulose–lignin ratio, but the positive tendency between hemicellulose–lignin ratio and methane yield was determined (r=0.66). Lower hemicellulose–lignin ratio was determined for *Helianthus annuus* L. samples in comparison with *Helianthus tuberosus* L., *Cannabis sativa* L., *Miscanthus sacchariflorus*, cv Szarvasi–1, and *Setaria italica* L.

Table 2. Cellulose (C), hemicellulose (HC) and lignin (L) content in dry matter (DM), HC/L and C/L ratio, total solids (gTS $kgFM^{-1}$)* and volatile solids (gVS $kgTS^{-1}$)* of different energy crops.

Energy crop	HC%	C %	L %	HC/L	C/L	gTS $kgFM^{-1}$	gVS $kgTS^{-1}$
Helianthus tuberosus L.	5.48	20.95	5.05	1.1	4.15	912	953
Miscanthus sacchariflorus	30.15	42.00	7.00	4.3	6.0	929	950
Energy grass cv Szarvasi–1	27.33	37.85	9.65	2.8	3.9	922	935
Setaria italica L.	31.61	33.02	5.34	5.9	6.2	918	924
Helianthus annuus L. 1**	5.18	34.06	7.72	0.7	4.4	907	911
Helianthus annuus L. 2**	7.29	27.39	8.28	0.9	3.3	905	881
Cannabis sativa L. 1**	10.83	55.00	7.15	1.5	7.7	924	944
Cannabis sativa L. 2**	10.60	53.86	8.76	1.2	6.2	925	951

* TS – total solids; FM – fresh matter; VS – volatile solids

** 1– crops harvested on 18 August; 2– crops harvested on 12 October

The accumulated methane production during 77 days of incubation at mesophilic temperature for perennial (Fig. 1) and annual (Fig. 2) energy crops was determined. Lignocellulose has been found to be slowly and often incompletely degraded under anaerobic conditions (Lynd et al., 2002). The initial degradation rate of carbohydrates between perennial crops was the lowest in the biomass of *Miscanthus sacchariflorus* (15 LCH_4 $kgTS^{-1}$ per day), but after the second day the degradation rate increased up to 19 LCH_4 $kgTS^{-1}$ per day and the high degradation rate (over 9 LCH_4 $kgTS^{-1}$ per day) continued for two weeks. The initial degradation rate of carbohydrates was the highest for the samples from perennial crop *Helianthus tuberosus* L. (105 LCH_4 $kgTS^{-1}$ per day), but after the 6th day the degradation rate decreased dramatically. In spite of different degradation rates, the total amount of CH_4 for these crops did not differ significantly (332 and 325 LCH_4 $kgTS^{-1}$, respectively).

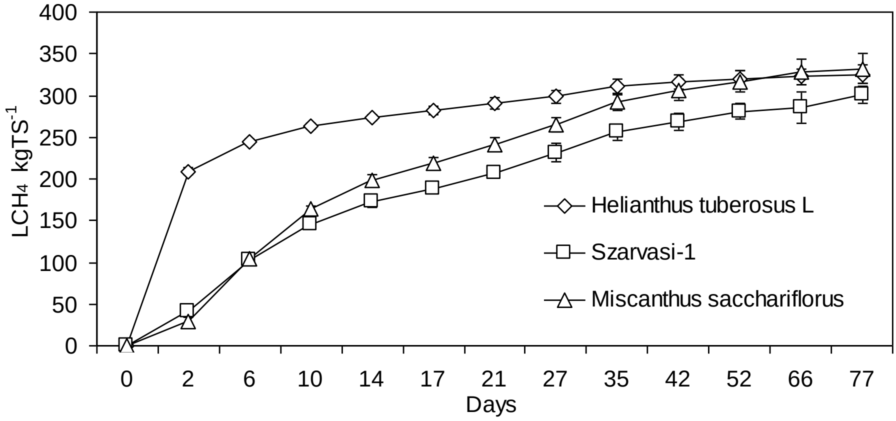

Figure 1. Accumulated methane production (LCH_4 $kgTS^{-1}$) during 77 days of incubation at mesophilic temperature for perennial energy crops.

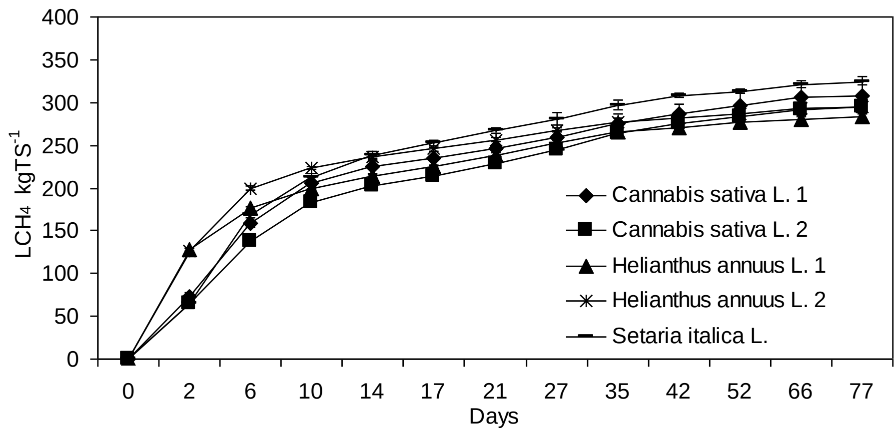

Figure 2. Accumulated methane production (LCH_4 $kgTS^{-1}$) during 77 days of incubation at mesophilic temperature for perennial energy crops.

*1– crops harvested on 18 August; 2– crops harvested on 12 October

Between the annual crops the initial degradation rate was the highest in the biomass of *Helianthus annuus* L. (63–64 LCH_4 $kgTS^{-1}$ per day), but after the 6th day the degradation rate decreased 3.5–5 times. The degradation behaviour of other annual energy crops was similar (initial degradation rate 32–36 LCH_4 $kgTS^{-1}$ per day; after the 10th day the degradation rate decreased below 6 LCH_4 $kgTS^{-1}$ per day). The highest total amount of CH_4 of annual energy crops was obtained from samples of *Setaria italica* L. (323 LCH_4 $kgTS^{-1}$), which significantly differed from the same data of *Helianthus annuus* L. (284–295 LCH_4 $kgTS^{-1}$).

High total methane yield was determined from samples of *Miscanthus sacchariflorus*, *Helianthus tuberosus* L. and *Setaria italica* L. (Fig. 3) because the development rate of these crops was slow and development stage more suitable for methane production. Therefore, postponing the harvest time was more suitable for southern derived plants whereby the biodiversity of energy crops improves the operational management for farmers (Klimiuk et al., 2010). Methane yield was influenced by harvest time also. Lignin content was higher and methane yield lower in later harvested *Cannabis sativa* L. In spite of higher lignin content, the methane yield of later harvested *Helianthus annuus* L. was higher than that of earlier harvested samples. This may have been caused by the oil composition; therefore, further field studies are needed. These results suggest that proper harvest time should be considered for optimal biogas production (Amon et al., 2007; Oslaj et al., 2010).

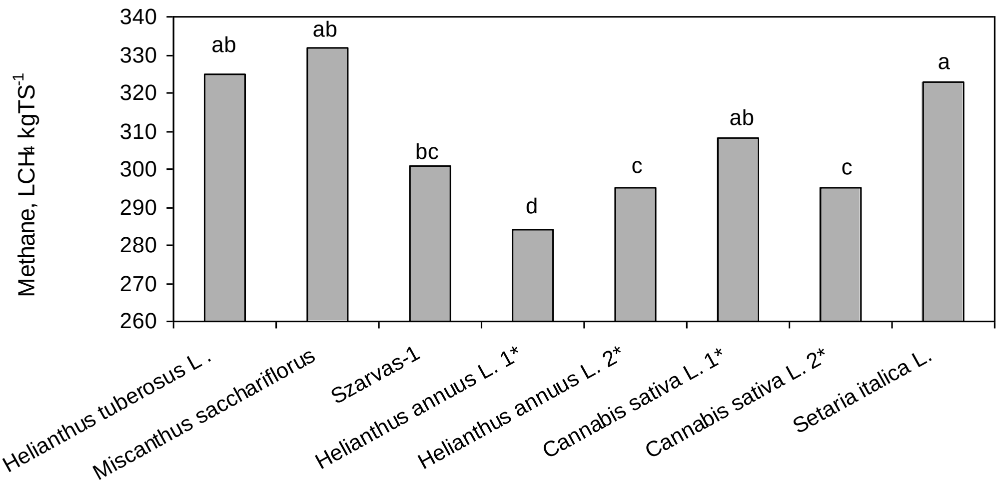

Figure 3. Total methane yield ($LCH_4\ kgTS^{-1}$) of annual and perennial energy crops (different letters note significant differences between columns).

*1– crops harvested on 18 August; 2 – crops harvested on 12 October

Our study indicated methane yield dependence on biomass lignin content. The correlation between methane yield and lignin content was significantly negative ($r= -0.70$; $P<0.05$). In comparison, hemicellulose and cellulose content did not influence methane yield production significantly. The efficiency of lignin conversion to biogas depends on pre–treatment and hydraulic retention time (Klimiuk et al., 2010).

A simple linear regression with lignin content as explanatory variable was performed to estimate total methane production during 77 days of incubation (Table 3, Fig. 4). Residual standard error (SE) of the model was 13.27 on 6 degrees of freedom. The expected average methane yield of biomass with lignin content 5% was estimated to be 325.61 (304.05–347.17) $LCH_4\ kgTS^{-1}$. In comparison, the same data with biomass lignin content of 10% was estimated to be 288.18 (264.89–311.47) $LCH_4\ kgTS^{-1}$. Therefore, the expected average decrease in the methane yield is 7.49 $LCH_4\ kgTS^{-1}$ with the increase of 1% lignin content in biomass. Lignin content has been included as an explanatory variable in regression models to analyse the biogas yield of selected cultures also in previous studies. Nallathambi Gunaseelan (2006) compared regression models with different variables, including lignin content and also the ratio of lignin and acid–detergent fibre.

Lignin has been declared to be one of the drawbacks of using lignocellulosic materials in fermentation of several studies (Martínez–Pérez et al., 2007; Taherzadeh & Karimi, 2008); therefore, pre–treatment (e.g. thermophysical procedures) is suggested to dissolve lignin from the biomass (Bauer et al., 2007). Substrates with very high lignin content are not biodegraded by bacteria during anaerobic digestion resulting in low biogas production and consequently low methane yield (Lübken et al., 2010). However, celluloses and hemicelluloses can be relatively easily bio–converted into methane and carbon dioxide (Amon et al., 2007). Degradability rates of cellulose and hemicelluloses depend on their lignin association. Crystalline form cellulases can be degraded more easily into propionate and butyrate than lignin–incrusted forms (Jördening & Winter, 2005).

Table 3. Regression model of total methane yield (LCH_4 $kgTS^{-1}$) during 77 days of incubation at mesophilic temperature (adjusted $R^2 = 0.40$, $P = 0.05$).

	Coefficient	SE of coefficient	P–value
Intercept	363.041	23.671	4.85e–06
lignin	–7.486	3.149	0.0549

SE – standard error

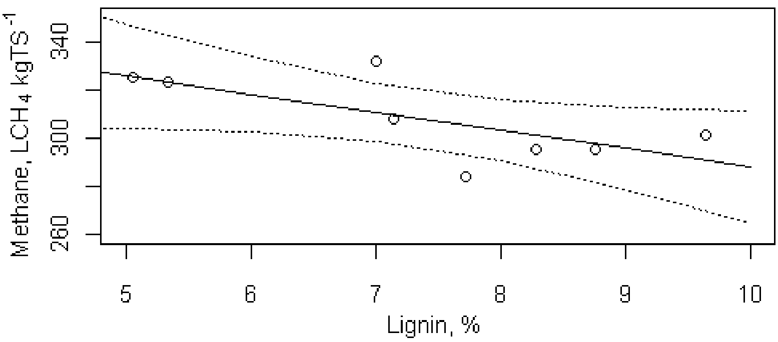

Figure 4. Regression model with confidence bands of total methane yield (LCH_4 $kgTS^{-1}$) during 77 days of incubation at mesophilic temperature.

CONCLUSIONS

1. The correlation between methane yield and lignin content was significantly negative. The expected average decrease in the methane yield is 7.49 LCH_4 $KgTS^{-1}$ with the increase of 1% lignin content in biomass.

2. The highest methane yield was obtained from *Miscanthus sacchariflorus*, *Helianthus tuberosus* L., and *Setaria italica* L. samples.

3. The alternative non–food crops from southern areas gave higher methane yield in Estonian conditions than *Cannabis sativa* L. and *Helianthus annuus* L., because

their development rate was slower, lignin content smaller and development stage more suitable for methane production. The variety of plant biomass improves the operational management of biogas plants and favours agro–ecosystem biodiversity.

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