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GREEN HARVESTS OF THREE PERENNIAL ENERGY CROPS AND THEIR CHEMICAL COMPOSITION.

Ivan Kron[1*], Pavol Porvaz[2], Alena Kráľová-Hricindová[1], Štefan Tóth[2], Ján Sarvaš[1], Matej Polák[1]

[1] Research and Educational Center for Bioenergy (RECB), University of Economy in Bratislava, Kapušany 568, SVK-08212, Kapušany, Slovakia

[2] National Agricultural and Food Center (NAFC) Lužianky - Agroecology Research Institute (ARI), Michalovce, Špitálska ul. 1723, SVK-07101, Michalovce, Slovakia

[*]Corresponding author

ABSTRACT

Aim: To analyze the chemical composition of three potential energy crops for bioenergy (biogas or bioethanol) production, namely Miscanthus x gigantheus, Sida hermaphrodita, Arundo donax during three phases of maturation.

Methods: The study consisted of two parts: field experiments (in short: preparation of soil, planting of energy crops, fertilization, and green harvests (28.7.2014, 15.8.2914, 8.9.2914, respectively) – three times in different stages of flowering) and laboratory determinations (dry matter content, CHNS(O) analysis, heavy metals and toxic elements determination, and heat combustion evaluation).

Results: Dry matter of energy crops increase with later harvest, the highest dry matter found for Miscanthus. CHNS analysis confirmed highest values of carbon for Miscanthus on contrary to heavy metal determinations with lowest metal contests for Miscanthus. Heat of combustion and calculated heating values correlated very well with carbon content of energy crops. Theoretical yield of biomethane was highest for Miscanthus as well.

Conclusion: From our study follows that the best energy crop for 2nd generation of biofuels from our choice is Miscanthus x giganteus, because of the highest content of dry matter, better elemental composition (CHNS(O) analysis), less toxic elements and heavy metals, the highest combustion heat, and theoretically would produce more biomethane in all three green harvests than the others.

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Keywords: Green harvests, *Miscanthus x gigantheus*, *Sida hermaphrodita*, *Arundo donax*, chemical analysis.

1. INTRODUCTION

Biofuels made from biomass are currently the only real direct substitute for fossil fuels in transport and are already incorporated into the infrastructure and fuel supply. First-generation biofuels, produced in Europe using the most economical production method, result in greenhouse gas emissions 35-50 % lower than the conventional fuels they replace. Directive 2003/30/EC on the promotion of the use of biofuels or other renewable fuels for transport sets for all member countries target for biofuels in the total energy consumption of motor fuels for 2010 to 5.75 %. Based on the Spring Summit 2007 for 2020 this value increased as an obligatory minimum target of 10 % to be achieved in a cost effective manner.

Such an objective is likely to be achieved only with the use of 2nd generation biofuels. The main difference between the current biofuels and 2nd generation biofuels is that they will not be produced only from crops and seeds. The entire plants, including plant waste, will serve as a source.

In the world the production of biofuels starts to apply non-traditional crops such as *Miscanthus x gigantheus*, *Sida hermaphrodita*, *Arundo donax*, with a high content of structural fiber [Lewandovski et al., 2003; Dahl and Obernberger, 2004; Ambrose et al., 2005; Angeliny et al., 2005; Porvaz et al., 2008; Prčík and Kotrla, 2015; Borkowska et al., 2009; Ge et al., 2011; Brosse et al., 2012; Wawro et al., 2013]. These crops have several advantages, namely they are perennial, growing on poor grade soils with a low cost of fertilization. They can be used for various purposes including biofuel production. Therefore they were chosen for our field and laboratory experiment for their chemical composition determination and later for bioethanol production.

Reasons for green harvesting: In the case of building a plant for bioethanol/biogas production, continuous supply of biomass is necessary during whole year. If only winter harvest would be preferred then a big storage place is required for biomass. Winter harvest has lowest humidity of the biomass but also some losses of biomass are possible, moreover, biomass cannot be silage. It was estimated that in winter, harvestable biomass can decrease 30 % to 50 % as stems can be broken with wind and weather [Heaton et al., 2014]. Hence we decided to focus on green harvests and the chemical composition of chosen energy crops.

1.1 Botanical taxonomy of energy crops

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Miscanthus x giganteus of Miscanthus sp. genus is a perennial tuft grass with low water-mineral needs, resistant to diseases and pests, which can be grown on poor grade soils. It is characterized with high growth of biomass, what leads to possible improvement of the cost-efficiency of the production of second generation biofuels. *Miscanthus* was first introduced from Japan and cultivated in Europe in the 1930s [Brosse et al., 2012]. The actual species of *Miscanthus x giganteus* is a hybrid bred from two other species i.e. diploid *Miscantheus sinensi* and tetraploid *Miscanthus sacchariflorus* and this hybrid can grow to the height of 3.5 m in one vegetation season [Wawro et al., 2013]. It is a non-traditional plant which is used as feed, technical crop and for soil protection, moreover, it is noninvasive [Brosse et al., 2012]. It can be grown under conditions of temperate climate zones [Strašil, 2009]. In Slovakia, farmers have already first experience with large-scale cultivation of *Miscanthus*, which is particularly suited to maize production area [Porvaz et al., 2008; Porvaz et al., 2012, Kotrla and Prčík, 2013]. *Miscanthus x gigantheus* is generally considered for a non-invasive species [Smith and Barney, 2014].

Sida hermaphrodita (L.) Rusby (or Virginia fanpetals, Virginia mallow) is one of the perennial species adjusted to over ten years of cultivation tolerant to quality of soil. This species comes from the United States of America [Borkowska et al., 2009]. It is unconventional fiber plant which use is as feed, pharmaceuticals, technical, and honey crop and for soil protection. Also it can be grown under conditions of temperate climate zones. The sum of temperatures needed for the flowering is 1550-1650 ° C, and for full maturity 2500 - 2700 ° C [Usťak, 2008].

Arundo donax (L.), also known as giant reed, is tall, perennial grass species of type C_3. It requires high intensity of solar radiation, high rainfall totals and land enriched by nitrogen [Lewandowski et al., 2003; Ambrose et al., 2005; Benton et al., 2006]. However, *arundo* is considered to be invasive species [Ambrose et al., 2005; Pilu et al., 2012].

1.2 Material and methods

1.2.1 Crop culture

An experimental station Milhostov is located in the central part of East Slovakian Lowland at an altitude of 101 m (48° 40' N; 21° 44' E) north-west of the town Trebišov. The experimental site belongs to warm and very dry continental climate of lowland region T 03 [Linkeš et al., 1996]. The average annual air temperature in the period 2006 - 2009 was 10.3 ºC and the average temperature in the growing season reached 17.3 ºC. Long-term annual rainfall (1961 - 1990) for Milhostov is 550 mm and during the growing period 348 mm [Mikulová et al., 2008].

On the territory of the experimental locality are soils of Gleyic Fluvisol (FM_G) type, resulting from long-term exposure of groundwater and surface water at very heavy alluvial sediments with adverse physical, hydro-physical and chemical properties. Agronomic properties of these soils

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are mainly due to a proportion of clay particles in the whole soil profile or in the subsoil, respectively. Gleyic Fluvisol in Milhostov are heavy or very heavy, clay-loamy soils with an average content of clay particles higher than 53 %. Topsoil has a lumpy texture with a high binding capacity, being hardly permeable in the whole profile. The soil profile at depth from 0.7 to 0.8 m is dark gray to yellowish-gray clay. The level of underground water is high. Soil and climate conditions of the experimental locality Milhostov were described [Kotorová & Šoltysová, 2011; Kotorová et al., 2011].

1.2.2 Field experiments

In 2013 the experiment has begun with planting energy crop rhizomes to a clamp 1.0 x 1.0 m. Technology of energy crop growing was ensure from the year of establishment to producing years by these working steps:

First year of growing – first crop year for *Miscanthus x giganteus, Arundo donax and Sida hermaphrodita*: Application of herbicide on the site (Roundup 3 *l*.ha^{-1}). Autumn – 25 cm deep plowing, shearing and harrow, application of fertilizers (NPK – 200 kg.ha^{-1}), plantation of rhizomes, herbicide treatment, line weeding, crushing of plant matter.

Second crop year and following: fertilization with nitrogen in a dose by experiment variant (divided nutrition nitrogen: 30 kg nitrogen before sowing, and 30 kg full tillering), harvest.

1.2.3 Plantation and chemical protection

Miscanthus x giganteus - vegetation in the second utilitarian year 2014

Date of plantation 18.5.2013. Herbicidal spray on 12.8.2013 - active substance: tembotrione 44 g/*l* safener and isoxadifen-ethyl.

Sida hermafrodita - vegetation in the second utilitarian year 2014

Date of plantation 19.5.2013. Herbicidal spray on 19.7.2013 - active substance: bentazone 480 g/*l*. Line weeding on 12.8.2013.

Arundo donax - vegetation in the second utilitarian year 2014

Date of plantation 20.5.2013. Herbicidal spray on 12.8.2013 - active substances: rimsulfuron 500 g and thifensulfuron-methyl 250 g.

1.2.4 Harvesting of energy crops

First harvest on 28.7.2014, second harvest on 15.8.2014, third harvest on 8.9.2014.

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Miscanthus x giganteus: First harvest - growth stage (BBCH) 51 – Beginning of spikelet ejection: the tip of spikelet emerges from the sheath or it breaks through laterally. Second harvest - growth stage (BBCH) 59 - end of spikelet ejection: the spikelet is completely visible. Third harvest - growth stage (BBCH) 65 - middle of flowering: 50 % of mature buds.

Sida hermafrodita: First harvest - beginning of butonization. Second harvest - beginning of flowering. Third harvest at full flowering.

Arundo donax: First harvest - growth stage (BBCH) 49 - tips of awns: awns are visible above the flag leaf ligule. Second harvest - growth stage (BBCH) 55 - middle of spikelet ejection: the base is still in the sheath. Third harvest - growth stage (BBCH) 61 - beginning of flowering: first stamens are visible.

1.2.5 Laboratory determinations

Samples of energy crops of each harvest were firstly used for determination of the dry matter content by an electronic moisture meter KERN MLB N (Germany). After drying at 120 °C for several hours to constant weight the plant material was milled with a knife mill RETSCH GM 200 (Germany) and homogenized by a mortar and piston. Samples of homogenized energy crops were used for CHNS(O) analysis with Thermo Scientific Flash 2000 (UK) and heat combustion with a calorimeter IKA C200 (IKA, Germany). Mineralization of 0.5 g homogenized plant material with a mixture of concentrated hydrogen peroxide and concentrated nitric acid (2 ml and 6 ml, resp.) was performed with microwave digestion system Ethos One Milestone (Italy). Solutions after digestion were diluted with demineralized water into total 50 ml volume and used for determination of cadmium, chromium, copper, lead, nickel and arsenic with atomic absorption spectrometer ZEEnit 700 P (Analytik Jena, Germany) and mercury with DMA 80 Milestone (Italy).

All samples were analyzed at least three times, means and standard deviations were calculated and tabled – see lower. Note: The samples of dry matter of energy crops are not fully homogeneous, in spite of homogenization, therefore relatively high standard deviations are found out in our determinations.

1.3 Results and discussion

All our results for the energy crops are shown in the Tables 1 – 7.

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Table 1: Harvest of energy crops and their (N = 3, mean ± S.D.) dry matter content.

	Harvesting and Dry Matter Content (in %)		
Crop	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Miscanthus x giganteus	27.57 ± 0.14	39.95 ± 0.16	48.99 ± 0.15
Sida hermafrodita	26.73 ± 0.13	35.02 ± 0.21	37.75 ± 0.13
Arundo donax	24.10 ± 0.07	33.16 ± 0.13	41.32 ± 0.21

In our field experiment three green harvests were performed within almost two months in three phenophases of selected energy crops. The green harvest (higher content of water than dry mass) could have some advantage in pretreatment and fermentation of plant material [McKendry, 2002]. In all nine specimens from harvests were determined firstly dry matter contents. Predictably, the contents steadily increased with time of harvest – see Table 1. The highest content of dry matter was recorded for *Miscanthus* in all three harvests in comparison to the other two plants. During two months the dry matter almost doubled. Practically the same observations of dry matter (DM) increase were made by Nassi o Di Nasso et al. 2011 for *Miscanthus* and *Arundo* from July to September on next year: increase of DM from 28 % to 53 % for *Miscanthus* and from 31 % to 45 % for *Arundo*, respectively. Small differences can be ascribed to different weather conditions in Pisa, Italy and Milhostov, East Slovakia.

During senescence and harvests in winter time the moisture of *Miscanthus* can be as low as 5 %. For the reason and low ash content is *Miscanthus* generally considered for a key candidate crop for biomass conversion to bioenergy [Dahl & Obernberger 2004; Brosse et al., 2012, Arundale et al., 2015; Purdy et al., 2015]. On the other hand, Angelini et al., 2005 evaluated *Arundo* yield in response to fertilization, harvest time and plant density. They found out that the crop yield increased by 50 % from establishment period to the second year of growth. Fertilization enhance dry matter yield mainly in the initial period. The biomass water content was affected by harvest time, decreasing from autumn to winter by approximately 10 %. Higher dry matter yields were achieved with density of 20,000 plants per ha than with 40,000 per ha, that one could explain by better drying at less dense of plants. On the other side, the Illinois study showed that environment has little effect on biomass composition of *Miscanthus* across soil types, nitrogen fertilization and harvest time [Arundale et al., 2015].

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Table 2: CHNS analysis of dry matter (N = 4, mean ± S.D.) of *Miscanthus x giganteus* related to the harvest time.

	Harvesting and CHNSO Content (in %)		
Miscanthus x giganteus	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Carbon	45.28 ± 0.66	44.52 ± 0.76	44.25 ± 1.03
Hydrogen	5.74 ± 0.09	5.60 ± 0.17	5.68 ± 0.15
Nitrogen	0.29 ± 0.35	1.00 ± 0.89	0.96 ± 1.65
Sulphur	0	0.02 ± 0.03	0.02 ± 0.03
Oxygen*	48.69	48.86	49.09

*Calculated value to 100 %

Table 3: CHNS analysis of dry matter (N = 4, mean ± S.D.) of *Sida hermafrodita* related to the harvest time.

	Harvesting and CHNSO Content (in %)		
Sida hermafrodita	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Carbon	43.74 ± 0.50	43.02 ± 0.92	40.94 ± 0.40
Hydrogen	5.68 ± 0.07	6.44 ± 0.67	6.08 ± 1.01
Nitrogen	0.77 ± 0.30	1.37 ± 1.03	1.62 ± 1.46
Sulphur	0	0.03 ± 0.04	0.10 ± 0.07
Oxygen*	49.81	49.14	51.26

*Calculated value to 100 %

Table 4: CHNS analysis of dry matter (N = 4, mean ± S.D.) of *Arundo donax* related to the harvest time.

	Harvesting and CHNSO Content (in %)		
Arundo donax	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Carbon	44.19 ± 0.58	42.80 ± 0.33	42.60 ± 1.08
Hydrogen	5.70 ± 0.06	6.13 ± 0.34	5.70 ± 0.34
Nitrogen	1.29 ± 0.62	1.40 ± 0.78	1.27 ± 1.31
Sulphur	0	1.53 ± 1.74	0.38 ± 0.27
Oxygen*	48.82	49.54	50.05

*Calculated value to 100 %

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Our elemental CHNS analysis of the energy crops showed slight decrease of carbon on contrary to nitrogen, sulfur and oxygen that increased with time of green harvest – see Tables 2 - 4. More nitrogen and sulfur probably means more proteins (amino acids). The highest contents of carbon and lowest contents of nitrogen and oxygen were recorded for *Miscanthus x gigantheus* in all three harvests in comparison to *Sida* and *Arundo*. According to Brosse et al., 2012 during winter harvest the major elemental composition based on dry matter in *Miscanthus* ranged from 47.1 to 49.7 % for carbon, from 5.38 to 5.92 % for hydrogen, and from 41.4 to 44.6 % for oxygen. Similarly, Bilandzija et al., 2016 determined also for *Miscanthus* carbon range from 46.50 to 50.05 %, hydrogen content from 3.57 to 4.15 %, nitrogen from 0.20 to 0.54 %, oxygen range from 45.26 to 49.31 % and sulfur around 0.07 % during three harvests (November, February and March). Our results for harvests during flowering of *Miscanthus* are in compliance for the content of hydrogen [Brosse et al., 2012], sulfur and nitrogen [Bilandzija et al., 2016], but lower carbon and higher oxygen contents were recorded in our case. Generally, the later harvest the lower content of carbon in all studied energy crops. However, the changes in elemental composition during the senescence of *Miscanthus* and *Arundo* were not reflected in variation of composition of cellulose, hemicellulose and lignin that were stable from July to February [Nassi o Di Nasso et al., 2011]. Time of harvest is also important for content of starch in rhizomes of *Miscanthus*, which is highest in November, while in winter time is depleted, especially in cooler regions and forming a problem in germination during spring [Purdy et al., 2015].

Determination of heavy metals and toxic elements is very important, because the elements can be released during hydrolysis and fermentation steps and inhibit the process. Arsenic was detected only in traces in a sample of *Sida hermafrodita* in the first harvest. The highest content of heavy metals of three studied plants was found in the samples of *Sida hermafrodita*, while the lowest in *Miscanthus x gigantheus* – see Tables 5 - 7.

Table 5: Heavy metal content (in mg/kg) of dry matter (N = 3, mean ± S.D.) of *Miscanthus x giganteus* related to the harvest time.

	Harvesting and Heavy Metal Content (in mg/kg)		
Miscanthus x giganteus	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Cadmium	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.12
Chromium	0.28 ± 0.21	0.20 ± 0.52	0.21 ± 0.60
Copper	3.50 ± 0.62	1.80 ± 0.83	1.86 ± 0.36
Lead	0.14 ± 0.03	0.14 ± 0.13	0.05 ± 0.18
Nickel	0.05 ± 0.48	0.02 ±0.20	0.02 ± 0.23
Mercury (in µg/kg)	0.5	0.4	0.4

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Table 6: Heavy metal content (in mg/kg) of dry matter (N = 3, mean ± S.D.) of *Sida hermafrodita* related to the harvest time.

	Harvesting and Heavy Metal Content (in mg/kg)		
Sida hermafrodita	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Cadmium	0.20 ± 0.03	0.23 ± 0.03	0.20 ± 0.02
Chromium	0.45 ± 0.23	0.26 ± 0.22	0.27 ± 0.48
Copper	6.50 ± 2.36	3.33 ± 0.88	4.79 ± 0.29
Lead	0.36 ± 0.21	0.24 ± 0.32	0.27 ± 0.19
Nickel	0.97 ± 1.86	0.64 ±1.98	1.30 ± 1.36
Mercury (in μg/kg)	1.0	0.8	1.2

Table 7: Heavy metal content (in mg/kg) of dry matter (N = 3, mean ± S.D.) of *Arundo donax* related to the harvest time.

	Harvesting and Heavy Metal Content (in mg/kg)		
Arundo donax	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Cadmium	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.02
Chromium	0.37 ± 1.03	0.24 ± 0.35	0.02 ± 0.14
Copper	6.70 ± 0.50	4.39 ± 0.61	3.21 ± 0.33
Lead	0.21 ± 0.11	0.11 ± 0.03	0.09 ± 0.13
Nickel	1.00 ± 0.72	2.05 ±2.14	0.59 ± 1.47
Mercury (in μg/kg)	1.2	1.2	1.2

Arundo and *Sida* accumulated copper almost twice better than *Miscanthus*. Higher accumulation of copper by *Sida* in comparison with other plants was confirmed by Antonkiewicz and Jasiewicz, 2002. Barbosa et al., 2015 tested *Arundo* and *Miscanthus* species (including *Miscanthus x giganteus*) for their tolerance and phytoremediation capacity in soils contaminated with zinc, chromium and lead. *Arundo* biomass production was significantly reduced only with 600 mg Cr kg^{-1}. Both crops showed the metal accumulation pattern in the order Zn > Cr > Pb. They concluded that *Arundo* and *Miscanthus* species are convenient for phytostabilization of heavy metals soil contamination.

Miscanthus sinensis also proved good extraction ability for chromium in heavy metal contaminated soil of a zinc antimony mine in China [Xu and Shaohong, 2015].

The heat of combustion (kJ/g or MJ/kg of dry matter) is the best parameter for consideration of bioenergy production from biomass. The highest values were recorded for *Miscanthus x*

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gigantheus, followed by *Arundo donax* and *Sida hermafrodita* in all three harvests – see Table 8. A bit higher values for *Arundo* and *Miscanthus* were recorded by Dahl and Obernberger, 2004, 19.8 and 19.6 MJ/kg of dry mass, respectively.

Table 8: Heat of combustion and calculated higher heating value (in parentheses) (in kJ/g or MJ/kg) of dry matter of energy crops related to the harvest time.

	Harvesting and Heat of Combustion (in kJ/g or MJ/kg)		
Crop	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Miscanthus x giganteus	18.583 (18.408)	18.055 (18.076)	17.671 (18.054)
Sida hermafrodita	17.440 (17.918)	17.054 (18.204)	16.318 (17.366)
Arundo donax	18.025 (18.042)	17.907 (17.930)	17.214 (17.584)

Biomass heating value is connected with the elemental composition of energy crops [Brosse et al., 2012], therefore the higher heating value (HHV) was calculated by the formula [Sheng and Azevedo, 2005]: HHV (MJ/kg) = 0.3137 C% + 0.7009 H% + 0.0318 O% - 1.3675. Values C%, H%, O% were taken from Tables 2 – 4 and HHV results are shown in Table 8 (in parentheses). Our calculated HHVs for *Miscanthus* agree very well with values (18.11 – 18.22 MJ/kg of dry mass) published by Bilandzija et al., 2016.

Carbon is the most important element of biomass for production of heat; accordingly it must correlate well with combustion heat (CH) and HHV. The correlation equation for combustion heat (N = 9): CH = 0.4629 C% - 2.5415, the correlation coefficient R = 0.897, while for higher heating value (N = 9) is: HHV = 0.2088 C% + 8.8757, the correlation coefficient R = 0.862. Both correlations are very significant ($P<0.001$), slightly better is the relationship between determined combustion heat and relative carbon content than the relationship of calculated higher heating value and relative carbon content in dry matter of energy crops.

Similarly can be calculated theoretical yield of biomethane per 1 kg of dried tested energy crops from the values obtained in CHNS analysis by [Frigon & Guiot, 2010] – see Table 9.

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Table 9: Theoretical yield of methane per 1 kg of dry matter (by [Frigon & Guiot, 2010])

	Harvesting and yield of methane (in m^3/kg of DM)		
Energy crop	I. harvest - 28.7.2014	II. harvest - 15.8.2014	III. harvest - 8.9.2014
Miscanthus x giganteus	0.186	0.174	0.171
Sida hermafrodita	0.165	0.160	0.130
Arundo donax	0.170	0.147	0.149

Our results show that from our chosen 2nd generation energy crops seems to be the best for production of bioethanol *Miscanthus x giganteus.* Its advantage is highest content of dry matter, less toxic elements and heavy metals (so less negative influence on fermentation process), and the highest combustion heat. Disadvantage of *Sida* is accumulation of toxic elements which is a threat for fermentation, while *Arundo* is invasive species. In spite of this all three energy crops will be tested also for production of bioethanol. Currently we work on hydrolysis and fermentation steps of the energy crops, which we intend to publish in a following part.

1.4 Conclusion

From our study follows that the best energy crop for 2nd generation of biofuels from our selection is *Miscanthus x giganteus*, because of the highest content of dry matter, better elemental composition (CHNS(O) analysis), less toxic elements and heavy metals, the highest combustion heat, and theoretically would produce more biomethane (and likely more bioethanol as well) in all three green harvests during flowering.

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