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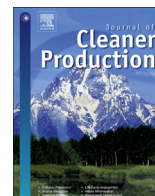
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## Green biomass to biogas – A study on anaerobic digestion of residue grass



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### ABSTRACT

Sustainable management in the biogas production via anaerobic digestion process intends the use of alternative biomass sources that are not competitive with food production. The aim of this study is to investigate the application of the abundant-quantity residue in more sustainable production of heat and electricity along with the production of the digested substrate as a fertiliser. The study has been divided into several sequential steps. First, the grass samples have been collected at the following locations: uncultivated land, river embankment and highway verge. The greatest grass yield has been determined for the riverbank grass, with an average value of 19 t/ha of fresh mass and 2.6 t/ha of dry mass. Next, the chemical characterisation of the collected residue grass and the laboratory batch mono and co-digestion tests with maize silage and cattle slurry have been conducted. The results show that all grass samples have satisfying digestive parameters (C/N ratio between 16.6:1 to 22.8:1) with the low presence of impurities, which makes them suitable for biogas production. The following biochemical methane potential in mono-digestion of residue grass has been recorded: uncultivated land (0.275 Nm<sup>3</sup>/kgTS), riverbank (0.192 Nm<sup>3</sup>/kgTS) and highway verge (0.255 Nm<sup>3</sup>/kgTS). The control of the process has been improved in co-digestion tests, by avoiding acidification in the first days of the operation. The estimation of kinetic parameters in mathematical modelling has shown that the degradation of residue grass shows some different parameters compared to the previous study. The model results for the gas phase show some small deviations compared to the experimental data. Based on the life cycle analysis results it can be concluded that there are perspectives in the use of residual grass compared to maize silage in the production of heat and electricity, especially in the improvement of ecosystem quality.

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## 1. Introduction

A recent study has shown that anaerobic digestion (AD) is likely to be one of the most promising technologies for biomass energy recovery, especially on farms (Massimo and Montorsi, 2018). Also, animal manure is better suited as an AD substrate instead of its direct use as a fertiliser. It contains significant concentrations of nutrients and pathogens (Neshat et al., 2017) and could cause contamination of ground waters and soil (Holm-Nielsen et al., 2009). Storing the manure in the open air results in methane and carbon dioxide emissions through the process of self-remediation (Burg et al., 2018). Using animal manure as a feedstock for the

AD, several negative impacts on the environment could be reduced; emissions of carbon dioxide, methane and nitrous oxide; reduction of waste, odour; destruction of pathogens (especially when the AD runs at thermophilic conditions) and better fertilisation effect (Bochmann and Montgomery, 2013). On the other hand, use of only animal manure in the AD has some disadvantages, and one of the major is low carbon to nitrogen ratio (C/N) (Neshat et al., 2017). Cattle manure appears to be a major substrate for biogas plants, especially in the intensive-farming countries (Franco et al., 2018). To increase relatively low biogas yield from mono-digestion of manure (10–20 m<sup>3</sup>/t of fresh manure) pretreatment methods could be applied, co-digestion with other biodegradable organic substrates or combination of both (Ormaechea et al., 2018).

Energy crops have been largely used as lignocellulosic biomass feedstock in the production of biogas via an AD in recent years. Abundant quantities of lignocellulosic biomass and respective

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biochemical methane potential (BMP) from biomass biodegradation, point to the promising feedstock in the production of energy-rich methane gas. It has been calculated that the annual global production of dry biomass exceeds  $2.00 \cdot 10^{11}$  t (Kumar et al., 2008) and thus there is a significant potential for lignocellulosic biomass to be investigated in the AD and sequentially used in biogas production. Biodegradation of different types of lignocellulosic biomass depends on the chemical structure, primarily on the cellulose content, hemicellulose, lignin and C/N ratio, as it has been presented for various organic substrates (Karthikeyan and Visvanathan, 2013).

Residue grass belongs to a group of lignocellulosic biomass and could be profitably used for the more sustainable production of bioenergy in biorefineries (Nimmanterdwong et al., 2017). Average production of  $500 \div 600$  m<sup>3</sup> of biogas per t of VS could be achieved from the AD of residue grass (Mattioli et al., 2017). Also, methane content of the generated biogas ranges between 52% and 56%, similar to maize silage (L.E.E. SÀRL, 2018), feedstock often used in biogas plants (Bull, 2008) while it could be used as fodder to feed ruminants. Analyses have shown that the higher biomass yields could be achieved in the low-nature quality areas and the nutrient-rich soils.

Among the promising type of residue grass in the AD supply chain is the riverbank grass (Boscaro et al., 2018). Fieldwork has shown that the average yield of green biomass on the riverbank was around 13 t/ha. The average dry matter (DM) content in the riverbank grass was 37% which gives the dry mass yield of around 4.8 t/ha. The overall results pointed to the conclusion that the energy recovery of grass biomass could decrease the dependency of the AD supply chain on the energy crops while obtaining a positive energy return (Meyer et al., 2014). Antagonistic and synergistic effects on biogas and methane production from batch anaerobic co-digestion of cattle and pig slurries with grass silage have shown that the replacement of cattle slurry with grass silage increased the biogas and methane yields (Himanshu et al., 2018).

Besides several experimental works on the AD, various studies based on mathematical modelling of the AD have been performed. Mathematical modelling of the AD of biodegradable matter describes the interactions between physical and biological mechanisms (Lauwers et al., 2013). Typically, Anaerobic Digestion Model No. 1 (ADM1) is applied for the mathematical description of the process. ADM1 describes the reactions occurring in an AD by assuming a perfect mixing and consequently homogenous reactor mixture. The components in the process are expressed regarding their Chemical Oxygen Demand (COD) or molar concentrations. Recent studies on modelling of the AD using ADM1 have been applied to several substrates: blackwater and rotten vegetable (Feng et al., 2006); grass silage (Koch et al., 2010); a mixture of municipal waste and grease (Nordlander et al., 2017); microalgae (Mairet et al., 2011) and many others. ADM1 is available in Matlab and Simulink and water-related simulation software such as WEST, BioWin and AQUASIM.

Life Cycle Assessment (LCA) is a useful tool for improving the biogas production chain, with the main focus on the environmental performance and eco-efficiency (Huttunen et al., 2014). There have been several LCA studies on biogas production, such as LCA study on co-digestion of fresh algae with animal manure (Cappelli et al., 2015). LCA-based mixed integer programming (MIP) mathematical model has been applied to investigate sustainability of the biogas production from environmentally harmful raw materials where it was shown that the integrated biogas production with included auxiliary facilities led to a significant eco-profit in the large-scale applications (Čuček et al., 2011). Evaluation of replacing energy crops with macroalgae at a real biogas plant has been performed using the LCA approach where sustainable energy

production and lower environmental effects have been obtained compared to energy crops, but only if microalgae are regionally accessible (Ertem et al., 2017).

The focus of this study is on the use of residue grass as a replacement for maize silage in the AD. The grass samples have been collected from the areas that do not compete with the food production: uncultivated land, the Sava riverbank in the city of Zagreb and highway verge. The study includes determination of the fresh and dry yield of residue grass biomass, chemical characterisation of residue grass, determination of biogas yield and biogas composition from the residue grass in the AD together with the application of ADM1 model to describe the AD and compare the modelling results with the experimental results. In the end, LCA has been used to determine the environmental effects of biogas production from residue grass in the production of heat and electricity.

It is worth noting that most of the studies in this area include experimental investigations, mathematical modelling and life-cycle analysis, each of them separately, or two of them combined. A novelty in this study is combining all three approaches to evaluate the use of the alternative substrate in the sustainable production of biogas and digestate.

## 2. Materials and methods

In this section, an overview of applied methods is presented. First, the grass yield has been evaluated, and the sampling procedure has been determined. After the samples have been collected and stored, elemental analysis and analysis of heavy metals have been performed. Before setting up a batch AD experiment, the preparation of feedstock and inoculum has been conducted. During the AD, biogas yield, biogas composition and reactor pH have been monitored. Finally, mathematical modelling of biogas production has been performed, and the results of the mathematical model and experimental process have been compared.

### 2.1. Grasslands and grass sampling

Three types of grasslands have been used for valorisation in this research: uncultivated land, riverbank and highway verge. Each of the grasslands is located nearby the capital city of Croatia, Zagreb. The chosen locations of grasslands are not suitable for food crops production or feed purposes, and thus their application in the AD is in accordance with the sustainability principles. The grass samples have been collected at the end of April 2018. A metal frame of the internal area of 2 m<sup>2</sup> has been used to surround the grass stems which were collected using scissors. On each of the examined grasslands, nine samples have been collected. For each of the samples, the length of the grass stems and the mass of collected grass per area has been measured. Grass cutting and measuring procedures have been conducted for each of the nine samples for each of the grasslands. After the grass has been collected from the grasslands, it was stored in plastic bags. Using a tabletop vacuum device the air was removed, and the samples have been weighted and further stored in the freezer at  $-15$  °C to preserve grass characteristics and composition.

### 2.2. Chemical analysis of residue grass

Chemical analysis of the collected residue grass consists of the determination of elemental composition of grass samples and their lower (LHV) and upper heating values (UHV), and determination of metal contents in analysed grass samples.

Proximate and ultimate analyses of the residue grass have been conducted in the Central Laboratory for Chemical Technology in the

**Table 1**  
Proximate and ultimate parameters of residue grass and applied test methods.

Parameter	Test method
Moisture	HRN EN ISO 18134-1:2015
Ash	HRN EN ISO 18122:2015
LHV	HRN EN ISO 18125-1:2017
UHV	HRN EN ISO 18125-1:2017
Sulphur	HRN EN ISO 16994:2015
Carbon	HRN EN ISO 16948:2015
Hydrogen	HRN EN ISO 16948:2015
Nitrogen	HRN EN ISO 16948:2015
Oxygen	HRN EN ISO 16948:2015

HEP Generation Ltd. in Croatia. Table 1 contains the analysed parameters and applied test methods.

The grass is a lignocellulosic biomass mainly composed of cellulose, hemicellulose and lignin (Paul and Dutta, 2018). Determining the elemental composition of dried grass samples, theoretical chemical oxygen demand ( $COD_{theoretical}$ ) of each sample could be calculated. Grass has been summarised as a molecule with the following empirical formula:  $C_aH_bO_cN_d$  (Gerike, 1984), where  $a$ ,  $b$ ,  $c$  and  $d$  present a number of carbon, hydrogen, oxygen and nitrogen atoms estimated by the elemental composition. When the molecular formula of grass samples has been estimated, the  $COD_{theoretical}$  could be calculated as (Koch et al., 2010):

$$COD_{theoretical} = \frac{16(2a + 0.5(b - 3d) - c)}{12a + b + 16c + 14d} \left[ \frac{kg_{O_2}}{kg_{C_aH_bO_cN_d}} \right] \quad (1)$$

As the calculation of the  $COD_{theoretical}$  is independent of their digestibility and due to the presence of lignin which is not readily digestible, the real COD is always lower compared to the theoretical one.

Metals in the residue grass have been further analysed due to the challenges they might present when digestate from the AD is used as a fertiliser (Fermoso et al., 2015). The analysis of heavy metals presence in the residue grass has been conducted at the School of Public Health “Andrija Štampar” in Zagreb, Croatia. The following metals have been analysed: lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni), manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu). The applied test method for all metals was *SOP-262-053 Edition 01* and the investigation technique AAS; ICP-MS.

### 2.3. Feedstock preparation

The following substrates have been used for the analysis: residue grass from the uncultivated land (RG1), residue grass from the riverbank (RG2), residue grass from the highway verge (RG3), maize silage collected from the biogas plant (MS) and cattle slurry collected from a small farm (CS).

The residue grass has been collected as described in Section 2.1, and further, it has been chopped into smaller pieces of approx. 3–6 cm in length. The inoculum and maize silage were collected from a biogas plant treating poultry manure and maize silage and operating under mesophilic conditions. Fresh cattle slurry has been collected from a small farm in the municipality of Šentilj. Once collected, inoculum and cattle slurry have been filtered through a coarse filter to remove large particles and to improve the homogeneity in the reactors. All the substrates have further been dried in five parallels in an oven at 105 °C until constant weight to determine the average total solids (TS) of each substrate.

### 2.4. Experimental setup

Anaerobic digestion has been performed in 250 mL batch reactors for 42 days in a heated bath. The temperature in the heated bath was maintained with SC 100 immersion circulator (Thermo Scientific™) at 39 °C which is in the mesophilic range. Filter flasks for vacuum use (Witeg) have been used as reactors and were sealed with silicone cream/PTFE septa (La-Pha-Pack) to maintain anaerobic conditions.

All the samples have been prepared based on the average dry matter (DM) content of samples in triplicates. In total, 9 g of total solids (TS) has been added to each reactor. The basic medium containing salts (Angelidaki et al., 2009) has further been added to substrate mixtures to reduce the DM concentration in reactors to 6%. Each filter flask was filled to a working volume in reactors of 150 g. Different types of residue grass have been placed in reactors as mono-substrates for anaerobic digestion (MRG1: residue grass from the uncultivated land, MRG2: residue grass from the riverbank and MRG3: residue grass from the highway verge). For comparison with residue grasses, maize silage has been analysed as a mono-substrate for anaerobic digestion (MMS).

Furthermore, riverbank grass and maize silage have been added as a co-substrate with the animal slurry in the 1:1 ratio based on a dry mass (C1 and C5). Additionally, residue grass from the riverbank was mixed with maize silage at different ratios on dry basis (C2 - 0.75:0.25, C3 - 0.5:0.5, C4 - 0.25:0.75) together with animal slurry in the 1:1 ratio to investigate if the grass could be an alternative substrate for food-competitive maize silage in the actual biogas plants. For all the batch assay the ratio between inoculum and substrates for anaerobic digestion was 1:1. Finally, the blank assays containing only inoculum and medium (IN) were set to subtract biogas and methane production in substrate assays. The setup of samples on TS basis is shown in Table 2.

After the addition of substrates and medium, and sealing the flasks, the reactors were flushed with inert argon gas 4.8 (Messer Group GmbH) for about 30 s to achieve anaerobic conditions. During anaerobic digestion, biogas production was measured daily, and bottles were hand-mixed daily for approximately 20 s. Biogas yield was measured by a water displacement method. Methane and carbon dioxide compositions in biogas were measured five times during the process (once a week) by the gas chromatograph Varian CP4900 using argon and helium as carrier gases and were recorded on a personal computer using Galaxie Workstation software. Twice a week around 3 mL of samples were removed from the reactors using a 10 mL syringe fitted with a needle and transferred to 15 mL vials to analyse pH (Smonkar et al., 2017). pH was measured using a wireless pH sensor (Pasco) which was connected to a tablet computer via Bluetooth and recorded via the SPARKvue app. After the analysis, the samples were returned to the flasks. The schematic of the batch digester and the biogas collecting apparatus is shown in Fig. 1.

**Table 2**  
Batch assay setup of samples on TS basis [g].

Reaction mixture	Inoculum	Residue grass	Maize silage	Cattle slurry
MMS	4.500	/	4.500	/
MRG1	4.500	4.500	/	/
MRG2	4.500	4.500	/	/
MRG3	4.500	4.500	/	/
C1	4.500	2.250	/	2.250
C2	4.500	1.687	0.563	2.250
C3	4.500	1.125	1.125	2.250
C4	4.500	0.563	1.687	2.250
C5	4.500	/	2.250	2.250
IN	4.500	/	/	/

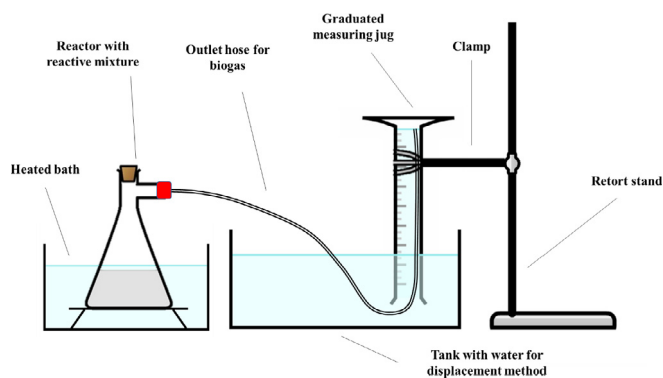


Fig. 1. Laboratory experimental set-up for anaerobic digestion.

### 2.5. Anaerobic Digestion Model No. 1

The ADM1 was published in 2002 by the IWA Task Group for mathematical modelling of anaerobic digestion (Page et al., 2008). The model is highly complex and includes 19 chemical and biological conversion processes with 24 dynamic state variables. Simulations and parameter estimation procedures have been conducted in Aquasim 2.0. The values of parameters used in the calculation have been adopted from literature (Batstone et al., 2008). The set of sensitive kinetic parameters in the ADM1 for the grass degradation has been chosen and presented in Table 6 in Section 3.3. These parameters have been estimated and fitted to the degradation of grass using the experimental data recorded in the laboratory.

### 2.6. Life Cycle Assessment (LCA) study

The Life Cycle Assessment (LCA) of the biogas production was conducted according to ISO 14040/14044 standards (International Standards Organization, 2006a, 2006b) using SimaPro v7.3.3 software. The study aimed to estimate and compare the environmental effects of the biogas production from co-digestion of riverbank grass with cattle slurry and maize silage in mass ratios presented in Table 2 and its usage in combined heat and power plant.

The system boundary includes all the processes regarding maize silage and grass collection and transportation, production of biogas in the anaerobic digestion plant and co-generation of heat and electricity in combined heat and power plant. Three different grass types (grass from the uncultivated land area, riverbank grass and verge next to the highway) were collected from uncultivated lands. The grass is assumed to be mowed and formed into round bales of 175 kg of DM each and transported to an AD plant, where a transport distance of 50 km has been assumed.

The functional unit for this study was defined as the production of “1 kWh of useful energy” (heat and electricity). The impact assessment methods selected were Impact 2002+ (Jolliet et al., 2003), the method that evaluates several midpoint categories grouped in four damage categories: Human health, Ecosystem quality, Climate change and Resources, and Global Warming Potential (GWP) calculated over 100 y time horizon (GWP100).

The data used in the study regarding the grass and maize silage quality and the biogas production by anaerobic digestion were obtained from laboratory analyses. All other data have been obtained from Ecoinvent v2.2 (Frischknecht et al., 2007) database. The results of the LCA analysis are shown in Section 3.4.

## 3. Results and discussions

In this section, the results from the residue grass characterisation and the batch AD process are presented. The results of the ADM1 are further shown which provide the view of kinetic parameters in the AD and show the comparison between the experimental and predicted behaviour of the process. In the end, the results of the conducted LCA provide the environmental impacts associated with a grass application in anaerobic digestion.

### 3.1. Residue grass characterisation

The results of the grass yield determination, the length of stems and the chemical composition of the examined fresh and dry grass are shown in Table 3.

Field measurements have shown that the greatest yield of fresh grass is present for the riverbank grass RG2. Other two samples have shown similar fresh grass yield, where the yield for RG3 appeared to be a bit higher compared to RG1. At the same time, by using the moisture content in grass samples, the yield of dry matter on grasslands is similar for RG2 and RG3. The higher moisture content of grass sample RG2 compared to samples RG1 and RG3 can be explained by the fact that the river bank area is occasionally flooded.

The analyses of residue grass types have shown significant differences in proximate parameters when expressed over the fresh matter. On the other side, when parameters were expressed on a dry basis, the values of proximate parameters of three grass samples (RG1, RG2 and RG3) were more similar. The reason for such phenomenon lies in the fact that all grass samples have shown significant variations in moisture contents. As expected, the highest moisture content has been determined for riverbank residue grass, grown in the partially flooded area. On the other side, residue grass collected on the highway verges has shown the lowest dry matter content, probably because it grows on the sloping terrain, where water drains more easily compared to the flat riverbank terrain.

The results from the ultimate analysis of grass samples for all elements except sulphur showed to be very similar for all the examined grass samples. Deviations in the term of sulphur content could be due to different positions of grasslands and the soil type on which the examined grass grows. Higher sulphur contents in residue grasses from the riverbank and highway verge are due to the sulphur presence in the Sava River (Kanduč and Ogrinc, 2007) and the uptake of sulphur dioxide emissions from vehicles by plants (WHO Regional Office for Europe, 2000).

The results of the metal presence analysis have shown that metal presence is the highest for the grass collected on the highway verges (RG3). Large traffic volumes and consequently high vehicle pollutant emissions are the probable cause. The grass from the uncultivated land has also shown the relatively high presence of heavy metals. The reason for such a trend could be found in the fact that the uncultivated land is located near the state road with a relatively high traffic concentration. Current studies of the presence of metals in roadside grass have been successfully conducted in Denmark (Meyer et al., 2014), the UK (Delafield, 2006), and Northern Germany (Werner, 2010). The differences in the results of the metal presence of roadside grass indicate that their presence is primarily a function of the traffic density and past activities in that area.

The lowest presence of heavy metals was found in the grass samples collected from the riverbank of the Sava River. Although the riverbank grass has shown the lowest share of heavy metals, the data were not drastically lower compared to the other grass samples, except for the iron presence. As the Sava riverbank is



**Table 3**  
Results from field measurements, proximate and ultimate analysis and heavy metal presence analysis of residue grass, fresh (dry) matter basis.

Characterisation	Parameter	RG1	RG2	RG3
Field measurements	Average yield [kg/m <sup>2</sup> ]	0.74 (0.14)	1.90 (0.26)	1.01 (0.23)
	Average stems length [m]	0.28	0.68	0.49
Proximate analysis	Moisture [%]	80.9 (/)	86.3 (/)	77.5 (/)
	Ash [%]	2.0 (10.4)	1.6 (11.2)	1.9 (8.4)
	LHV [MJ/kg]	1.48 (18.08)	0.25 (17.23)	2.07 (17.61)
	UHV [MJ/kg]	3.69 (19.34)	2.53 (18.45)	4.24 (18.85)
Ultimate analysis [%]	Carbon	8.9 (47.1)	6.3 (44.7)	10.4 (46.2)
	Hydrogen	1.1 (5.8)	0.8 (5.6)	1.3 (5.7)
	Nitrogen	0.54 (2.84)	0.31 (2.18)	0.46 (2.03)
	Oxygen	8.5 (44.2)	6.3 (47.2)	10.3 (45.9)
	Sulphur	0.017 (0.089)	0.039 (0.278)	0.033 (0.146)
Metal presence analysis [mg/kg]	Lead	0.019 (0.10)	0.010 (0.07)	0.081 (0.36)
	Cadmium	0.002 (0.01)	0.001 (0.01)	0.002 (0.01)
	Mercury	0.004 (0.02)	0.003 (0.02)	0.005 (0.02)
	Chromium	0.124 (0.65)	0.064 (0.47)	0.173 (0.77)
	Nickel	0.145 (0.76)	0.095 (0.69)	0.196 (0.87)
	Manganese	1.459 (7.64)	0.486 (3.55)	1.928 (8.57)
	Zinc	1.119 (5.86)	0.682 (4.98)	2.520 (11.20)
	Iron	10.390 (54.40)	2.617 (19.10)	21.060 (93.60)
	Copper	0.711 (3.72)	0.393 (2.87)	1.024 (4.55)

occasionally flooded (Gilja et al., 2010), heavy metals from the river accumulate in the soil and grass. As the Sava River springs in Slovenia where it passes through an area that has been strongly industrialised in the past, the presence of heavy metals in the river is not unexpected. Several mines, car, chemical and pharmaceutical industries, as well as the nuclear plant in Slovenia have contaminated the river in the past (Žibret and Gosar, 2017). The past activities related to mining in that area have thus caused significant pollution of the Sava River and its banks.

Table 4 further presents the estimated empirical formula of grass samples and theoretical oxygen demand, determined by Equation (1).

The range of the theoretical oxygen demand of grass according to the chemical composition is limited between 1.2 ÷ 1.6 kgO<sub>2</sub>/kgTS (Koch et al., 2010). The results of this study are fluctuating around the lower limit. RG2 sample has shown the lowest theoretical oxygen demand, due to the low content of oxidable compounds and higher oxygen content, in comparison to the other two samples. An important factor for anaerobic digestion, carbon to nitrogen ratio (C/N), has the following values: 16.6:1 (RG1); 20.5:1 (RG2) and 22.8:1 (RG3). It has been determined that the grass show C/N values between 10:1 to 25:1 (Steffen et al., 1998). Ultimate analysis has given valuable data which show that the residue grass collected on different grasslands has the potential to serve as feedstock in anaerobic digestion.

Significant yield, favourable biodegradability and low content of impurities indicate that the use of residue grass could be attractive in the bioenergy production.

**Table 4**  
Estimated empirical formula and theoretical oxygen demand of the analysed grass samples.

Parameter	RG1	RG2	RG3
<i>a</i>	19.3	23.9	26.5
<i>b</i>	28.3	35.6	38.9
<i>c</i>	13.6	19.0	19.8
<i>d</i>	1.0	1.0	1.0
COD <sub>theoretical</sub>	1.23	1.13	1.19

### 3.2. Laboratory batch test

In this study, the stress has been put on the examination of the gas phase (biogas) because of organic matter degradation. The results presented in this section give the view of the generated biogas and biomethane quantity expressed regarding the biochemical biogas potential (BGP) and biochemical methane potential (BMP). Also, the pH values of reaction mixtures (digested substrates) have been monitored over time. The impact of substrate properties on the pH value in anaerobic digestion is shown in Fig. 2 where the average values for each sample (analysis has been performed in triplicates) are presented.

Each of the pH profiles for analysed samples shows a common trend; in the initial days, the drop of pH values occurred due to the generation of acids, and after the rise of pH values was observed due to degradation of acids and the biogas generation. All grass mono-digestion samples, shown in Fig. 2 a), have shown a similar behaviour of the pH values over time; only the MRG2 sample has shown a little bit lower pH values compared to others. The pH values for mono-digestion of grass silage with the inoculum ratio of 1:1 were in the range between 7.31 and 8.00. The results of the conducted experiments were in line with the previous studies (Abu-Dahrieh et al., 2011), with some slight deviations that could have been the result of different substrate and inoculum type. Mono-digestion of maize silage (MMS), see Fig. 2 a), has shown much lower pH values compared to grass samples (with a minimum of 6.5 on the 8th day of the AD). That resulted in a significant decrease in the biogas production after five days of operation. As methanogenesis and thus the biogas production is the most efficient in the pH range between 6.5 and 8.2 (Mao et al., 2015), in order to avoid inhibitory effects, the pH value was raised when it reached the value close to 6.5. Sodium hydroxide (10 mL of solution with pH of 13) has been added on the 8th day of the AD to each parallel of MMS sample. After the addition of a strong base, a significant rise of the pH to approximately 7.7 has occurred, as shown in Fig. 2 a). After a few days, the process returned to the usual production of biogas.

Co-digestion samples, shown in Fig. 2 b), have not shown inhibitory effects because the animal slurry serves as a buffer and in that way controls the pH in the system and prevents the occurrence

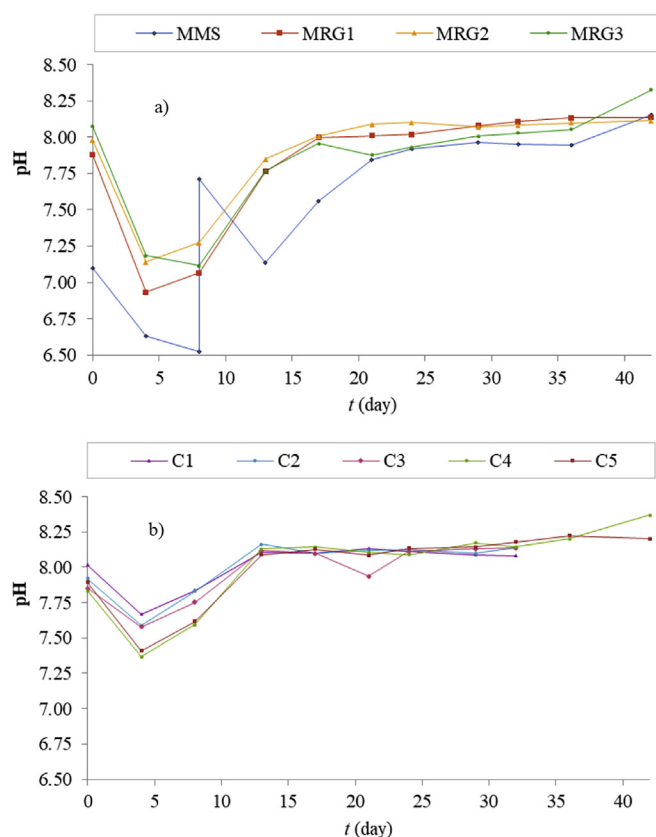


Fig. 2. Profiles of pH values in digested substrates during a) mono- and b) co-digestion.

of inhibition in the process (Husted and Husted, 1995). The missing data for the samples C3, C4 and C5 after the 31st day of operation are the result of removing the flask content from the reactor when the biogas production stopped.

Table 5 shows average data on BGP and BMP of the analysed samples after 40 days of operation under mesophilic conditions.

All grass samples (MRG1, MRG2 and MRG3) have shown both lower BGP and BMP compared to the mono-digestion of maize silage (MMS), which was expected. The riverbank grass (MRG2) has shown the lowest potential for biogas and biomethane production, which could be related to the lowest COD value as shown in Table 4. Also, the higher COD value—the higher BGP and BMP trend has been observed for the grass samples RG1 and RG3. Even though the sample RG2 has shown the lowest production of biogas, it has been selected for further analysis in co-digestion tests with maize silage and cattle slurry since it has shown the highest yield on the grasslands (Table 3). Therefore, the potential of replacing the part of maize silage by riverbank grass has been investigated in the samples C1 to C5. The results point to the expected situation, as the share of maize silage in the feedstock increases, both increase the BGP and BMP. In general, it can be stated that the riverbank grass gives the lower quantity of the biogas compared to maize silage. At the same time, it is non-competitive with food production, and as a residue material it can be cheaper feedstock compared to maize

silage, and thus it could reduce the operating cost of biogas plants. In terms of the environmental impacts of residue grass application in the biogas production at larger scale, the results are presented in Section 3.4.

### 3.3. ADM1 model predicted data for gas phase in grass mono-digestion

Substrate parameters for ADM1 have been based on the previous research (Koch et al., 2010) with the following composition assumed: proteins ( $f_{Pr\_Xc}$ ) = 0.187; lipids ( $f_{Li\_Xc}$ ) = 0.033; carbohydrates ( $f_{Ch\_Xc}$ ) = 0.401, and inerts ( $f_{Xi\_Xc}$ ) = 0.379. To estimate the sensitive kinetic parameters of grass degradation, the following recorded data have been used: methane and carbon dioxide content in biogas and the biogas production for grass mono-digestion sample RG2 shown in Table 6.

In the parameter estimation procedure, it is important to find the optimal set of parameters for a model structure that will result in a good data fit. The set of parameters shown in Table 6 includes the hydrolysis step, as it has been recognised as an important step in the degradation of lignocellulosic biomass. Other parameters have been selected due to the following facts; acetate degrades directly to methane in the methanogenesis step, and hydrogen is a compound in anaerobic degradation that is generated in the hydrolysis and acetogenesis step, but at the same time consumed by bacteria in the acidogenesis and methanogenesis step. The results of the parameter estimation procedure show that both disintegration and hydrolysis steps for lignocellulosic biomass are slower compared to the default values in the model, which was expected. Furthermore, for the degradation of acetate default and the estimated value of half-saturation constants ( $K_S$ ) do not differ significantly, but the estimated kinetic parameter for the Monod maximum specific uptake rate constant ( $k_m$ ) is significantly lower compared to the default value. Combined, the model assumes that in the methane generation from degrading acetate has a lower rate compared to the default assumption. On the other side, both higher estimated values of half-saturation constants and Monod maximum specific uptake rate constant in comparison to the default values cannot point to the conclusion whether the hydrogen uptake, in general, has higher or lower rate. Using the estimated parameters shown in Table 6, the share of methane in the biogas and the BGP values have been estimated for all grass samples as shown in Table 7. The ADM1 model considers that the biogas is composed of methane, carbon dioxide and hydrogen (Batstone et al., 2002). The laboratory measurements of the biogas composition give the share of methane and carbon dioxide. Due to the fact that the hydrogen share in the biogas is typically measured in ppm (Gaida, 2014), the assumption that the biogas is hydrogen-free has been made. Therefore, all the results for measured and estimated data are fitted to 100% content of methane and carbon dioxide in biogas.

The highest methane content in biogas has been recorded for mono-digestion of the grass sample RG1 (MRG1) – grass collected on the uncultivated land. As it is shown in Table 5, MRG1 exhibits also the highest BMP and BGP compared to the other grass samples (MRG2 and MRG3). To present deviations between the experimental data and ADM1 data, the relative error has been determined, as it is shown in Fig. 3.

Table 5

Measured biochemical biogas and biochemical methane potentials of the analysed samples.

Parameter	MMS	MRG1	MRG2	MRG3	C1	C2	C3	C4	C5
BGP [Nm <sup>3</sup> /kgTS]	0.4744	0.4361	0.3482	0.4131	0.2888	0.3211	0.3268	0.3861	0.4029
BMP [Nm <sup>3</sup> /kgTS]	0.2896	0.2750	0.1921	0.2552	0.1724	0.1965	0.1952	0.2514	0.2521

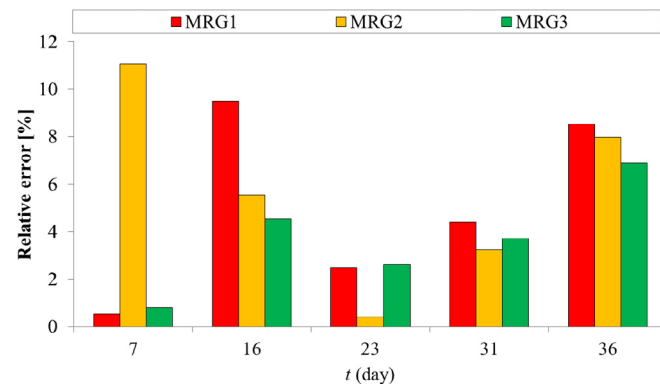
**Table 6**  
Estimated kinetic parameters in the grass degradation.

Parameter	Initial values (default) Batstone et al. (2002) Batstone et al. (2002)	Estimated by MRG2 experimental data	Unit
$k_{dis}$	0.50	0.17	1/d
$k_{hyd\_Ch}$	10	7.07	1/d
$k_{hyd\_Li}$	10	4.31	1/d
$k_{hyd\_Pr}$	10	6.29	1/d
$k_{m\_Ac}$	8	1.70	kgO <sub>2</sub> /(kgO <sub>2</sub> ·d)
$k_{m\_H2}$	50	70.2	kgO <sub>2</sub> /(kgO <sub>2</sub> ·d)
$K_{s\_Ac}$	0.15	0.12	kgO <sub>2</sub> /m <sup>3</sup>
$K_{s\_H2}$	$7 \cdot 10^{-6}$	$4.7 \cdot 10^{-4}$	kgO <sub>2</sub> /m <sup>3</sup>

Parameters shown in Table 6 present:  $k_{dis}$  – disintegration constant,  $k_{hyd}$  – hydrolysis constant  $k_m$  – Monod maximum specific uptake rate constant,  $K_s$  – half-saturation constant.

**Table 7**  
Results of measurements of methane content in biogas and ADM1 estimated values.

Period [day]	MRG1		MRG2		MRG3	
	Measured	ADM1	Measured	ADM1	Measured	ADM1
7	38.1	38.3	42.4	37.8	38.3	38.0
16	75.9	68.7	73.8	69.7	72.6	69.3
23	76.3	74.4	73.9	74.2	72.1	74.0
31	77.0	73.6	74.6	72.2	75.6	72.8
36	77.5	70.9	75.4	69.4	75.3	70.1



**Fig. 3.** The relative error values between measured and modelled methane content data in the gas phase.

There are no significant fluctuations in the methane content in biogas around the 23rd day of the AD process, while some fluctuations occurred at the start of the process and its end. To obtain a better fit of the experimental results to the model data, more frequent measurements on the gas phase should be conducted, preferably once a day or even twice a day.

The comparison between simulation and experimental data has been investigated in various studies. The threshold for a maximum relative standard error to 10% has been set (Poggio et al., 2016). Some examples of the previous studies: batch and semi-continuous anaerobic digestion of green and food waste has been performed and 10 (maximum 26.4%) and 2% (maximum 9.7%) average standard errors have been obtained (Poggio et al., 2016); for batch anaerobic digestion of agro-waste it has been shown that the correlation for several types of waste is very good while for some the simulation data showed higher values than experimental data (Galí et al., 2009). In case of a semi-continuous process, the relative error has been up to 9%; anaerobic digestion of cane-molasses vinasse has been studied and a mean absolute relative error ranging from 1% to 26% has been obtained (Barrera et al., 2015).

The production of biogas predicted by the ADM1 model, expressed as BGP, is shown in Table 8.

According to the results shown in Table 8, it can be stated that the ADM1 correctly describes the production of biogas in the mono-digestion process for the RG2 sample. Since the experimental data for MRG2 have been used to estimate the kinetic parameters in the ADM1, such results were expected. On the other side, the ADM1 results of BGP for mono-digestion of the RG1 and RG3 have shown higher deviation, around 20%. Although these fluctuations appear to be significant, when modelling the phenomena in the organic system as the ones examined, then compared to the inorganic systems, the error values are higher.

#### 3.4. Environmental impacts of residue grass application in the anaerobic digestion

The environmental impact analysis has been performed for nine analysed samples (all studied reaction mixtures except inoculum) with the ratios between substrates as shown in Table 2. For the LCA study it was assumed that the biogas produced is used for heat and electricity generation. The following two impact categories have been considered: GWP100 expressed as carbon dioxide equivalents (CO<sub>2</sub>-eq) to indicate the effects on climate change, and a single score characterisation expressed in  $\mu$ Pt to determine contributions of four damage categories; Resources, Climate change, Ecosystem quality and Human health. The results are shown in Fig. 4 and Fig. 5.

The results by the single score characterisation identify the ecosystem quality category as a category that makes a significant difference among all studied cases (Fig. 4). Negative results should be interpreted as an environmental benefit. Compared to maize silage, the grasses grow naturally without using any agricultural inputs and without cultivating the soil, and therefore, the results in Aquatic ecotoxicity, Terrestrial ecotoxicity and Land occupation (all are part of the Ecosystem quality category) show beneficial effects to the ecosystem quality. Comparing only the results obtained from the processes with co-digestion (C1–C5), it can be noted that the ecosystem quality arising from the process C1 and carried out with the residue grass and cattle slurry is 3.8 times environmentally better than the process C5, carried out with the maize silage and cattle slurry. The results in terms of greenhouse gas (GHG) emissions are shown in Fig. 5.

**Table 8**  
Results of BGP predicted by the ADM1 and error value compared to the experimental data.

Parameter	MRG1	MRG2	MRG3
BGP [Nm <sup>3</sup> /kgTS]	0.3578	0.3515	0.3465
Relative error [%]	21.9	0.9	19.2



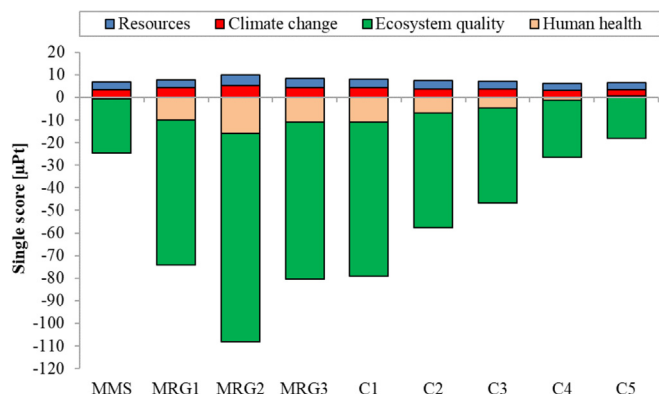


Fig. 4. The single score results of the life cycle impact assessment.

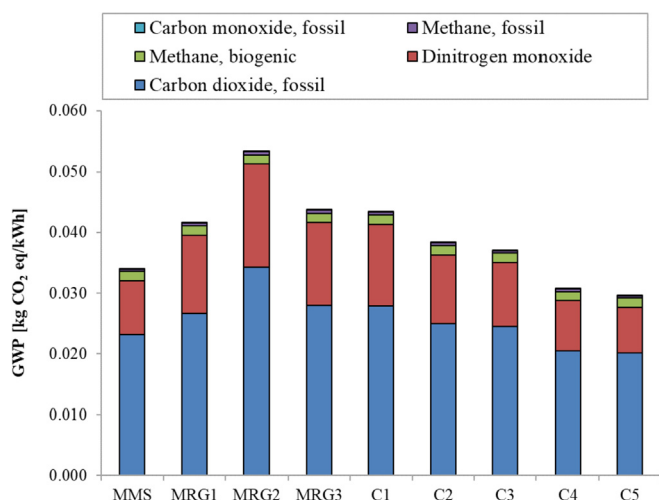


Fig. 5. Global warming potential (GWP) results.

Most of the emissions belong to carbon dioxide from fossil fuels used for agricultural machinery and grass and maize silage transportation. GHG emissions related to grass collection are the result of quite high energy inputs (fossil fuels) for collecting and baling of grass. Compared to the GHG emissions from maize silage, all studied grass types have lower biogas yield potential which increases the emissions for transportation since more grass needs to be transported to the AD plant to produce the same amount of energy. For that reason, the process C1 resulted in 32% higher GHG emissions than the process C5. It should be noted that the benefit of using grass from the uncultivated lands for biogas production instead of its natural decomposition on the field, resulting in avoiding GHG emissions, was not considered in this study. Also, GHG emissions related to land use changes were not considered.

#### 4. Conclusions

Investigations of residue grass utilisation in anaerobic digestion have been successfully carried out. Based on the grass yields and analysis of the presence of chemical compounds in grass samples on the examined grasslands it could be concluded that the position of grassland influences the grass properties and consequently behaviour during anaerobic digestion. Even though riverbank grass has shown the highest grass yield, it has also shown the lowest quality and production of the biogas, in comparison to the other two grass types. Monodigestion of maize silage has shown the

greatest yield of biogas, but on the other side, it has shown that issues regarding process control exist, especially in terms of the pH regulation. Analysis of the co-digestion samples points to the conclusion that cattle slurry increases the degradation of riverbank grass residue. Co-digestion processes stopped producing biogas earlier than mono-digestion processes, after 30 days of operation instead of after 42 days. That phenomenon could be analysed in more details in further analyses.

Modelling of the gas phase in the anaerobic digestion has given the view of the rate of chemical reactions which occur during the process. Especially the first stages of digestion, disintegration and hydrolysis are attractive for further observation due to the estimated kinetics parameters. This work has shown that the disintegration and hydrolysis of biomass occur at lower rates of reactions compared to initial assumptions. The investigation has also shown the importance of knowing the feedstock composition for mathematical modelling by using mechanistically inspired model, in this study the ADM1 model.

Investigation of mono- and co-digestion processes could be extended by applying different pre-treatment methods to improve the digestion of green biomass. The LCA analysis has provided the results which should be explained carefully due to the complexity of the analysis and quality and quantity of the data that are to be used. In general, the residue grass has shown lower BMP compared to the maize silage which leads to the increase of the required quantity of grass to produce the same amount of energy as when using maize silage. The residue grass has the potential to serve as a replacement for maize silage in the production of heat and electricity, and therefore some further investigations should be aimed at the way to increase the digestibility of grass.

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#### Nomenclature

##### Abbreviations

AAS	Atomic absorption spectroscopy
Ac	Acetate
ADM1	Anaerobic Digestion Model No. 1
BGP	Biochemical biogas potential
BMP	Biochemical biomethane potential
C	Co-digestion
C/N	Carbon to nitrogen ratio
Ch	Carbohydrates
COD	Chemical Oxygen Demand
dis	Disintegration
DM	Dry matter
EROEI	Energy return on energy invested index
GHG	Greenhouse gas
GWP	Global Warming Potential
hyd	hydrolysis
ICP-MS	Inductively coupled plasma - mass spectrometry
IN	Inoculum
LCA	Life Cycle Assessment

LHV	Lower heating value
Li	Lipids
M	Mono-digestion
MS	Maize silage
Nm <sup>3</sup>	Normalized cubic meter (for gases: 101,325 Pa and 0 °C)
OLR	Organic load rate
Pr	Proteins
RG	Residue grass
TS	Total solids
UHV	Upper heating value
VFA	Volatile fatty acids
VS	Volatile solids
Xc	Composite material
Xi	Inerts

### Symbols

<i>a</i>	Number of carbon atoms [–]
<i>b</i>	Number of hydrogen atoms [–]
<i>c</i>	Number of oxygen atoms [–]
<i>d</i>	Number of nitrogen atoms [–]
<i>f<sub>Ch_Xc</sub></i>	Carbohydrates from composite material [–]
<i>f<sub>Li_Xc</sub></i>	Lipids from composite material [–]
<i>f<sub>Pr_Xc</sub></i>	Proteins from composite material [–]
<i>f<sub>Xi_Xc</sub></i>	Inerts from composite material [–]
<i>k<sub>dis</sub></i>	Disintegration constant [1/d]
<i>k<sub>hyd_Ch</sub></i>	Hydrolysis constant for carbohydrates degradation [1/d]
<i>k<sub>hyd_Li</sub></i>	Hydrolysis constant for lipids degradation [1/d]
<i>k<sub>hyd_Pr</sub></i>	Hydrolysis constant for proteins degradation [1/d]
<i>k<sub>m_Ac</sub></i>	Monod maximum specific uptake rate constant for acetate [kgO <sub>2</sub> /(kgO <sub>2</sub> ·d)]
<i>k<sub>m_H2</sub></i>	Monod maximum specific uptake rate constant for hydrogen [kgO <sub>2</sub> /(kgO <sub>2</sub> ·d)]
<i>K<sub>s_Ac</sub></i>	Half saturation coefficient of acetate [kgO <sub>2</sub> /m <sup>3</sup> ]
<i>K<sub>s_H2</sub></i>	Half saturation coefficient of hydrogen [kgO <sub>2</sub> /m <sup>3</sup> ]

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