Enhancement of Biogas Production by Co-Digestion of Maize Silage with Common Goldenrod Rich in Biologically Active Compounds

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This paper analyses the suitability of common goldenrod plants as mono- and co-substrates for biogas production. Furthermore, the role of bioactive compounds included in the biomass of this plant species was investigated. The results showed that the common goldenrod species produced lower biogas and methane yields than maize silage. However, the methane fermentation of their mixture resulted in approximately 9.5% higher biogas yield and 16.6% higher methane yield compared to the theoretical yields estimated based on two mono-digestions. A statistically significant increase in biogas production efficiency resulted from more favorable C/N ratio and the influence of bioactive compounds contained in common goldenrod. The addition of goldenrod crude extract caused an approximately 30% increase in the biogas yield of maize silage. This effect may be associated with a positive impact of biologically active substances on microorganisms or with a decrease in redox potential of the fermenting mass.

Keywords: Solidago virgaurea; Common goldenrod; Co-digestion; Crude extract; Bioactive compounds; Biogas production; Methane fermentation

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INTRODUCTION

Species of the genus *Solidago* L. (Goldenrod) are perennial plants found throughout Poland, growing mainly on fallows, wastelands, and meadows. Some species, particularly *S. canadensis* (Canadian goldenrod) and *S. gigantea* (Giant goldenrod), are classified as invasive plants. They were introduced to Europe in the 17th century, and now they displace native flora as a dominant species (Pliszko and Zalewska-Gałosz 2016). They may reach high biomass yield *ca*. 16 Mg ha^{-1} and calorific value *ca*. 16 MJ kg^{-1}. They are also popular plants for honey bees (Ciesielczuk *et al.* 2016). Moreover, the raw material of *Solidago*, including the herbs of *Solidago canadensis* (Canadian goldenrod), *Solidago gigantea* (giant goldenrod), and *Solidago virgaurea* (common goldenrod) is used in treatment of various diseases, particularly in disorders of the urinary tract (Radusiene *et al.* 2015).

Pharmacological activity of their preparations is mainly caused by the presence of various bioactive compounds with antioxidant and free-radical scavenging abilities (Apati *et al.* 2002). The most biologically active substances are contained in *S. virgaurea*, a native European species. Dominant compounds observed in this species are rutin and chlorogenic acid, but astragalin, quercetin, rosmarinic acid, and virgaureasaponins are also detected (Bader *et al.* 1992; Apati *et al.* 2002; Rosłoń *et al.* 2014). Herrmann and Janke (2001) indicated that rutin does not have any negative influence on methane production in bioreactors. Broudiscou *et al*. (2000) investigated the effect of dry extract of *S. virgaurea* on methanogenesis in ruminants and stated that its addition to wethers' diet enhanced methane fermentation. For ruminants, methane production is an adverse process, harmful to both these animals and the environment. In contrast, a maximized methane yield is desirable for biogas processes (Popp *et al.* 2016). Neither the effect of dry extract nor individual compounds from goldenrod herb on biogas production in bioreactors has been investigated.

Several publications have documented the biogas yields from invasive varieties of *S. canadensis* and *S. gigantea* (Seppälä *et al.* 2013; Ciesielczuk *et al.* 2016). These studies stated that these species are productive and cheap substrates that are worthy of interest. However, there are no reports about the biochemical methane potential of *S. virgaurea*. Generally, to achieve the highest profitability in biogas production, low-cost substrates with high methane potential are selected. In addition to organic waste, both cultivated and wild perennials are increasingly considered for this purpose. Their desirable features are high yield and low soil requirements. To avoid competition with land for food and feed production, fallows for cultivation of perennials could be considered in view of their large areas, which were estimated at 8.3 Mha (million hectare) in EU-15 by year 2030 (Seppälä *et al.* 2013). Such lands are overgrown mostly by invasive plants (such as goldenrods), which are characterised by great tolerance to habitat conditions. As stated by Young *et al.* (2011), insufficient research has been conducted on existing (non-cultivated) bioenergy sources (such as invasive plant species) from non-crop agricultural land. Oleszek *et al.* (2014) reported that the biomass of wild varieties of grasses such as reed canary grass could be a good substrate if it was fertilized and systematically harvested.

The aim of this study was to evaluate the suitability of *Solidago virgaurea* (common goldenrod) as a mono- and co-substrate for biogas production. Furthermore, the role of bioactive compounds included in this plant biomass was clarified. It was hypothesized that the common goldenrod, as well as other *Solidago* spp., may improve biogas production because they contain biologically active compounds whose positive impact on the methane fermentation process in a rumen has been stated previously.

EXPERIMENTAL

Research Materials

The goldenrod herb (Herba *Solidaginis*) and maize silage (*Zea mays* var. *Ułan*) were used in this study as substrates for methane fermentation. Chopped and dried biomass of common goldenrod was obtained from the herb company of Kawon-Hurt (Gostyń, Poland). Maize was cultivated in the experimental station of Institute of Soil Science and Plant Cultivation in Osiny, Poland.

The experiment was conducted in random sub-blocks with 4 replications. Nitrogen, phosphorus, and potassium (NPK) fertilization was applied in dosages of 120, 26, and 67 kg ha^{-1}, respectively.

The biomass was collected in October 2014, separately from each of four plots. All parts were combined, fragmented into 1 cm pieces, and ensiled. Silage was prepared in sealed, plastic barrels of 5 L volume with a silage additive in the form of lactic acid bacteria, and then stored in the dark.

Experimental Procedure

The experiment included four steps. First, the chemical composition of tested raw materials was determined. The maize silage (selected as reference feedstock, due to its wide application in biogas plants in Europe) and common goldenrod herb were fermented separately, in a mono-digestion, and as co-substrates at a ratio of 1:1 (based on volatile solids (VS)). Next, a methanolic extract of goldenrod herb was prepared. Lastly, a lyophilized extract was used as an additive in the methane fermentation process of maize silage at the concentration of 100 ppm in the fermenting mass. Table 1 presents the amounts of tested substrates and crude extract used in the experiments.

Table 1. Amounts of the Goldenrod Herb, Maize Silage, and Crude Extract Used in the Particular Experiments (g FM and g VS)

Experiment	Goldenrod Herb		Maize Silage		Crude extract
	g FM*	g VS	g FM	g VS	g
1st Mono-digestion	0	0	50	16	0
2nd Mono-digestion	18	16	0	0	0
Co-digestion	9	8	25	8	0
Crude extract addition	0	0	50	16	0.08
*FM – fresh matter, VS – volatile solids					

Chemical Analysis

The chemical analysis of total solids (TS), volatile solids (VS), crude ash (CA), total nitrogen (N_{tot}), crude protein (CP), and organic carbon (C_{org}) was conducted as described by Oleszek *et al.* (2016). Briefly, TS, VS, and CA were determined using a gravimetric method after drying at 105 °C and 550 °C, respectively. The contents of N_{tot} and CP were analysed by Kjeldahl's method (Kjeldahl 1883). The analysis of C_{org} was performed on a TOC-V CPN analyzer with a solid sample module (Shimadzu, Kyoto, Japan).

Crude fat was determined by extraction with hexane. Crude fibre fractions (neutral-detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL)) were evaluated with van Soest and Wine's method (Van Soest and Wine 1967). Cellulose (CEL) and hemicellulose (HCEL) contents were calculated by subtracting ADF from NDF and ADL from ADF, while the non-fiber carbohydrates (NFC) were estimated by using Eq. 1,

$$NFC = 100\% - (CP + CF + NDF + CA) \quad (1)$$

where NFC is non-fiber carbohydrates (% TS), CP is crude protein (% TS), CF is crude fat (% TS), NDF is neutral detergent fiber (% TS), and CA is crude ash (% TS).

Crude Extract Preparation

The plant material (about 30 g) was defatted with chloroform in a Soxhlet apparatus until complete discoloration occurred. The crude extract from *S. virgaurea* (common goldenrod) was prepared as described by Pawelec *et al.* (2013). Briefly, the defatted and dried material was extracted under reflux with 70% methanol (3 x 250 mL) for 2 h. The extract was filtered, and the solvent was removed under reduced pressure (40 °C) using a rotary evaporator (Heidolph, Schwabach, Germany). The crude extract was suspended in water and freeze-dried.

Batch Assays

The batch assays were performed according to VDI 4630 (2006) by using one-litre eudiometers in a water bath. The parameters of methane fermentation were as follows: temperature of 37 °C, pH 7, VS concentration of 6%, substrate to inoculum ratio (S/I) of 1:2 (based on the VS), and total weight of fermenting mass of 800 g.

Anaerobic conditions were ensured by blowing nitrogen gas across the reactor before and shortly after filling. The fermented mass was mixed once a day for half a minute. Also, every day the biogas volume was determined by the liquid displacement method (Oleszek and Tys 2013).

Methane concentration was measured daily, using the automated analyzer GFM 400 series (Gas Data, Coventry, UK). The process was performed until the daily volume was lower than 1% of the previous total biogas volume. The obtained values of biogas yields were converted into standard conditions (1013 hPa, 273 K).

To assess the effect of co-digestion on biogas yield, methane yield or methane content, the modified equation of Poulsen and Adelard (2016) was used,

$$\Delta X = \left(X_{mix} / (0.5(X_{maize\ silage} + X_{goldenrod\ herb})) - 1\right) \times 100\% \quad (2)$$

where X is the biogas yield, methane yield or methane content, and ΔX is relative change compared to what would be expected based on mono-digestion. The coefficient of 0.5 follows from the proportion of both substrates in the mixture (1:1).

Statistical Analysis

Statistical analysis was performed in STATISTICA 12 software (Stat Soft Inc, Tulsa, OK, USA). The chemical composition data and biogas and methane yields were expressed as the mean ± standard deviation (SD) of three independent replicates. The influence of goldenrod herb and its crude extract addition on biogas and methane yields of maize silage was determined by one-way ANOVA. The significance of differences between the measured parameters was examined using post-hoc Tukey's test. The level for accepted significance was $p < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition

The chemical compositional analysis showed significant differences between both tested plant materials (Table 2). The chemical composition of maize silage was typical for the substrate and was similar to the results of other studies (Schittenhelm 2008; Negri *et al.* 2014). The goldenrod herb was characterized by higher TS but displayed lower VS than maize silage. Moreover, it contained more N_{tot} and less C_{org} than maize. Consequently, it resulted in a much lower C/N ratio.

There were no significant differences in CF, NDF, and cellulose content. In contrast, a significant variation was stated in the case of ADF, ADL, hemicellulose, and NFC content. The results showed that goldenrod herb contained much more ADL and less NFC than maize silage, indicating that it is more difficult to digest substrate.

Table 2. Chemical Composition of Goldenrod Herb and Maize Silage

Parameters	Goldenrod Herb	Maize Silage
TS (wt.% FM)	96.87 ± 0.16[a,*]	33.50 ± 0.41[b]
VS (wt.% TS)	92.90 ± 0,02[a]	96.35 ± 0.01[b]
$C_{org.}$ (wt.% TS)	44.63 ± 0.12[a]	49.92 ± 0.37[b]
$N_{tot.}$ (wt.% TS)	1.70 ± 0.01[a]	1.37 ± 0.02[b]
C/N (wt.% TS)	26.22 ± 0.11[a]	36.54 ± 0.86[b]
CP (wt.% TS)	10.64 ± 0.07[a]	8.54 ± 0.15[b]
CF (wt.% TS)	3.40 ± 0.16[a]	3.20 ± 0.02[a]
CA (wt.% TS)	7.10 ± 0.02[a]	3.65 ± 0.01[b]
NDF (wt.% TS)	45.20 ± 2.94[a]	43.22 ± 0.04[a]
ADF (wt.% TS)	29.50 ± 2.20[a]	23.19 ± 0.24[b]
ADL (wt.% TS)	7.30 ± 1.37[a]	3.25 ± 0.08[b]
Cellulose (wt.% TS)	22.13 ± 4.25[a]	19.94 ± 0.31[a]
Hemicellulose (wt.% TS)	15.80 ± 1.14[a]	20.03 ± 0.24[b]
NFC (wt.% TS)	33.66 ± 3.13[a]	41.39 ± 0.13[b]

TS, total solids; FM, fresh matter; VS, volatile solids; CP, crude protein; CF, crude fat; CA, crude ash; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; NFC, non-fiber carbohydrates.
*Mean values with different superscript letters within row differ significantly in Tukey test ($p<0.05$).

Comparison of Mono- and Co-Digestion

The less favorable chemical composition of goldenrod herb was reflected as lower biogas and methane yields than maize silage (Table 3). Although the biogas yield of the mixture was lower than maize silage alone, it was significantly higher than the average biogas yields of two mono-substrates (predicted biogas yield of the mixture). Furthermore, the methane content was higher in biogas obtained from co-fermentation than both mono-fermentations, although the difference was not significant. The value of relative changes in biogas and methane yield (ΔX) indicated that the combination of two substrates caused approximately 9.5% and 16.6% increase of biogas and methane yields, respectively. The methane content in biogas was also raised by approximately 7.2%.

Table 3. Results of Mono- and Co-Fermentation of Common Goldenrod Herb and Maize Silage

Parameters	Goldenrod Herbs	Maize Silage	Goldenrod and Maize Mixture (1:1)	Average for Both Mono-substrates	ΔX*
Biogas yield ($dm^3\ kg^{-1}$ VS)	265 ± 13[a]	480 ± 33[b]	408 ± 17[c]	373 ± 16[d]	9.5%
Methane yield ($dm^3\ kg^{-1}$VS)	127 ± 6[a]	241 ± 31[b]	214 ± 22[b]	184 ± 17[c]	16.6%
Methane content (vol. %)	48 ± 1[a]	50 ± 3[a]	53 ± 4[a]	49 ± 2[a]	7.2%

ΔX, relative changes in biogas yield, methane yield, and methane content compared to the expected values from mono-digestion
*Mean values with different superscript letters within row differ significantly in Tukey test ($p<0.05$).

Figure 1 presents daily biogas yields of two tested mono-substrates and their 1:1 mixture. This figure shows the kinetics of biogas production and indicates that some specific phases can be separated.

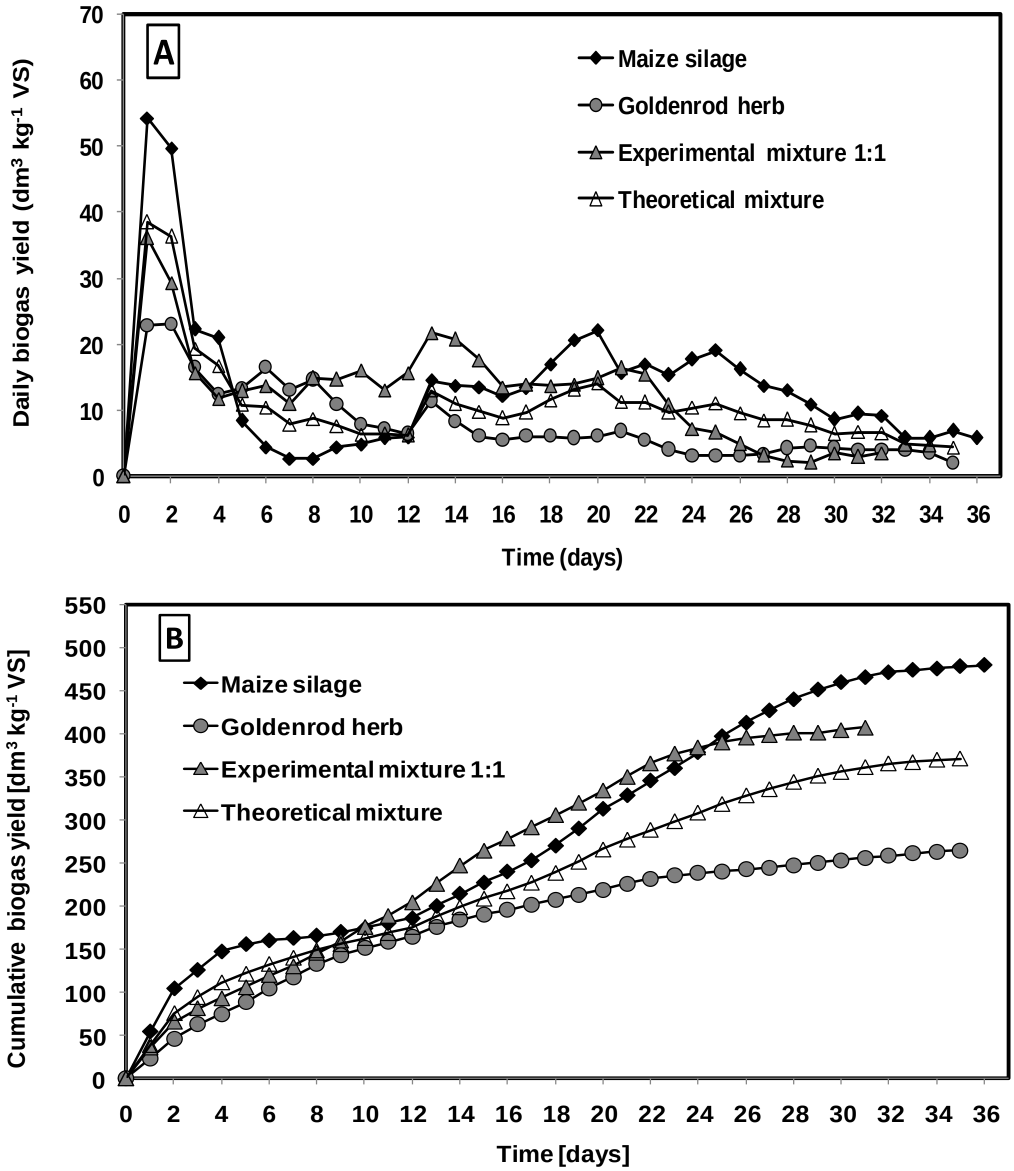

Fig. 1. Daily (A) and cumulative (B) biogas yield of maize silage, goldenrod herb, and their theoretical and experimental mixture.

The beginning of each process contained an intense peak. At this time, the highest biogas yields were measured in all tested samples. Notably, the highest peak was noted for maize silage. After this peak, biogas production declined.

The scale of the drop differed significantly between substrates and was the greatest for maize silage. It was probably caused by the accumulation of volatile fatty acids and the decrease in pH following the rapid hydrolysis of simple compounds occurring in large numbers in maize silage (Oleszek *et al.* 2016). At about 13th day of fermentation, a second peak was observed, which was of the lowest scale for the goldenrod. After that, the methane fermentation of this substrate neared its end, while there were more high daily yields for maize silage.

The second peak observed was probably associated with the digestion of less decomposable compounds, which required longer periods for hydrolysis (Zhou *et al.* 2014). The lack of meaningful yields during this time for goldenrod herb indicated that the biomass was digestible only to a small extent.

Moreover, in Figure 1, the predicted biogas yield of the mixture of maize and goldenrod was also presented as the average of two mono-fermentations (theoretical mixture). This result was next compared with the experimental biogas yield of the 1:1 mixture of maize and goldenrod. The peaks of both processes coincided but were higher for the experimental mixture. Furthermore, the methane fermentation of the experimental mixture was much more stable, free of sharp declines as in the case of maize silage. Likewise, the fermentation process of the mixture was finished sooner. These results proved the positive influence of the co-digestion of these substrates.

There are some examples of synergy during the co-digestion of substrates. Co-fermentation is a feasible method for overcoming the drawbacks of mono-fermentation and the favoritism of positive interactions, *i.e.*, macro-, microelements, and moisture balance, or dilute inhibitors and toxic compounds (Mata-Alvarez *et al.* 2014). Consequently, “1 + 1 > 2” can be achieved, which means that the mixture may produce more biogas than that produced in both mono-fermentations. Seppälä *et al.* (2013) reported that the methane yield of a mixture of brown knapweed and liquid cow manure was 105% of the methane yield calculated based on the yield of mono-substrates, which was probably due to a more balanced nutrient composition in the fermenter. Zhou *et al.* (2014) noticed that in the case of co-digestion, a suitable C/N ratio is very important. In their study, the mixture of food waste and corn stover at a C/N ratio of 20:1 had a higher methane yield than the ratios of 30:1 and 25:1. The substantial role of C/N ratio was also confirmed by Zhao *et al.* (2014), who obtained higher biogas and methane yields (26.39% and 24.79% increases, respectively) through the 1:1 co-fermentation of rice straw and municipal sewage sludge. In the present study, the C/N ratio was slightly too high for maize silage (36.54). Goldenrod herb addition caused a decrease in the C/N ratio of the mixture, which affected biogas production.

Effect of Crude Extract Addition

One of the reasons for high biogas yield of the mixture may be the presence of biologically active compounds in the goldenrod herb. The present study showed that the biogas and methane yields of maize silage was much higher after the addition of goldenrod crude extract compared with the control sample (Table 3). Also, the addition of crude extract caused an increase in methane content, although the difference was not statistically significant.

Table 4. Results of Addition of Crude Extract of Common Goldenrod

Parameters	Extract-free Control	100 ppm Extract
Biogas yield (dm^3 kg^{-1} VS)	447 ± 56^a	583 ± 55^b
Methane yield (dm^3 kg^{-1}VS)	229 ± 32^a	312 ± 24^b
Methane content (vol. %)	51 ± 3^a	54 ± 1^a
*Mean values with different superscript letters within row differ significantly in Tukey test ($p<0.05$).		

Common goldenrod includes numerous compounds with antioxidant and reducing activities, including flavonoids, phenolic acids, and saponins (Bader *et al.* 1992; Apati *et al.* 2002; Rosłoń *et al.* 2014). Many of these substances have a positive impact on biological membranes (Covaliov *et al.* 2012). The increase in biogas production from grape waste have been explained by the fact that bioactive compounds in this substrate manifest themselves as anti-oxidants, anti-hypoxants, anti-mutagens, and membrane-protectors. Biologically active substances stabilize the cell membranes of microbes, reduce the peroxide oxidation of lipids, and prevent the deterioration of cell membranes. As a consequence, the acceleration of biochemical methanogenesis and increase in biogas yield occurred. Prabhudessai *et al.* (2009) showed that the addition of 100 ppm caffeine increased biogas production by 16% compared with the control, most likely because caffeine serves as a stimulant, increasing microbial activity. Singh *et al.* (2001) investigated the influence of microbial stimulants of Aquasan® and Teresan® containing steroid saponins on methane production. These addition increased the biogas yield of cattle dung and kitchen waste. Goldenrod contains significant quantities of saponin compounds (Bader *et al.* 1992).

The above-mentioned reports concern the effect of bioactive compounds on the activity of microorganisms involved in methane fermentation. However, Demir *et al.* (2009) showed that the crude extract of common goldenrod exhibited antioxidant activity and relatively high reducing power. Reducing ability decreases the redox potential, which is very important for anaerobic processes.

In sum, the reasons for the positive impact of goldenrod crude extract are not completely clear. Further research is needed to find the particular compounds or substances responsible for this effect. Moreover, studies on methanogens are recommended to determine the influence of the extract compounds on their growth and proliferation.

CONCLUSIONS

1. Compared with maize silage, common goldenrod was an inferior substrate for biogas production.
2. Methane fermentation of the mixture resulted in an approximately 16% higher methane yield than the average result of two mono-substrates, which may indicate synergy during co-fermentation.
3. The enhancement of biogas production resulted from improvement of C/N ratio or from the action of bioactive compounds contained in common goldenrod.
4. The addition of goldenrod crude extract caused an improvement in the biogas production of maize silage.
5. Further research is needed to resolve which compound in the crude extract is most responsible for the increase in biogas yield and whether it is associated with positive impact on microorganisms or with decrease in redox potential.

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