ANAEROBIC FERMENTATION OF SPRING FRESH WILD PLANTS

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Abstract. Noticeable climate change around the world has led to the search for more and more low-carbon technologies. Biogas is also produced in Latvia and is used to produce electricity and heat. The use of biomethane in transport needs to be further developed to replace diesel and petrol engines more quickly. In the spring, biogas producers sometimes have difficulty securing good quality raw materials. The raw materials procured in the previous year are not always left in sufficient quantities. It happens that long-term storage of raw materials also breaks down. The amount of manure, which is mainly used by Latvian biogas plants, cannot usually be increased. There is a shortage of raw materials and gas yields are falling. Biomethane yields can be increased by substances that catalyze the anaerobic fermentation process. They can also be found in spring plants. The use of spring plants such as dandelions (taraxacum), which grow over large areas, could improve the situation. The methane potential of dandelions and three other plants was investigated in this study. Plants such as celandine (Chelidonium majus), European twist (Cuscuta europaea) and elderberry (Sambucus nigra) have specific effects. Elderberry and celandine have a healing effect, but the European twist is a parasitic plant. Therefore, it seemed interesting to find out whether these plants will somehow specifically affect the activity of bacteria in the process of anaerobic fermentation. Fourteen bioreactors were filled with fresh plants collected in the field and in the garden on the same day. The two bioreactors were filled with inoculum only. Anaerobic fermentation took place at 38°C and lasted from 10.05 to 7.06. The methane potential was 0.486 $L \cdot g^{-1}{}_{DOM}$ for dandelions, 0.440 $L \cdot g^{-1}{}_{DOM}$ for elderberry leaves, 0.488 $L \cdot g^{-1}{}_{DOM}$ for European twist and 0.403 $L \cdot g^{-1}{}_{DOM}$ for celandine. All the plants tested are very useful as feedstocks for methane production in anaerobic fermentation. However, expectations that Sambucus nigra, Chelidonium majus and Cuscuta europaea will show some catalytic properties for anaerobic fermentation were not met.

Key words: anaerobic fermentation, methane, dandelions, celandine, European twist, elderberry.

Introduction

Noticeable climate change around the world has led to the search for more and more low-carbon technologies. Biogas is also produced in Latvia and is used to produce electricity and heat. The use of biomethane in transport needs to be further developed to replace diesel and petrol engines more quickly. In spring, biogas producers sometimes have difficulty securing good quality raw materials. The raw materials procured in the previous year are not always left in sufficient quantities. It happens that long-term storage of raw materials also breaks down. The amount of manure, which is mainly used by Latvian biogas plants, cannot usually be increased. There is a shortage of raw materials and gas yields are falling. There is an urgent need to investigate the suitability of various inexpensive renewable biomass resources for energy production [1]. Also, it is necessary to explore the possibilities to improve the anaerobic digestion process with the help of various catalysts [2]. Biomethane yields can be increased by substances that catalyze the anaerobic fermentation process. They can also be found in spring plants. Sambucus nigra is one of the most famous medicinal plants in the world. According to the US Department of Agriculture, Sambucus nigra has almost twice the anthocyanin concentration of any other berry. Anthocyanins are plant-based dyes, the most powerful natural antioxidants – substances that work to protect cells from possible damage caused by free radicals, as well as stimulate the body's immune system. Elderberry berries also contain ascorbic acid, carotene, rutin, alkaloids – sambucine, tannins, organic acids, essential oils, etc. In countries where elderberry cultivation is traditional, wine, soft drinks, jams, etc. are made from these berries. In traditional medicine, elderberry (*Sambucus nigra* L.) has long been used to treat viral diseases, colds and flu. Today's clinical trials have also shown that it is effective in fighting flu types A and B, as well as the symptoms of colds that have been halved with elderberry juice concentrate in as short as 3 days. In the garden celandine is a nasty weed that blooms in early spring before the lawn and the first flowers. Celandine preparations are a popular remedy in folk medicine. Infusions have long been used as a biliary, sedative, analgesic and diuretic. It is used against various diseases characterized by smooth muscle spasms: gastritis, colitis, inflammation of the liver, gallbladder and bronchi, also as a means of lowering and soothing blood pressure [3]. In the past, it was used topically for the treatment of skin tuberculosis, warts, corns, eczema, ringworm and against brown spots on the skin. It was also used to eliminate polyps in the colon. The plant's yellow milk juice is still

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used externally to kill warts. Dried celandine leaf tea strengthens the immune system and is useful in the fight against cancer. However, it should be noted that celandine is poisonous and should not be used for more than a month.

Cuscuta europaea as a parasitic and dodder plant, also has interesting properties. These phytochemicals possess antimicrobial, anticancer and antioxidant potentials and can be used as a potential drug for the treatment of various diseases [4]. Cuscuta europaea is wrapping around itself over the host plant after attachment with host. Cuscuta makes haustorial connection with the vascular tissue of the host plant. This haustorium is able to penetrate the xylem and phloem of the host plant and attached with tissues of the host plant [4]. Dodder is a herb. People use the parts that grow above the ground to make medicine. Dodder is used to treat urinary tract, spleen, psychiatric, and hepatic disorders. It is also used for cancer, depression, and pain. Researchers from the Eotvos Lorand University obtained from cuscuta japonica methane yield 465-571 $mL \cdot g^{-1}$ vs [5].

Scientists in Chile and Spain have also studied the use of dandelions for methane production [6]. "Dandelion residue anaerobic degradation (biomethanization) for biogas production is the ending process. Inulin as the main compound in the tuberous root was selected as substrate and tested at different concentrations (5, 10 and 15 g $TS \cdot L^{-1}$). The methane production yield (Y_{CH4}), specific maximum methane production rate (SR_{MAX}) and the first-order hydrolysis constant (k_H) were affected by the substrate concentration. Bicarbonate was found not to be determinant for the overall biomethanization process because no significant effect over Y_{CH4} was achieved in the presence or absence of bicarbonate; between 0.19 to 0.23 $m^3 \cdot kg^{-1}$ VS added. However, k_H and SR_{MAX} were affected by the presence of this inorganic compound. Without the addition of bicarbonate, k_H and SR_{MAX} decreased as the TS increased. These results will be key for dandelion biorefinery proof-of-concept under the proposed conditions."

Plants such as celandine (*Chelidonium majus*), European twist (*Cuscuta europaea*) and elderberry (Sambucus nigra) have specific effects. Elderberry and celandine have a healing effect, but the European twist is a parasitic plant. Therefore, it seemed interesting to find out whether these plants will somehow specifically affect the activity of bacteria in the process of anaerobic fermentation, hoping to reveal some catalytic properties of the process.

Materials and methods

Similar methods were used in the study as described in the work of other researchers [8-10]. First, 500 g of inoculum was charged up to each bioreactor. Then all raw materials were shredded in a shredder ALKO 2400R New Tec. R2-R5 bioreactors were filled in 20 g leaves of elderberry (*Sambucus nigra*). R6-R9 bioreactors were filled in 20 g dandelions (*Taraxacum*). R10-R12 bioreactors were filled in 20 g European twist (*Cascuta europaea*). R13-R15 bioreactors were filled in 20 g celandine (*Chelidonium majus*). In bioreactors, the raw materials were thoroughly mixed with the inoculum. Digestate from a permanently operating 110 L bioreactor, in which cattle manure was used as raw material, was used as inoculum. The batch mode AD process was ongoing at temperature 38 ± 0.5 ºC. Biogas released was collected in gas bags for further measurements of gas volume and elemental composition. Biogas and methane volumes and gases composition were measured during the AD process at regular time intervals. The AD process was provided until biogas emission ceases (28 days). The obtained experimental data were processed using appropriate statistical methods. Dry organic matter (DOM) content was determined by weighting and drying of the initial biomass samples, in the equipment Shimazu at 105 ºC and then placed in the oven ("Nabertherm" type) at 550 ºC. All the components were carefully mixed together and refilled in bioreactors. All bioreactors were placed into heated thermostat at the same time before starting of anaerobic digestion. Gas released from each bioreactor was collected in a storage bag positioned outside the thermostat. Gas volumes were measured using the flow meter (Ritter drum-type gas meter). The composition of gases, including oxygen, carbon dioxide, methane, and hydrogen sulphide, was measured by help of the gas analyser (model GA 2000). The substrate pH value was measured before and after finishing off the AD process, using a pH meter (model PP-50) with accessories. Scales (Kern, model KFB 16KO2) was used for weighting of the total weight of substrates before and after the AD process.

Results and discussion

The raw materials were sampled and analyzed before being loaded into the bioreactors. The results of the analyzes are shown in Table 1.

Table 1

Analyses of raw material samples before anaerobic digestion

Bioreactor	pH	TS, %	TS, g	Ash, %	DOM, %	DOM, g	Weight, g
R1, R16	7.66	2.88	14.40	46.15	55.85	8.04	500
R2-R5		22.65	4.53	7.71	92.29	4.18	20
R2-R5 + In		3.64	18.93	35.45	64.55	12.22	520
R6-R9		15.14	3.028	21.32	78.68	2.382	20
R6-R9 + In		3.35	17.428	40.20	59.80	10.422	520
R10-R12		10.90	2.18	16.25	83.75	1.825	20
R10-R12 + In		3.19	16.58	40.49	59.51	9.866	520
R13-R15		10.99	2.198	21.48	78.52	1.726	20
R13-R15 + In		3.19	16.598	41.16	58.84	9.766	520

Note: R – bioreactor, In – inoculum, TS – total solids, DOM – dry organic matter

All raw materials have a high content of dry organic matter. It is the highest in elderberry leaves. The results show that the ash content is high in the inoculum and, after mixing with the raw materials, is still high in the bioreactor. The yield of methane and biogas from the tested feedstocks from each bioreactor is shown in Table 2. In data shown in this table, the results from bioreactors R1 and R16 have already been taken away.

Table 2

Biogas and methane yields

Bioreactor/ Rawmaterial	Biogas, L	Biogas, $L \cdot g^{-1}_{DOM}$	Methane, aver.%	Methane, L	Methane, $L \cdot g^{-1}_{DOM}$
R1 In	0.2	0.025	6.8	0.014	0.002
R16 In	0.2	0.025	4.70	0.009	0.001
R1, R16 average	0.2	0.025	5.75 ± 1.05	0.012 ± 0.0025	0.0015 ± 0.0005
R2 Sn	3.2	0.766	44.16	1.413	0.338
R3 Sn	3.0	0.718	54.73	1.642	0.392
R4 Sn	4.0	0.957	51.63	2.055	0.492
R5 Sn	4.9	1.172	46.02	2.255	0.539
R2-R5 average	3.775 ± 0.675	0.903 ± 0.161	49.14 ± 4.045	1.844 ± 0.314	0.440 ± 0.075
R6 Ta	2.1	0.882	53.05	1.014	0.468
R7 Ta	2.0	0.839	54.30	1.086	0.455
R8 Ta	3.3	1.385	39.55	1.305	0.548
R9 Ta	2.6	1.092	43.50	1.131	0.475
R6-R9 average	2.5 ± 0.45	1.050 ± 0.189	47.60 ± 6.075	1.272 ± 0.086	0.486 ± 0.031
R10 Ce	2.1	1.150	38.81	0.815	0.446
R11 Ce	2.4	1.314	38.96	0.935	0.512
R12 Ce	2.2	1.205	42.23	0.929	0.508
R10-R12 average	2.233 ± 0.111	0.917 ± 0.061	40.00 ± 1.487	0.893 ± 0.052	0.488 ± 0.028
R13 Cm	1.9	1.101	39.00	0.741	0.390
R14 Cm	1.7	0.984	35.29	0.600	0.347
R15 Cm	2.1	1.217	38.90	0.817	0.473
R13, R14, R15 average	1.9 ± 0.133	1.101 ± 0.078	37.73 ± 1.627	0.719 ± 0.08	0.403 ± 0.046

Note: Ta – taraxacum, Sn - Sambucus nigra, Cm - Chelidonium majus, Ce - Cascuta europaea, In – inoculum.

The average biogas and methane yields from each raw material for dry organic matter are shown in Fig.1. They are very good. They are better, as Chilean researchers show in their study. However, such a comparison, when the soil and other conditions are not known, is not very objective.

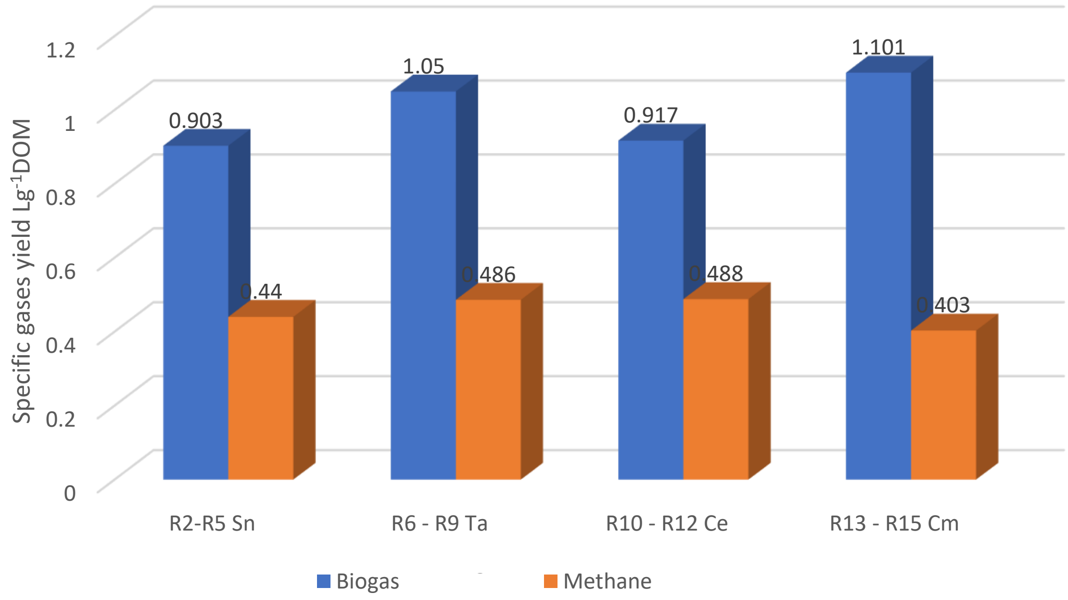

Fig. 1. **Average yields from each raw material**

Average methane content from each raw material is shown in Fig.2

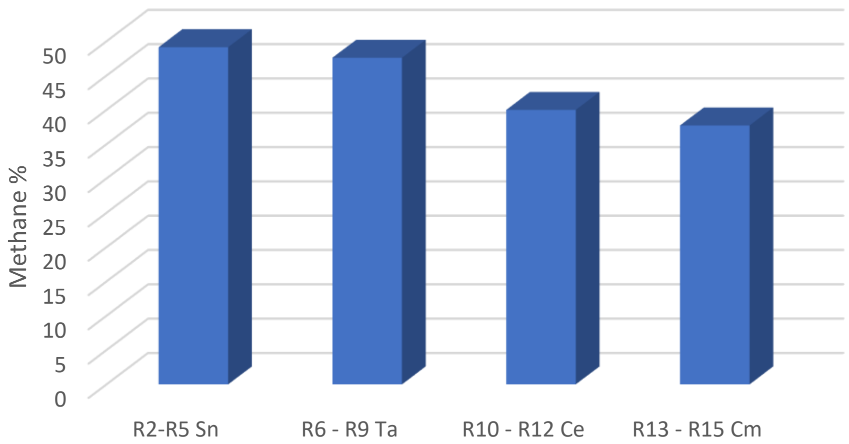

Fig. 2. **Average methane content from bioreactors filled with each raw material**

The average methane content of individual feedstocks is relatively low, but it should be noted that this is the case of the batch mode AD process. The maximum methane content was reached on the seventh day after the start of the process: Sn – 66.8%; Ta – 59.0%; Ce – 56.6%; Cm – 54.3%.

Conclusions

1. From Sambucus nigra the methane yield obtained was 0.440 $L \cdot g^{-1}{}_{DOM}$
2. From Taraxacum methane the yield obtained was 0.486 $L \cdot g^{-1}{}_{DOM}$
3. From Cascuta europaea the methane yield obtained was 0.488 $L \cdot g^{-1}{}_{DOM}$
4. From Chelidonium majus the methane yield obtained was 0.403 $L \cdot g^{-1}{}_{DOM}$
5. The plants tested in the study can be used very well for methane production, but no specific process catalytic properties were observed.

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Conceptualization, V.D.; methodology, V.D.; software, V.D.; validation, V.D; formal analysis, V.D and I.P..; investigation, V.D., and I.P.; data curation, V.D., I.P. and I.S.; writing – original draft preparation, V.D.; writing – review and editing, V.D.; visualization, V.D., I.P. and I.S.; project administration, V.D. All authors have read and agreed to the published version of the manuscript.

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