IMPROVEMENT OF ANAEROBIC FERMENTATION OF MECHANICALLY PRETREATED LIGNOCELLULOSIC BIOMASS

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Abstract. The aim of the study is to improve anaerobic fermentation (AF) of lignocellulose biomass by mechanical pre-treatment of willow biomass and by addition of used cooking rapeseed oil, trace element selenium and by application a low-voltage electric field during the AF process in batch mode at temperature 38 °C. Experimental setup includes 2 bioreactors of 0.75 L volume filled with 0.5 l inoculums (control) and 22 bioreactors filled with 0.50 L inoculums, 12 g milled or pelletised willow biomass, with added rapeseed oil (1 mL or 2 mL) or selenium (15 µg) in different groups of bioreactors. One group of bioreactors was equipped with electrodes for application of low-voltage (1.00 V) electric field. AF process was provided for a 28-day period until gases emission ceases. Methane yield from unpelletized willow biomass was 0.13 $L \cdot g^{-1}_{DOM}$ and was 0.4 $L \cdot g^{-1}_{DOM}$ with 2 mL rapeseed oil added. Methane yield from willow pellets was in the range from 0.22 $L \cdot g^{-1}_{DOM}$ without additives to 0.361 $L \cdot g^{-1}_{DOM}$ for the combination of willow pellets with 2 mL rapeseed oil and selenium 15 µg under influence of 1.0 V low voltage electric field. Addition of the trace element 15 µg selenium in pelletised willow substrate plus 1 mL oil gave specific methane yield 0.29 $L \cdot g^{-1}_{DOM}$ or higher by 48%, compared to pelletized willow biomass plus 1 mL oil without selenium. Methane formation was higher from biomass with average particle size 3 mm compared to biomass with average particle size 9 mm. Using the electric field in the substrate provides an additional amount of methane with energy, which is 32% more than the energy consumed.

Keywords: lignocellulose biomass; willow; anaerobic fermentation; sunflower oil; selenium.

Introduction

Word economics faces with the problem of depletion of fossil fuels, as limited resources are available and it is foreseen that all oil reserves will run out by 2052, even if the population growth rate will be constant. The green transition could lead to additional jobs in some sectors compared to the business-as-usual scenario. An increase in the demand for raw materials needed to build renewable energy power plants could lead to job growth in energy-intensive transforming sectors (up to 2.4%); and an increased demand for biofuels and renewable materials could lead to job growth in agriculture and forestry (up to 2.2%) in 2050 [1].

Pollution, largely from burning fossil fuels, kills up to seven million people annually, with low and middle-income countries carrying the highest burden. This includes exposure to toxic fumes from using wood, coal or dung as the primary cooking fuel [2]. So, there is of importance to avoid burning not only the fossil fuels, bet also burning of the biomass with high content of lignocellulose, e.g., woody biomass and willow. Lignocellulose is the most abundant biopolymer available on the earth as waste biomass. Lignocellulose-degrading enzymes, namely, cellulases, hemicelluloses, and ligninases, play a crucial role in converting lignocellulose into sugars and biofuels [3].

The global area of willow is estimated to be 9 million ha, which is largely natural (95%) and only 5% planted (400 000 ha). The production of biomass from willow plants requires the use of 0.018 MJ of non-renewable energy to produce 1 MJ of renewable energy in the form of wood fuel [4].

Willow can be used as animal feed additive to reduce methane emission from ruminants. A 79-day rotational grazing experiment was conducted to compare effects of grazing willow (*Salix* spp.) fodder blocks, a combination of small trees (*i.e.,* 1.0 m) and herbage, or perennial ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) control pasture on breath methane (CH_4) emissions, concentrations and solubility of CH_4. Compared to control pasture, grazing willow fodder blocks reduced CH_4 emission/kg metabolic body weight ($BW^{0.75}$) by 20% [5].

Improvement biodegradability of willow is important for biogas production in the AF process. The conversion of lignin to monocyclic hydrocarbons as a consumer chemical and fuel is a highly desirable target for biorefineries [6].

Lignin modification is recognized as a valid aspect for the successful purification of lignocellulosic biomass, and the literature focuses on the treatment and processing of lignin by enzymes [7].

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Due to the recalcitrant properties of lignocelluloses, the hydraulic retention time (HRT) is increased and thus a lower organic loading rate (OLR), and also a high amount of dilution water has to be used, which makes their fermentation economically less feasible. These reasons limit the utilization of willow saw-dust, willow wood chips, or other woody biomass in industrial biogas plants [8].

Biogas production from willow biomass was investigated previously, as it was necessary to conduct research with various biomass resources available in Latvia in order to be able to use alternative methods if necessary [9].

The purpose of this research is to investigate possibilities to find methods to increase biogas and methane production from mechanically pre-treated willow biomass by means of mechanical crushing, pelletising, and adding of oil and selenium additives, and by exposure of substrates to a low voltage electric field.

Materials and methods

Unprocessed biomass samples were harvested from naturally occurring willow stand in the area under power line in October 2021. Willow biomass stem 2-3 diameters without branches were cut into pieces, ground and sieved to obtain biomass particles with an average size of 9 and 3 mm, and part of the obtained biomass was granulated.

Additive materials were cooking rapeseed oil 1 mL or 2 mL (obtained from local confectionery) and the trace element selenium 15 µg that were added in some substrates according to the experimental plan. Some bioreactors were arranged with graphite electrodes [10] and connection to 1.0 V DC source was provided to investigate the effect of electric field on the AF process.

The total solids (TS) content of the samples was determined by drying the sample in a thermostat-balance (type MOC-120H) 105 °C; accuracy of weight measurements ± 0.001 g. The dry matter content (DOM) of the organic matter was obtained by incineration of the samples in a muffle furnace (model Naberthem B170) at 550 °C and using a standard method for the determination of moisture and ash content. Dry matter content (DM) and organic dry matter content (DOM) was calculated using standard mathematical operations.

The composition of the substrates was prepared according to the experimental plan and poured into twenty-four reactors with a volume of 0.75 L. The samples were grouped into ten different groups. A single reactor filling (batch) method was used for AF processing of substrates during this study. The following substrates were used: inoculum (IN) obtained from manure digestate from continuously working 110-litre anaerobic reactor; granular and non-granular willow mass with particle sizes of 3 mm or 9 mm; rapeseed oil and the trace element selenium.

All 24 bioreactors with substrates were placed in thermostats at temperature 38 ± 0.5 ºC to perform the anaerobic fermentation (AF) process. A low voltage (1 V) DC electric field was used to stimulate the AF process in two reactors. The AF process was provided 28 days in all reactors. Biogas was collected in special gas sampling bags (Tedlar type) placed outside the thermostat. Gas volume and composition analysis was performed regularly by help of a gas flow meter (Ritter type) and gases analyser (Gasboard 3200L), respectively.

After 28-day fermentation period, all substrates were weighed, and the dry and dry organic matter contents were determined using the above methods and standard mathematical methods to calculate the decomposition coefficient of the added biomass during the AF process. Substrate pH was measured in the substrates before and after the AF process using a pH meter (model JENCO 692, accuracy ± 0.01).

The biogas and methane volumes produced by inoculums in control reactors were subtracted from biogas or methane volume produced in every bioreactor with willow biomass according to the given method [11]. The average effectiveness of application of low voltage electricity was calculated by comparison of the energy utilised for 1.0 V low voltage field maintenance in the 28-day period vs surplus methane energy obtained in the result of the low voltage field application for substrate in the bioreactor.

Results and discussion

Groups of bioreactors with the same substrate, and the results of the analysis of substrates and components before anaerobic fermentation are shown in Table 1.

Table 1

Raw materials in reactors before AF process

Reactor	Raw material	Weight, g	TS, %	TS, g	ASH, %	DOM, %	DOM, g
R1; R16	IN	500.00	2.21	11.03	0.61	1.60	8.00
R2-R4	IN + W3	512.00	4.45	22.79	0.84	3.61	18.48
R5-R7	IN + W9P	512.00	3.73	19.09	0.67	3.06	15.65
R8-R10	IN + W9P + O1	512.85	3.89	19.94	0.67	3.22	16.50
R11-R13	IN + W9P + Se	512.00	3.73	19.09	0.67	3.06	15.66
R14, R15	W9P + O1 + Se	512.85	3.89	19.94	0.67	3.22	16.50
R17, R18	IN + W3P	512.00	3.82	19.54	0.64	3.18	16.28
R19, R20	IN + W3 + O2	513.70	4.77	24.49	0.84	3.93	20.18
R21, R22	IN + W3P + O2	513.70	4.13	21.24	0.64	3.50	17.98
R23, R24	IN + W3P + O2 + Se + e1V	513.70	4.13	21.24	0.63	3.50	17.98
-	W3	12.00	98.1	11.8	10.70	87.36	10.48
-	W3P	12.00	71.0	8.5	1.96	69.01	8.28
-	W9P	12.00	67.2	8.1	3.44	63.79	7.65
-	O1	0.85	99.8	0.8	0.10	99.69	0.847
-	O2	1.69	99.8	1.7	0.10	99.69	1.693

Note: IN – inoculum, where W3 – Willow powder, 3 mm particles; W3P - willow pellets from 3 mm particles; W9P - willow pellets, particle 9 mm particle; ASH - ashes; TS - total solids; DOM - dry organic matter; R1-R16 - bioreactors with inoculum only (control bioreactors); R2-R4 - a group of bioreactors with a similar substrate; Weight - biomass added in each reactor; e1V - low voltage 1.0 V DC source, Se - 15 µg selenium, O1- 1 mL (0.85 g) rapeseed oil, O2 – 2 mL (1.7 g) rapeseed oil.

The raw material analysis shows that small willow biomass particles 3 mm have the highest ash content 10.8%, compared to willow pellets, which can be explained by the influence of the biomass sieving process, where foreign matter, e.g., sand, dust particles were separated through the sieve in first order, compared to willow biomass particles. Negligible ash content was determined for rapeseed oil, as oil contains lot of substances volatilised during the aching process of the sample.

Crushed willow biomass with the particle size 3 mm had highest organic matter content in dry matter 87.3% compared to pelletized willow biomass. This evidence can be explained by partial evaporation of volatile solids due to relative high temperature of biomass during the pelletisation process.

The dry organic matter content of the substrates before anaerobic fermentation (AF) ranged from 3.06% for the group of substrate IN + W9P (inoculums plus pellets made of willow biomass with an average particle size of 9 mm) to 3.93% for the group of substrate IN + W3 + O2 (inoculum plus willow biomass with an average particle size of 3 mm plus rapeseed oil 2 ml).

Average specific biogas and methane volumes (in litres per 1 g dry organic matter added in bioreactor) were strongly varying between the groups of bioreactors, Fig. 1.

Substrate IN + W3 (inoculum and unpelletized willow biomass) showed the lowest average specific biogas 0.29 $\mathrm{L \cdot g^{-1}_{DOM}}$ and methane 0.13 $\mathrm{L \cdot g^{-1}_{DOM}}$ yield. This yield was lower, as compared to biogas 0.256 $\mathrm{L \cdot g^{-1}_{DOM}}$ and methane 0.172 $\mathrm{L \cdot g^{-1}_{DOM}}$ obtained from willow (variety Ingers) in earlier experiments [9]. Low biogas and methane yield from inoculum and unpelletized willow substrate can be explained by the possible contamination of the sieved willow biomass with soil or dust particles, as indicated by the high (10.7%) ash content in this group of substrates.

Addition of 2 ml rapeseed oil (or 13% of added biomass) in the group of bioreactors (IN + W3 + O2) provided the highest average specific biogas volume 0.79 $\mathrm{L \cdot g^{-1}_{DOM}}$ or specific methane yield 0.41 $\mathrm{L \cdot g^{-1}_{DOM}}$ that was 2.7 and 3.2 times higher, respectively, compared to the substrate (IN + W3) without oil additive.

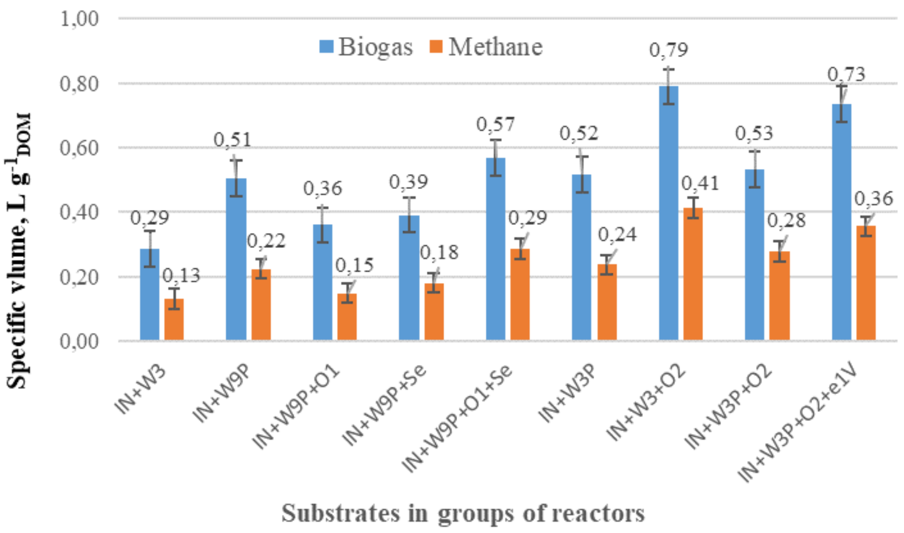

Fig.1. **Specific biogas and methane volumes**

Using of pelletized willow biomass (IN + W3P) resulted in specific biogas 0.52 L·g$^{-1}_{DOM}$ or methane 0.24 L·g$^{-1}_{DOM}$ yields that were higher by 44% or 46%, respectively, compared to non-pelletised willow biomass. This value is lower compared to biogas 0.63 L·g$^{-1}_{DOM}$ or methane 0.29 L·g$^{-1}_{DOM}$ obtained earlier from straw pellets [12].

Addition of the trace element selenium in willow pellets substrate (IN + W9P + Se) gave specific biogas 0.57 L·g$^{-1}_{DOM}$ or methane 0.29 L·g$^{-1}_{DOM}$ yields that were higher by 9% or 17%, respectively, compared to pelletised willow biomass (IN + W3P).

Average specific methane 0.22 L·g$^{-1}_{DOM}$ volume from substrate (IN + W9P) with pellets made of willow biomass of 9 mm particles was by 8% lower, compared to specific methane volume released from willow biomass pellets made of 3 mm particles. This evidence can be explained by higher available surface area for microbial activity in substrate with pellets made of 3 mm particles. A similar trend was reported for wheat straw where increase of specific methane by 38% was observed if the average size of wheat straw particles decreased from 1.5 mm to 0.75 mm [13].

Average specific methane 0.29 L·g$^{-1}_{DOM}$ yield from the combined substrate (IN + W9P + O1 + Se) was 48% compared to the substrate (IN + W9P + O1) and was higher by 38% compared to the substrate (IN + W9P + Se) respectively. This evidence can be explained by the enriched nutrient composition in the combined substrate, which was favourable for microbial activity.

Average specific biogas 0.73 L·g-1DOM or methane 0.36 L·g-1DOM yield from combined substrate with added selenium under the influence of 1.0 V electric field (IN + W3P + O2 + Se + e1V) was higher by 22% compared to the substrate (IN + W3P + O2) without the trace element selenium or electric voltage. The average low-voltage electrical efficiency was 35.3% obtained by comparing the energy 0.456 Wh used to maintain a low-voltage electric field over a 28-day period with a surplus energy 6.754 Wh calculated by multiplying of surplus methane volume 0.69 LCH4 by a lower calorific value of biomethane 9840 Wh·L^{-1} [14].

Further investigations on the influence of trace elements and parameters of the electric field are needed for optimisation of anaerobic fermentation of lignocelulostic biomass.

Average methane content in biogas from different substrates is shown in Fig. 2.

The highest average methane content 52% was observed in biogas substrate IN + W3 + O2 or IN + WP3 + O2 containing non-pelletised or pelletised willow biomass particles in the size 3 mm with addition of 2 ml of cooking rapeseed oil.

The lowest average methane content 39% was observed in biogas from the group of substrates (IN + W9P + O1) containing willow biomass pellets with addition of 1 ml rapeseed oil. The low methane concentration in biogas can be explained by unfavourable conditions for microorganisms especially methanogens responsible for production of methane. Such conditions can be caused by thermally treated both cooking oil or pellets and/or by dust or dirt inclusions released from working surfaces of the granulator, e.g., by iron particles from wearing parts of the granulator.

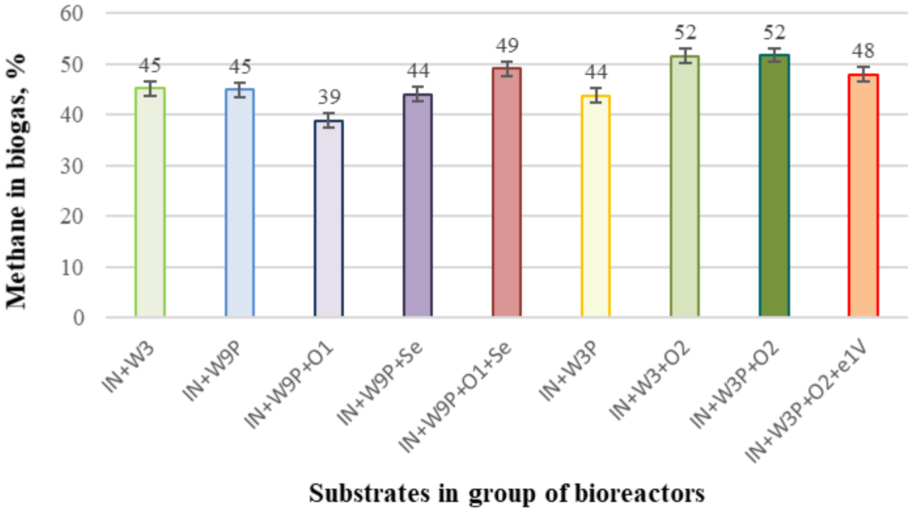

Fig.2. **Methane content in gases from reactors**

Conclusions

1. The lowest specific volume of methane 0.13 $L \cdot g^{-1}_{DOM}$ was released from the inoculum and the ungranulated willow biomass substrate of 3 mm particles, and highest specific methane yield of 0.41 $L \cdot g^{-1}_{DOM}$ was released from granulated willow biomass made from 3 mm particles and 2 ml cooking rapeseed oil additive. Low specific volume of gases from ungranulated willow biomass can be explained by contamination of substrate with soil or dust particles, as indicated by the high ash content in this substrate.
2. Pelletization of willow biomass provided specific methane 0.24 $L \cdot g^{-1}_{DOM}$ that was higher by 46% compared to non-pelletised willow biomass.
3. Combination of willow pellets plus 1 mL rapeseed oil, plus selenium 15 µg gave specific methane volume 0.29 $L \cdot g^{-1}_{DOM}$ which was by 48% higher compared to the willow pellet substrate plus 1 mL of rapeseed oil additive.
4. The highest methane content 52% in biogas was observed in biogas released from substrate with inoculum and unpelletized willow biomass in the size 3 mm plus 2 mL rapeseed oil, and in biogas from substrate containing pelletised willow biomass 3 mm particles with addition of 2 mL cooking rapeseed oil.
5. Combination of willow pellets of 3 mm particles with 2 mL rapeseed oil and selenium 15 µg under the influence of the low voltage 1.0 V electric field provided methane yield 0.36 $L \cdot g^{-1}_{DOM}$ which was higher by 22% compared to substrate without 15 µg selenium and electric field.
6. Using the electric field in the substrate consumes 4.578 Wh of energy, which is 32% less compared to the energy consumed for maintenance of the electric field in substrate. Further research will be needed to test the effects of different contents of trace elements and electric field parameters on the AF process.

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Author contributions

Experimental setup preparation, formal analysis, writing – review and editing: I.P.; Conceptualization, methodology, funding acquisition: V.D.; Experimental investigation, formal analysis, writing: M.K;

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