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Broiler house litter processing by anaerobic digestion using enzymes

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ABSTRACT
Sawdust is used as litter for broiler chickens. Mostly they are taken from the treatment of low-value deciduous trees. Keeping broilers for 40-50 days on a litter of sawdust, all the time, absorbs moisture well and mixes with bird droppings. When the broilers are moved to the slaughterhouse, these littered manures are removed from the barn and stored in stacks. They can be used to fertilize fields but only once or twice a year when the fields are fertilized, so they need to be spread over large areas and must be covered due to unpleasant odors. The other alternative is to use this manure with litter to produce energy, preferably biomethane. Sawdust is rich in lignin, cellulose and hemicellulose and is therefore slowly and incompletely broken down by bacteria. This study investigated the effects of the use of the enzymes alpha amylase and xylanase as well as the biocatalyst Metaferm on the mixture of bird droppings and sawdust actually produced in broiler houses. The 14 bioreactors of the laboratory were filled with raw materials and the appropriate additives were added. An average of 0.349 Lg^{-1}dom methane was obtained from bioreactors where alpha amylase was added, but 0.368 Lg^{-1}dom methane was added to xylanase. From the bioreactors where the biocatalyst Metaferm was added average yield was 0.329 Lg^{-1}dom of methane. Addition of enzymes improved methane production, but the addition of biocatalyst Metaferm showed no improvement compared to control bioreactors.
Keywords: *anaerobic digestion; bird droppings; sawdust; methane; alpha amylase; xylanase.*

1. INTRODUCTION

Bedding for broiler breeding is required. Chopped straw is commonly used, but lately, sawdust of deciduous trees is increasingly used in Latvia. Sawdust without resin admixtures absorbs moisture well. Broilers are kept on such litter for 40 to 50 days. During this time, a mixture of bird droppings and sawdust is formed in the barns. When the cycle ends, the mixture is removed from the barn and stored in stacks. It is used as fertilizer when possible. The mixture of manure and bedding emits an unpleasant odor so long-term storage is highly undesirable. Bird manure and sawdust mixture can also be used for biogas production. However, sawdust microorganisms decompose slowly and methane production is relatively low. As we have verified above [1, 2], the separation of lignocellulolytic materials, straw, was enhanced by the addition of enzymes. The purpose of this study was to determine whether and to what extent the addition of enzymes improves methane production from a mixture of bird droppings and sawdust.

There are many studies in the literature on biogas production from poultry manure and litter [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14], but only a few [15, 16] when biogas was produced in a mixture with sawdust. Miah [16] evaluated a mixture of rice hulls, sawdust and chicken excreta of broilers mixed with the co-substrate cow dung and poultry droppings for the production of biogas. "Four laboratory scale reactors, R1, R2, R3 and R4, were set up with different proportions of waste poultry litter, cow dung and poultry droppings and had a 6% total solid concentration. Digestion was carried out for 50 days at room temperature, 32 ± 3°C. Volatile solid degradation and specific gas production in the four reactors was 46%, 51.99%, 51.96%, 43% and 0.263, 0.469, 0.419, 0.221 l/g, respectively, based on the volatile solid (VS) feed. The methane yields were 71%, 72.5%, 72.6% and 70%, respectively. The COD reductions were 46.1%, 50.76%, 48.23% and 45.12%, respectively. A kinetic analysis showed that the anaerobic digestion of poultry litter with a co-substrate followed first order kinetics. Among the experimental reactors, R2 (25% cow dung, 75% poultry litter) gave the optimum results: a VS reduction of 51.99%, a specific gas yield of 0.469 l/g and a methane yield of 72.5%'' (Miah et al., 2016) [16]. Anaerobic treatment of relatively dry biomass (W=30-55 %) has an advantage, compared to the traditionally used wet biomass, due to lowered expenses of the transportation of raw or finished materials [14]. "With the help of laboratory scale 5 l digesters biogas production was investigated from different biomass in anaerobic fermentation in the batch process. Inoculum from cow manure finished fermentation process was added in each digester to facilitate the anaerobic fermentation process. The lowest average yield of biogas 185 l kg^{-1}vsd was obtained from fresh sawdust. The methane yield (percentage of methane) obtained from different biomass was the following: fresh sawdust 83 l kg^{-1}vsd broiler manure with slaughterhouse waste 185 l kg^{-1}vsd (52 %); and grain mill wastes 132 l kg^{-1}vsd (50 %)'' [15]. Co-digestion of poultry droppings with other substrates offers better gas yield and quality potential [10]. An Iranian research study [3] "evaluated codigestion of poultry litters and straw and found highest gas yield (0.12 m^3 CH_4/kg_{VS}) and highest methane content (70.2%) at loading rates of 3.0 kg_{VS}/m^3-d, HRT of 15 days and operating temperatures of 35°C (95°F) (Baebee, 2013) [3], but that yield and quality dropped off significantly at higher loading rates and at lower temperatures.'' The removal of ammonia from anaerobically digested manure was evaluated in this study [13]. "Firstly, the hydrolysis experiments were performed and the effects of temperature, total solids (TS) content and retention time were investigated. The results showed that 90% of the organic nitrogen in chicken manure (CM) can be converted into ammonia via biological hydrolysis within 3.6 days at 35 °C and 10–12.5% TS content'' [13]. It is unclear whether dry fermentation AD of poultry litter offers more yield and quality potential than more traditional wet AD. This study evaluated the effect of the substrate:inoculum ratio and digestate recirculation conditions on the biogas and methane yield, free ammonia concentration and solid phase agronomic quality (after treatment) of poultry litter solid state anaerobic digestion (SSAD)[14]. "Three laboratory-scale reactors containing 3 kg of poultry litter each were operated at mesophilic conditions (37°C) and seven experimental runs were performed at retention time of 30 days each. There were three substrate:inoculum ratios (1:1; 1:1.66, and 1:3) and three daily recirculation intervals (2, 3, and 4 times per day), and each recirculation event lasted 15 min. The highest biogas and methane yields were 183 $L.kg^{-1}$VSadd and 74 $L.kg^{-1}$VSadd, respectively, and they were obtained at the substrate:inoculum ratio of 1:3 with a digestate recirculation frequency of four times a day "[14]. "Poultry droppings (PD) make an excellent and abundant raw material for anaerobic co-digestion (AD) because of its high nitrogen content. Two sets of comparative assays were conducted on the anaerobic co-digestion of PD with two lignocellulosyc co-substrates (LCSs), namely wheat straw (WS) and meadow grass (MG), under five different mixing ratios to optimize substrate composition and C:N ratio for enhanced biogas production. All digesters were run simultaneously under a mesophilic temperature of 35 ± 1 °C with an identical volatile solids (VS) concentration. The results showed that the co-digestion of PD with LCSs was significantly higher in terms of biogas yield and bio-methane potential (BMP) than those obtained by mono-digestion of PD and LCSs. Co-digestion of PD and MG produced a higher cumulative biogas production, biogas yield and BMP than from respectively PD and WS. The highest methane contents found were 330.1 and 340.1 Nl kg^{-1} VS after digestion for 90 days at a mixing ratio of, respectively, 70:30 (PD:WS) with a C:N ratio of 32.02 and a mixing ratio of 50:50 (PD:MG) with a C:N ratio of 31.52. The increases were 1.14 and 1.13 times those of the LCSs alone, respectively (Rahman et al., 2017)'' [5]. "Anaerobic co-digestion of poultry droppings (PD) and briquetted wheat straw (BWS) with alkali additive in the form of KOH (BWS_{add}) or without any additive (BWS_{raw}) was conducted using continuously stirred tank reactors (CSTRs) under both mesophilic (35°C) and thermophilic (53°C) conditions. The aims of the study were to compare 1) co-digestion of PD and BWS versus mono-digestion of PD; 2) co-digestion of PD and BWS with or without additives; and 3) mesophilic and thermophilic anaerobic digestion (AD). Co-digestion of PD and BWS was superior to mono-digestion of PD in terms of gas production. Co-digestion of PD with BWS_{add} at thermophilic temperatures resulted in a higher methane volumetric yield per kg substrate compared to mesophilic conditions. With and without additive, co-digestion with BWS produced 8% and 11% higher yields at thermophilic conditions than at mesophilic conditions. Co-digestion of PD with BWS_{add} resulted in, respectively, 14% and 27% more methane produced at mesophilic and thermophilic conditions over mono-digestion of PD. When mono-digesting PD, the mesophilic temperature was superior to the thermophilic since methane yield was higher in the mesophilic temperature regime''[6]. Lignocellulosic residues are relatively recalcitrant to bioconversion during anaerobic digestion (AD) for biogas production. "Pre-treatments with cellulolytic enzymes or diluted alkali can facilitate biomass hydrolysis and enhance the process. Both pre-treatments require low energy and chemical inputs, without accumulation of inhibitor. Milled wheat straw was pre-treated with hydrolytic enzymes or with diluted NaOH before AD. The enzymatic pre-treatment only increased max by 14 %. However, the same increase was observed with heat-inactivated enzymes, thus it was merely caused by the bioconversion into methane of the organic compounds contained in the enzymatic preparations. Moreover, all the pre-treatments determined a holocellulose conversion into reducing sugars lower than 4 %'' [17]. "The hydrolysis of lignocellulose is assumed to be the rate-limiting step in the anaerobic fermentation process. One possibility to increase natural polymer degradation and concomitantly energy efficiency is the addition of exoenzymes to biogas facilities to enforce the primary degradation steps for biogas production. The influence of added enzymes on the viscosity of the biomass was tested'' [17]. "Only a marginal effect was obtained, when applying a tenfold higher concentration of added enzymes as proposed for practical use. The same result was achieved when commercially available enzymes were added to technical-scale fermentations using corn silage as monosubstrate. Various inhibition factors show studies'' [18, 19]. "The chemical complexity of the supplied feedstocks (either various compounds or complex molecule structures) directly affects the diversity of the biogas microbiome and hence the process performance'' [19].

The performance of chicken-manure-based AD at gradient organic loading rates (OLRs) in a continuous stirred tank reactor (CSTR) was investigated 150 days [20]. "The results showed that the biogas yield increased with increasing OLR, which was based on the volatile solids (VS), before reaching up to 11.5 g VS/(L·d), while the methane content was kept relatively stable and maintained at approximately 60%. However, when the VS was further increased to 11.5 g VS/(L·d), the total ammonia nitrogen (TAN), pH, and alkalinity ($CaCO_3$) rose to 2560 $mg·L^{-1}$, 8.2, and 15,000 $mg·L^{-1}$, respectively, while the volumetric biogas production rate (VBPR), methane content, and VS removal efficiency decreased to 0.30 $L·(L·d)^{-1}$, 45%, and 40%, respectively "[20].

"Metaferm created and produced in Latvia are substances, which induce biological processes. Metaferm contains multi enzymes, microelements and B group vitamins as well growing stimulators. Our previous studies show that the use of catalyst Metaferm has a positive effect on methane yield in anaerobic fermentation process of some biomass" [1, 2].

2. MATERIALS AND METHODS

The methodology described below and similar to German VDI 4630 (VDI 4630 2006), and Angelidaki et al., 2009 [21] guideline and the German Methodenhandbuch Energetische Biomassenutzung [22] were used for the present study.

Average samples of poultry manure and sawdust mixture (PMS) were taken and it's the chemicals compositions were determined in the LUA laboratory according to the standardized methodology ISO 6496:1999. For each group of raw materials an average sample was taken and the total dry matter, organic dry matter and ashes content were measured.

The average sample of each biomass was taken. The standard methods were used for analyses. The same amount (500,0g) of inoculum was filled in 16 bioreactors. Volume of each bioreactor was 0,75 L, The inoculum was digestate from continuos in laboratory working with small organic loading rate bioreactor (110 L volume). Only inoculums were filled in bioreactors (R1, R16) to determine how much biogas can be extracted from inoculum.
The others bioreactors were filled in with inoculums and biomass sample (20.0 g) with or without enzymes or catalyst Metaferm (see Table 1).

Poultry manure and sawdust mixture (20.0 g) were filled in bioreactors R2-R15 and in bioreactors R2–R5 added 1 ml alpha amylase, in bioreactors R6-R9 added 1 ml xylanase and in bioreactors R10-R13 added 1 mL Metaferm. Bioreactors were filled with substrate and placed in a heated chamber (SNOL model). Gas from each bioreactor was directed into a separate storage gas bag located outside the heated chamber.

For each biomass sample was determined dry matter (DM) and dry organic matter (DOM). DM % and weight was determined with the help scales Shimazu (accuracy ± 0,001) at 105°C and DOM% and weight with the help furnace Nabertherm burning the samples at 550°C. Before anaerobic digestion samples of biomass were prepared, carefully mixed all bioreactors were sealed and put in heating chamber SNOL (accuracy ± 0,1°C). Gases were collected in storage bags for each bioreactor located outside the heating chamber. From storage bags with the help gas analyser GA2000 (accuracy ± 0,025L) methane, carbon dioxide, oxygen and hydrogen sulfide contents were measured. pH value in every bioreactor before and after anaerobic digestion process was determined with the pH meter PP-50 (accuracy ± 0,02). Total weight of inoculum before digestion and digestate after digestion process in each bioreactor were measured with the scales Kern KFB16KO2 (accuracy ± 0,2 g). Batch mode (single filling) anaerobic digestion process was continued until biogas emmision occured (46 days). The volumes of biogas and methane measured from each bioreactor were converted to normal volume.

3. RESULTS

The data on sample analysis and on the amount of biogas and methane produced was estimated for all 16 bioreactors, and average results were calculated.

The results of raw material analyses before anaerobic digestion are shown in Table 1.

Biogas and methane volumes in liters obtained from each bioreactor are presented in Fig.1.

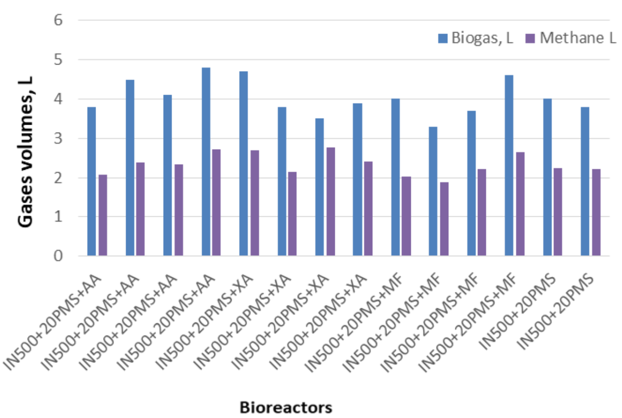

Figure 1. Biogas and methane gases volumes obtained from bioreactors.

Specific biogas and methane gases volumes obtained from bioreactors R2–R15 are presented in Fig. 2.

Weight of raw material in Table1 is provided with error value depending on the accuracy of respective weight measuring instrument used. Weight of total solids (TS) and dry organic matter (DOM) in Table1 is provided with accuracy ± 0.001 g.
Dry matter content in control bioreactors was low, because inoculum was digestate from bioreactor working with low organic loading rate.

As it can be seen from the Table 1 poultry manure and sawdust total biomass has a relatively high dry matter and organic dry matter content. This is explained due to the fact that the sawdust is dry.

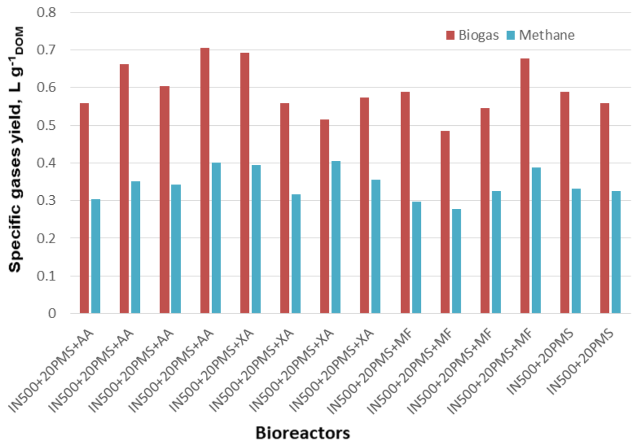

Figure 2. Specific biogas and methane yields from poultry manure and sawdust mixture.

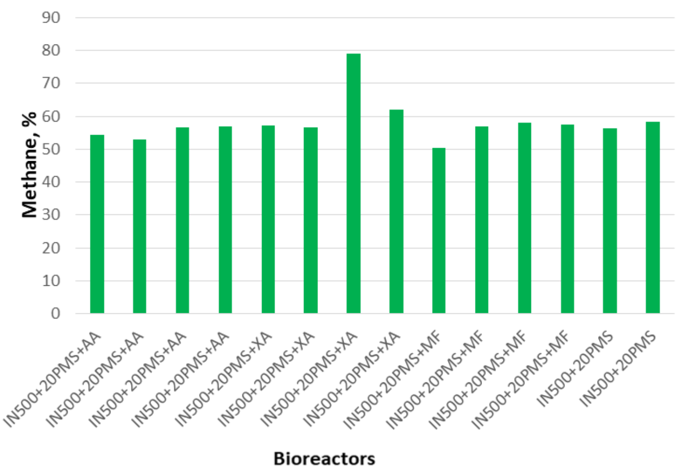

Figure 3 Methane content from bioreactors with poultry manure and sawdust mixture and enzymes.

Such raw material, containing a lot of organic dry matter, is well suited for biogas production. Biogas and methane yields from poultry manure and sawdust mixture with added enzymes are shown in Table 2.

As shown in Table 2, addition of the enzyme xylanase gave the best methane yield 0.368 L g^{-1}_{DOM}. Addition of the enzyme alpha amylase yielded methane 0.349 L g^{-1}dom, but Metaferm 0.322 L g^{-1}_{DOM}.

The average methane content (Fig. 3) in biogas compare with other biomass was average. It can be explained by the fact that poultry manure and sawdust mixture C: N was favourable for bacteria. The second reason is that there is a lot of lignin and cellulose in the sawdust, so more CO_2 is formed. Addition of enzymes xylanase methane content increased.

Table 1. Results of analysis of raw materials

Bioreactor	Raw material	Weight g	pH	TS %	TS g	ASH %	DOM %	DOM g
R1; R16	IN	500±0.2	7.86	4.18	20.9	24.18	75.82	15.84
R2–R5	20PMS	20±0.001		39.67	7.93	14.35	85.65	6.79
R2–R5	20PMS+1mlAA+IN	521±0.2		5.54	28.85	21.48	78.72	22.66
R6–R9	20PMS+1mlXA+IN	521±0.2		5.54	28.85	21.48	78.52	22.66
R10–R13	20PMS+1mlMF+IN	521±0.2		5.54	28.85	21.48	78.52	22.67
R14–R15	20PMS+IN	520±0.2		5.55	28.83	21.50	78.50	22.64

Abbreviations: TS – total solids; ASH – ashes; DOM – dry organic matter; IN – inoculums; PMS – poultry manure and sawdust mixture; AA – alpha amylase; XA – xylanase; MF – Metaferm

Table 2. Biogas and methane yields from poultry manure and sawdust mixture with added enzymes

Reactor	Raw material	Biogas, L	Biogas, L g^{-1}_{DOM}	Methane, aver. %	Methane L	Methane, L g^{-1}_{DOM}
R1	IN500	0.40	0.026		0.0290	0.002
R16	IN500	0.20	0.013		0.0008	0.0001
R1-R16		**0.30**	**0.02**		**0.015**	**0.001**
R2	IN500+20PMS+AA	3.80	0.559	54.31	2.064	0.304
R3	IN500+20PMS+AA	4.50	0.662	52.89	2.380	0.350
R4	IN500+20PMS+AA	4.10	0.603	56.73	2.326	0.342
R5	IN500+20PMS+AA	4.80	0.706	56.77	2.725	0.401
Aver. R2–R5 ±st.dev.		**4.30±0.44**	**0.633±0.065**	**55.18±1.91**	**2.374 ±0.272**	**0.349±0.040**
R6	IN500+20PMS+XA	4.70	0.692	57.17	2.687	0.395
R7	IN500+20PMS+XA	3.80	0.559	56.55	2.149	0.316
R8	IN500+20PMS+XA	3.50	0.515	78.88	2.761	0.406
R9	IN500+20PMS+XA	3.90	0.574	61.97	2.417	0.356
Aver. R6–R9 ±st.dev.		**3.97±0.51**	**0.585±0.075**	**63.64±10.44**	**2.504±0.279**	**0.368±0.041**
R10	IN500+20PMS+MF	4.00	0.589	50.37	2.015	0.297
R11	IN500+20PMS+MF	3.30	0.486	56.97	1.880	0.277
R12	IN500+20PMS+MF	3.70	0.545	58.11	2.208	0.325
R13	IN500+20PMS+MF	4.60	0.677	57.37	2.639	0.388
Aver. R10–R13 ±st.dev.		**3.90±0.55**	**0.574±0.080**	**55.71±3.58**	**2.186±0.331**	**0.322±0.048**
R14	IN500+20PMS	4.00	0.589	56.33	2.253	0.332
R15	IN500+20PMS	3.80	0.559	58.18	2.211	0.325
Aver. R14–R15 ±st.dev.		**3.90±0.14**	**0.574±0.021**	**57.26 ±1.31**	**2.232±0.030**	**0.329±0.005**

Note: Biogas and methane values for bioreactors 2–15 with fresh source biomass are provided with already subtracted average biogas and methane values obtained from reactors 1 and 16.
Abbreviation: L g^{-1}_{DOM} – litres per 1 g dry organic matter added (added fresh organic matter into inoculum

4. CONCLUSIONS

The average specific methane yield from poultry manure and sawdust mixture was 0.329 L g^{-1}_{DOM}. The result is good, similar that obtainable from maize silage. The average specific methane yield from poultry manure and sawdust mixture is better than from cow manure.

The addition of xylanase increased the specific methane yield of 11,19%. It is more advantageous to use this enzyme.

The addition of alpha amylase increased the specific methane yield 6,08%.

The addition of Metaferm decreased the specific methane yield of 2,2%. Using this biocatalyst for poultry manure and sawdust mixture cannot be economically.

The results of the study show that poultry manure and sawdust mixture can be used as raw materials for the production of methane. Addition of both enzymes improved methane yield.

In future studies, it would be desirable to clarify the effect of different pre-treatment (treatment with acids, bases, crushing degree) methods on the anaerobic fermentation of investigated biomass.

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