Ciência Rural, Santa Maria, v.52:4, e20200760, 2022 http://doi.org/10.1590/0103-8478cr20200760

ISSNe 1678-4596
AGRIBUSINESS

Anaerobic co-digestion of swine manure and forage at two harvesting ages

Juliana Dias de Oliveira[1*] Ana Carolina Amorim Orrico[1] Brenda Kelly Viana Leite[1] Alice Watte Schwingel[1] Marco Antonio Previdelli Orrico Junior[1] Marcio Romeiro de Avila[1] Janaina Freire Machado[1] Amanda Maria Domingos Ferreira Dias[1] Isabelly Alencar Macena[1] Wellington dos Santos[1]

[1]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados (UFGD), 79804-970, Dourados, MS, Brasil. E-mail: juliana.oli1997@hotmail.com. [*]Corresponding author.

ABSTRACT: *The co-digestion of swine manure with vegetable waste is an alternative that can increase the production of biogas and methane generated by the isolated digestion of manure. However, recommendations that are based on the best ratio between manure and forage, as well as the age of harvest, are still scarce in the literature. This study was conducted to evaluate inclusions (0, 25, 50, 75 and 100%) of the total solids (TS) of Elephant grass (Pennisetum purpureum Schum) harvested at two ages medium age (MA) at 45 days of growth and advanced age (AA) at 90 days in co-digestion with swine manure, using an entirely randomized design in a 5x2 factorial scheme. Batch digesters were used and biogas production was monitored for 12 weeks. There was influence of forage age (P <0.05) on the degradation of solids and neutral detergent fiber, with higher values for the substrates containing MA forage. The highest CH_4 yields were obtained by the substrates containing MA forage in the inclusion of 27.7 and 31.6%, being 253.7 and 222.2 L of CH4 per Kg of total or volatile solids. The age of the forages influenced the onset and persistence of biogas production, being advantageous only in the inclusion of 25% of MA forage. The AA forage inclusion is not recommended for co-digestion with swine manure.*
Key words: *biogas, fiber degradation, methane, solids reductions.*

Co-digestão anaeróbia dos dejetos de suínos e forragem em duas idades de colheita

RESUMO: *A co-digestão dos dejetos suínos associados a resíduos vegetais é uma alternativa que pode elevar as produções de biogás e metano alcançadas pela digestão isolada do dejeto. No entanto, recomendações que se baseiem na melhor proporção entre dejeto e forragem, assim como na idade de colheita ainda são escassas na literatura. O objetivo do trabalho foi avaliar inclusões crescentes (0, 25, 50, 75 e 100% dos sólidos totais (ST) de capim elefante (Pennisetum purpureum Schum.) colhido em duas idades de corte mediana (MA) aos 45 dias de crescimento e avançada (AA) aos 90 dias, empregado em co-digestão com o dejeto suíno, adotando-se delineamento inteiramente ao acaso, em esquema fatorial 5x2. Foram utilizados biodigestores batelada de bancada, sendo as produções de biogás acompanhadas durante 12 semanas. Houve influência da idade da forragem (P<0,05) na degradação de sólidos e fibra em detergente neutro, com maiores valores para os substratos que continham a forragem MA. Os maiores rendimentos de CH_4 foram alcançados pelos substratos que continham forragem MA na inclusão de 27,7 e 31,6 %, sendo de 253,7 e 222,2 L de CH_4 por Kg de ST ou sólido volátil adicionado. A idade das forragens influenciou o início e a persistência das produções de biogás ao longo do período de digestão, sendo vantajosa apenas na inclusão de 25% de forragem com MA.*
Palavras-chave: *biogás, degradação de fibra, metano, redução de sólidos.*

INTRODUCTION

Brazil is the 4th largest pig producer in the world and has produced 3.974 million tons of meat in 2020 (ABPA, 2021). This production is associated with an increasing amount of waste generated in the swine chain. According to PINTO et al. (2014), a pig intended for meat production can produce an average of 2.3 kg of manure per day, which considering its slaughter age at 150 days, would lead to a total of 345 kg of manure produced by each bred pig.

Anaerobic digestion of swine manure is one of the most commonly used recycling and treatment techniques, and presents excellent fermentation conditions, reaching high potential to generate methane and superior quality of the resulting biofertilizer, because in the swine manure there is availability of nutrients for digestion, due to the animal feed. According to ORRICO JÚNIOR et al. (2010), the isolated digestion of swine manure, fed by diets varying energetic concentrate between corn and sorghum, can result in production of up to 130 liters of biogas per kilogram of manure added with a hydraulic retention time of 90 days. However, it is known that biogas yields depend on factors acting in isolation or together, such as the animal production

Received 08.13.20 Approved 07.21.21 Returned by the author 08.12.21
CR-2020-0760.R2
Editors: Leandro Souza da Silva Frederico Costa Beber Vieira

phase, which influence the quantities of manure generated (PINTO et al. 2014); the diet composition, which will modify the constitution of waste and, especially its organic load (D´AQUINO et al. 2019). These factors will result in an increase or reduction of hydraulic retention time (HRT) required for efficient waste digestion (KINYUA et al. 2014).

However, the isolated digestion of swine manure can bring some undesirable conditions for the degradation of the material, such as excessive initial fermentation of the soluble constituents and acidification of the environment, and also due to higher nitrogen contents in swine manure, excessive concentrations of ammoniacal N (WANG et al. 2016). These limitations can be minimized with the co-digestion process, that is when two or more residues are combined to improve the digestion conditions, thus bringing better adjustment of nutrients to the substrates that will be degraded, allowing greater stability of the medium to be achieved.

In this way, the use of the forage surplus from animal production, generated mainly by the waste in the feeders, can be associated with swine manure resulting in a positive response in biogas production (SARITPONGTEERAKA et al., 2018). The forage has a higher amount of carbon than allied to the nitrogen present in the manure, resulting in a balance in the C:N ratio of the raw material (XIE et al., 2011), favoring the performance of microorganisms to obtain a higher rate of degradation, thereby improving the production of biogas (XIE et al., 2017). As related by WU et al. (2010) who observed an increase in the volumetric production of biogas using agricultural residues in associations with swine manure, reaching biogas production of 6.6 L per day with the inclusion of corn stalks and 7.2 L per day with oat straw, and when the authors used only swine manure it did not exceed 2 L per day.

Nonetheless, some factors must be considered so that co-digestion between swine manure and forage can represent an alternative to maximize the biogas generation, such as the age of the plant, which is a parameter that interferes with the availability of carbohydrates due to accumulation of cell wall components with forage growth. In a study by RODRIGUEZ et al. (2017), the neutral detergent fiber (NDF) values of *Penninsetum purpurem* increased from 51.9 to 64.4% of dry matter (DM) in 60 days of forage growth, indicating the accumulation of cell wall as the plant age advances.

The phenological stage of the plant directly influences the production of biogas and methane since the access of microorganisms to the substrate is restricted with the advanced age of the plant. However, the high concentration of fibrous content can improve methane production if the substrate remains in digestion for a longer time, making possible the degradation of these constituents. McENIRY & O´KIELY (2013) conducted a test with grasses, harvested in different months of the year. They did not verify the influence of the five different forage species for methane production. They just confirmed the effect of the forage harvest time on methane yields (253 and 225 L CH_4/kg of SV, for grasses harvested in May and July, respectively), to be 11% higher for younger grasses.

The characterization of the forage species, as well as the part of the plant that will be digested, is fundamental for the expected biogas and methane yields. Each part of the plant has its composition. The stem, for example, has a higher concentration of fibers, when compared to the leaves. The harvest time tends to collaborate so that the values are even more contrasted between these two parts (PAHKALA & PIHALA, 2000). In a study conducted by JIANG et al. (2016) significant differences were reported in the lignin composition of the wheat straw. They compared the stem and leaves, which presented lignin concentrations of 23.8% and 16.6% respectively.

The particle size must also be considered, as it may hinder or benefit microorganisms access due to the available contact area (WALL et al. 2016), as well as the proper adjustment of the ratio between forage and manure, which can increase or decrease favorable conditions to degradation (PRAPINAGSORN et al. 2017).

Based on the above, the following hypotheses were admitted: (1) that the addition of forage in co-digestion with pig manure will result in higher yields of biogas and methane; (2) that the yields of biogas and methane will be influenced by the harvest age of the plant, as well as by the proportion of its inclusion in the substrates. The objective of this work was to evaluate the influence of forage age and its inclusions, in co-digestion with swine manure, on the production of biogas and methane, in addition to the degradation of the solids and fibrous constituents during the process.

MATERIALS AND METHODS

The research was carried out at the Experimental Warehouse and Agricultural Waste Management Laboratory, belonging to the Faculty of Agricultural Sciences of the Universidade Federal da Grande Dourados (UFGD), located in

Dourados-MS, Brazil, Latitude 22° 13 ‘18’ ‘South and longitude 54 ° 48 ‘23’ ‘West.

The experiment was carried out in a completely randomized design, which consisted on a factorial scheme composed of 2 forage ages (medium and advanced) and 5 doses of forage inclusion (0 (control), 25, 50, 75, and 100%) in the substrates, as substitution of residues, containing 3 repetitions per treatment (biodigesters).

The manure used for the loading of the biodigesters was collected from a commercial pig breeding farm where the growth phase is developed. The animals were housed in collective pens and fed with an ad libitum diet, formulated according to the recommendations of ROSTAGNO et al. (2005). Elephant grass (*Pennisetum purpureum* Schum.) cultivated in the agrostological field belonging to UFGD was used as forage. This forage was collected at two ages, being considered at medium age (MA) the forage with 45 days of growth and advanced age (AA) at 90 days of growth. The experimental scheme used in this research is shown in figure 1.

Manure and forage were individually characterized by determining the hydrogen potential (pH), total solids (TS), volatile solids (VS), carbon (C), nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF) and chemical oxygen demand (COD) (Table 1).

An initial TS content equal to 3.5% was adopted for the formulation of the substrates and loading of the biodigesters. The inoculum represented the amount of 10% of the TS substrates and was previously prepared from the fermentation of swine manure, in order to accelerate the onset of substrate degradation. In order to adjust the amounts of each component (manure, forage, and inoculum), the original TS concentrations were considered and water was used for dilution. The harvested forage was chopped into particles with an average size of two centimeters, to then be part of the composition of the substrates, being homogenized in an industrial mixer, and determined: pH, COD, and the contents of TS, VS, C, N, NDF, and ADF.

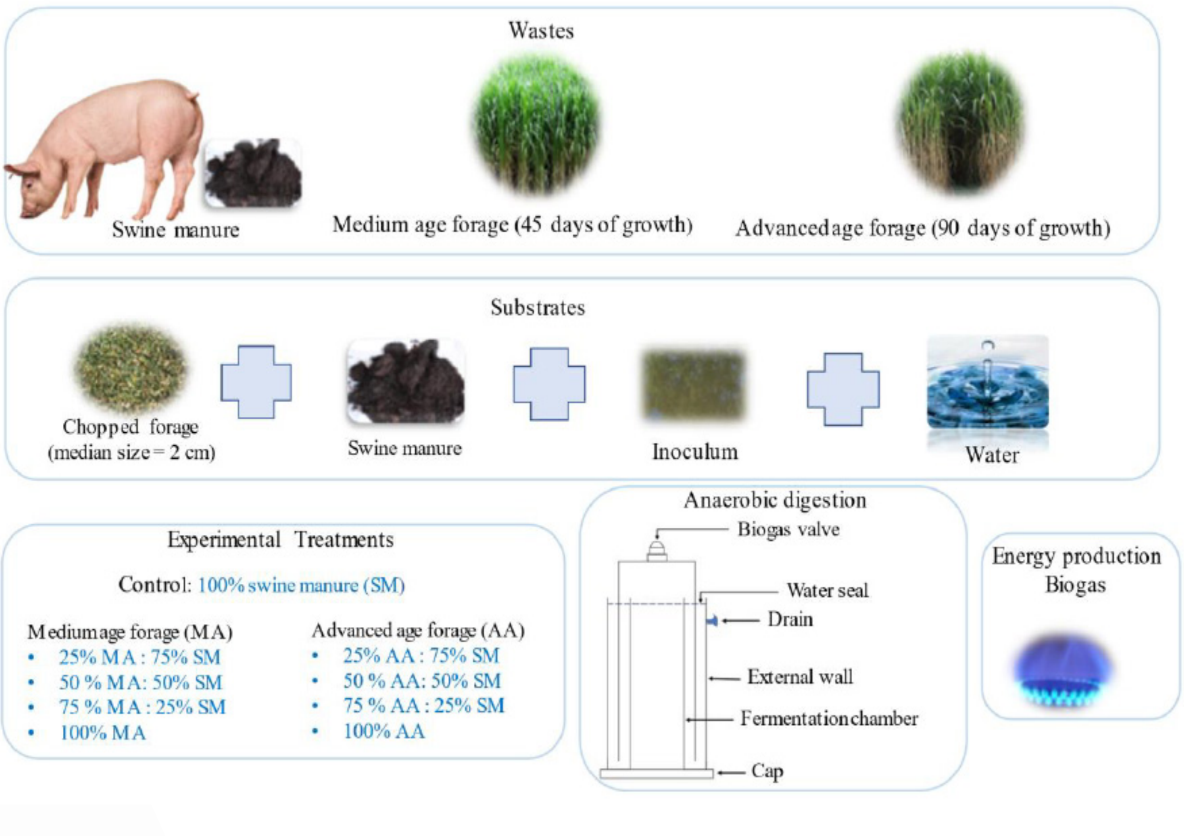

Figure 1 - Representation of the anaerobic co-digestion process of swine manure with different grass inclusions at two harvesting ages.

Table 1- Initial characterization of forages, swine manure and inoculum.

Material	--pH--	---TS---	---VS---	---N---	---NDF---	---ADF---	-----COD (g O_2 /L)-----
		---------------------------------(%)---------------------------------					
Swine manure	6.55	22.57	84.87	4.27	30.97	20.21	902.5
Medium age forage	5.31	35.39	92.36	0.87	66.17	38.35	1,331.2
Advanced age forage	5.58	45.93	92.83	0.74	67.33	41.28	1,661.5
Inoculum	-	3.84	69.74	3.55	19.49	9.06	436.3

TS: total solids, VS: volatile solids, NDF: neutral detergent fiber, ADF: acid detergent fiber, COD: chemistry oxygen demand.

The substrates were placed in containers with a capacity of 1.5 liters of fermentation content, using 27 batch bench digesters, consisting of two PVC cylinders, one of which was used as a "water seal" and the other as gasometer, for the biogas storage, having a discharge valve to release the biogas produced. The biodigesters were stored in a covered shed, protected from sun and rain. During the experimental period, biogas production was monitored daily and methane levels were measured. The measurements of biogas production were carried out by the vertical displacement of the gasometers, being the volume produced calculated from the gasometer area and the height of its displacement, correcting this value for normal conditions of temperature and pressure (KUNZ et al. 2019). For the analysis of the biogas composition, the GA - 21 Plus gas analyzer from Madur Electronics was used, equipped with sensors to determine the O_2, CO_2, and CH_4 concentrations. The specific productions of biogas and methane were calculated considering the total volumes produced and the amounts of TS and VS added. Reductions of TS, VS, and fibrous constituents were calculated considering the quantities of these constituents at the beginning (affluent) and at the end (effluent) of the fermentation process.

The analyzes of TS, VS, COD, and pH were performed according to the methodology described by APHA (2012). The contents of NDF and ADF were measured according to the methodology proposed by DETMANN et al. (2012). The N was determined by the micro Kjeldahl method as described by DETMANN et al. (2012). C contents were quantified using the LECO elementary analyzer model TruSpec CN628 (LECO INSTRUMENTS, ST JOSEPH, MICHIGAN, USA).

The results were subjected to analysis of variance, considering as sources of variation the age of forage and their inclusion, tested with 5% probability. The effect of the forage inclusion was evaluated by orthogonal contrasts, considering linear or polynomial behaviors according to the significance values presented. The effects of the forage maturation were compared by the Tukey test when a difference was observed.

RESULTS AND DISCUSSION

The inclusion of grass influenced the reductions of solids and NDF in the substrates, providing greater degradation ($P < 0.05$) of these constituents when using forage at a medium age compared to the one at an advanced age (Table 2). This beneficial influence provided by the use of younger forage is probably associated with its composition (Table 1), with lower levels of NDF and ADF, which possibly also reduced the participation of lignin in the substrates, in addition to higher concentrations of N. It may have improved the balance of the C:N ratio, especially in doses with higher grass inclusions.

The smallest reductions of solids and NDF in the substrates prepared with grass at an advanced age, is possibly related to the characteristics of the plant cell wall, as the age of the plant increases, the fibrous constituents tend to increase their concentration, mainly lignin. The average levels of 4.01 and 16.07% of lignin in elephant grass were reported by XIE-MING et al. (2011) when evaluating the composition of forage with 30 and 90 days of growth. This rapid increase in the proportion of lignin occurs due to the intense growth of the plant. This complex structure will act as a limiter in the degradation of polysaccharides, such as cellulose and hemicellulose. The generation of simple sugars, which benefit the fermentation process, is prevented, thus declining biogas production.

As described by CHANPLA et al. (2018), who found different concentrations of lignin for

Table 2 - Biogas productions (normal temperature and pressure conditions) and solids and fibrous constituents reductions during co-digestion of two ages of forages and swine manure.

Harvest Forage Age	---Biogas production (liter/kg added)---		-----------------------------Reduction (%)---------------------------			
	--------TS--------	----------VS----------	------TS------	------VS------	-----NDF-----	------ADF------
Medium	241.21	270.66	56.99	63.15	65.29	59.86
Advanced	206.36	234.22	50.10	52.84	50.21	58.35
p value	0.01895	0.02389	0.02885	0.02815	0.00894	0.84079
CV (%)	20.93	20.12	14.97	20.47	24.71	28.92

TS: total solids, VS: volatile solids, NDF: neutral detergent fiber, ADF: acid detergent fiber. CV: coefficient of variation.

Napier grass comparing the harvest period of 35, 45 and 55 days, with lignin levels being 3.8, 3.9 and 6.1% of DM, respectively. Lignin is mainly degraded under aerobic conditions, while under low oxygen or anoxic conditions it can remain intact for long periods, being able to create a barrier against hydrolytic enzymes, according to its higher participation in the substrate, resulting in less efficient conversions of organic material into biogas (LI et al., 2018).

There was no influence of the grass age ($P > 0.05$) for the ADF reductions in the substrates. It can be possibly explained because the forages, regardless of the age of harvest, presented high levels of ADF in their compositions. The higher concentrations of ADF may be related to the higher growth rate of this grass species, which, due to its large size, allows the deposition of cell walls, and also lignin, earlier, as reported by TOSI et al. (1999). Thus, the degradation of the ADF fraction may have posed resistance to microbial attack and slowness in its reduction, and the period of 80 days of substrate retention may have been insufficient to state the effect of forage age in reducing this fraction. Some studies such as the one by KANG et al. (2020) were carried out aiming the improvement in the degradability of *Penninsetum* with the use of sodium chlorite. The authors reported an enhance in the biogas production, when there was a reduction in the lignin content of the forage, resulting in an increase in the degradability of the substrates from 59.6 to 86.4%, and an increase in methane production by 38.3%.

Although this possibility of pre-treatment contributes to the greater degradation of the substrates, there is a greater demand for investments and also further research focusing in the development of the co-digestion process (ABRAHAN et al. 2020; KUMAR et al. 2019).

The reductions in solid and fibrous constituents are important as indicators of yields for biogas and methane production, as well as for the quality of the biofertilizer that will be resulted from the process. Therefore, evaluating the reductions presented by the substrates, in association with the production of biogas and methane during digestion, it can be inferred that it is a safe way to indicate the condition that must be chosen to maximize the results. For the results of specific biogas production (Figure 2) there was an interaction effect ($P < 0.05$) between the forage age and the inclusion dose in the substrates. Thus, there is a beneficial effect, with quadratic behavior (Figure 3) for substrates that contained forage at a medium age and linear decreasing behavior for substrates containing forage at an advanced age. Therefore, for biogas production, it was only beneficial to include grass at a medium age, and for the advanced age, isolated digestion of swine manure showed higher yields (Figure 2).

Possibly most of the disagreement between the researches that are available in the literature which refer to the use or not of forages on substrates in co-digestion with animal manure, are associated with the forage fraction composition. It may correspond to the forage species, or within the same species and, the harvesting age of the plant. In a study conducted by HARYANTO et al. (2017), the authors carried out the co-digestion of elephant grass with bovine manure, however, they did not find a beneficial effect on biogas production to recommend the inclusion of the forage in the substrate. It may happen because the authors worked with substrates containing high concentrations of fibrous constituents, as they associated a forage that has accelerated growth with the ruminant animal manure, which already contains a higher proportion of roughage in the diet, compared to non-ruminants. As a result, the authors concluded that co-digestion was inefficient to improve biogas yields and that the isolated digestion of the manure

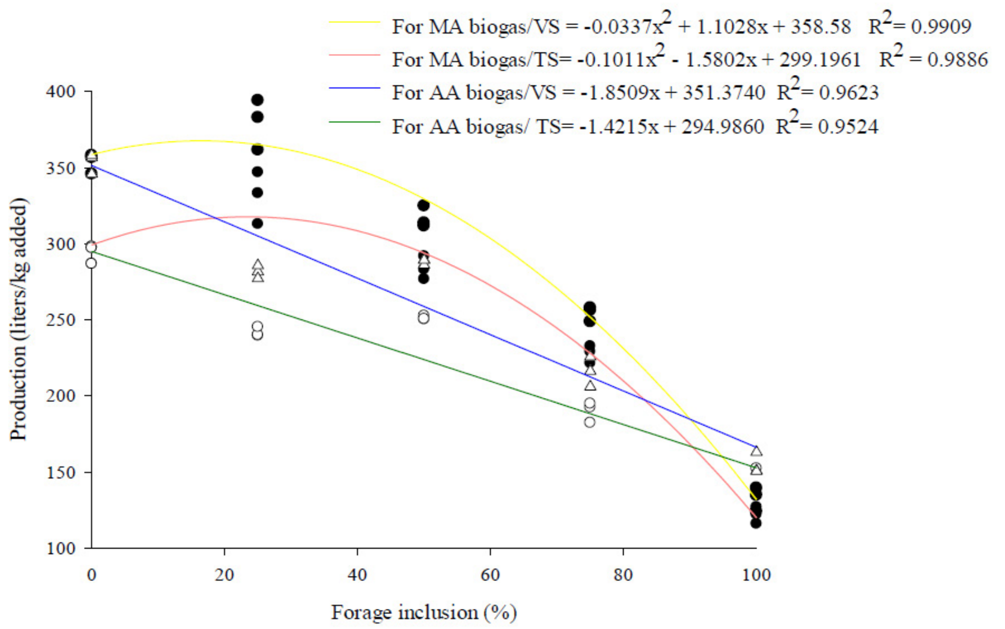

Figure 2 - Biogas yield (liters/kg added) in substrates containing swine manure and two ages of forage (MA– medium age and AA- advanced age) in anaerobic co-digestion. (p-value to all equations <0.001). TS: total solids, VS: volatile solids.

resulted in higher yields. Similar behavior was described by MOSET et al. (2017), who observed a reduction of 6% in methane production when substrates prepared with grasses and cattle manure were digested; although, the initial characteristics of the substrates and the quality of the forage were not adequately described by the authors.

The methane concentration is one of the most determining components to indicate the efficiency of the process, since it is a gas with fuel capacity and can be used in rural properties in order to supply or reduce the energy demand of these production units (KUPRYS-CARUK et al., 2019). In this sense, specific methane productions are important, because by expressing their yield according to a specific component that has been added to the digestion environment, they allow us to assess under which conditions the digestion process is more efficient. As seen for specific biogas production, methane yields (Figure 3) have benefited from the inclusion of medium age grass but was hampered by grass at an older age.

For the substrates containing the medium age grass, the maximum yields of methane were achieved with the inclusions of 27.7 and 31.6%, considering the potentials for each kilogram of VS or TS added, which would result on the productions of 253.7 and 222.2 liters of methane, respectively. With the inclusion of grass at an advanced age, the highest yields were with the condition of non-inclusion (control), and for each 1% of forage inclusion, there would be a reduction of 1.13 liters of methane for each kilogram of SV added.

The benefits of including grass in methane concentrations have also been verified by LIANHUA et al. (2020) when they co-digested the swine manure with *Penninsetum* in the proportion of 25:75, respectively, and verified yields 5% higher in this inclusion of forage compared to when only 50% was added. MAO et al. (2017) found higher methane yields when it was added 30% of rice straw to the swine manure in anaerobic digestion, compared with the isolated digestion.

This is a determining factor for recommending the inclusion of forage in swine manure because the forage addition to the substrates should only be indicated if there is an improvement in the process yield. That is why, it is important to consider the forage quality and prioritize harvests at younger ages, when they have a lower concentration of fibrous constituents so that these carbohydrates are accessible to microorganisms for digestion and consequent conversion into biogas.

The distribution of biogas production with the inclusion of grass is an additional concern since

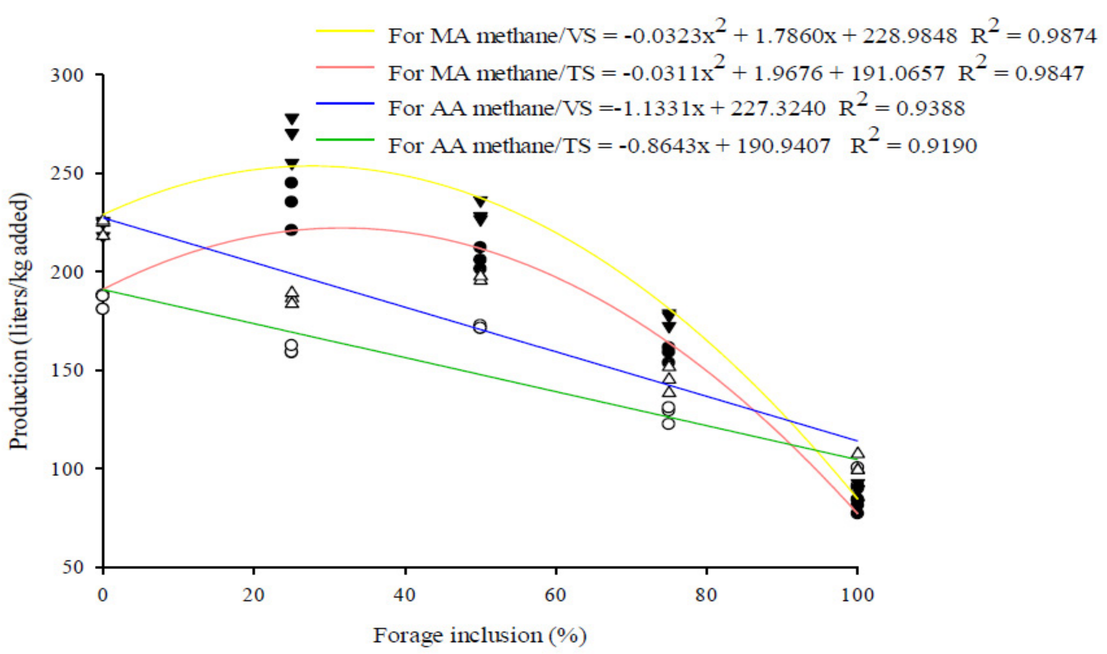

Figure 3 - Methane yield (liters/kg added) in substrates containing swine manure and two ages of forage (MA– medium age and AA- advanced age) in anaerobic co-digestion. (p-value to all equations <0.001). TS: total solids, VS: volatile solids.

fibrous constituents can delay the process. However, if there is a synchrony between the availability of the carbohydrate and protein fractions, mainly present in the co-digesting residues, the result may benefit and even advance and maintain biogas production for longer. Thus, with the intention of interpreting these results, in figures 4 (A and B) and 5 (A and B), the distribution of biogas production over the digestion period was demonstrated, as well as the time that is necessary for the achievement of the biogas production potential.

For the distribution, it is observed that with the inclusion of medium age grass (Figure 4A) at 25 and 50% levels, the biogas production started along with the control and was persistent for longer and at higher volumes with the inclusion 25% when compared to the control. With the inclusion of the grass at an advanced age (Figure 4B) it is possible to observe that despite the inclusion of 50 and 75% levels of grass present the initial biogas production along with the control, only in isolated digestion did the production persist for longer, which resulted in the highest volumes of biogas. Regarding the potentials of biogas production over time, it can be seen in figure 5A that until the 6th week of digestion, the inclusions of 25 and 50% of grass at a medium age showed a potential that was similar to the control; however, after this period, the inclusion of 25% excelled and reached higher potentials with less digestion time, in addition the potential reached by the control with 12 weeks of production was reached with 10 weeks for the substrates containing 25% of grass at a medium age. For the inclusion of the grass at an advanced age (Figure 5B), the control group reached potentials that were up until the 4th week of production, and were accompanied by 50 and 75% levels of grass inclusions; however, after that period all inclusions presented values lower than the control, and the maximum potentials with the inclusion of grass occurred in the proportions of 25 and 50% and corresponded to the potentials presented by the control in the 9th week of digestion.

Possibly the age of the grass, and the consequent composition, directly influenced the time needed to start the biogas production and the persistence of these productions, as well as the reach of the volumes produced, being advantageous, according to these parameters, only the inclusion of medium age grass, in the 25%. In all digested substrates, the pH values remained within the ideal range for the good development and performance of microorganisms, which is 7.0, which can vary from 6.6 to 7.6.

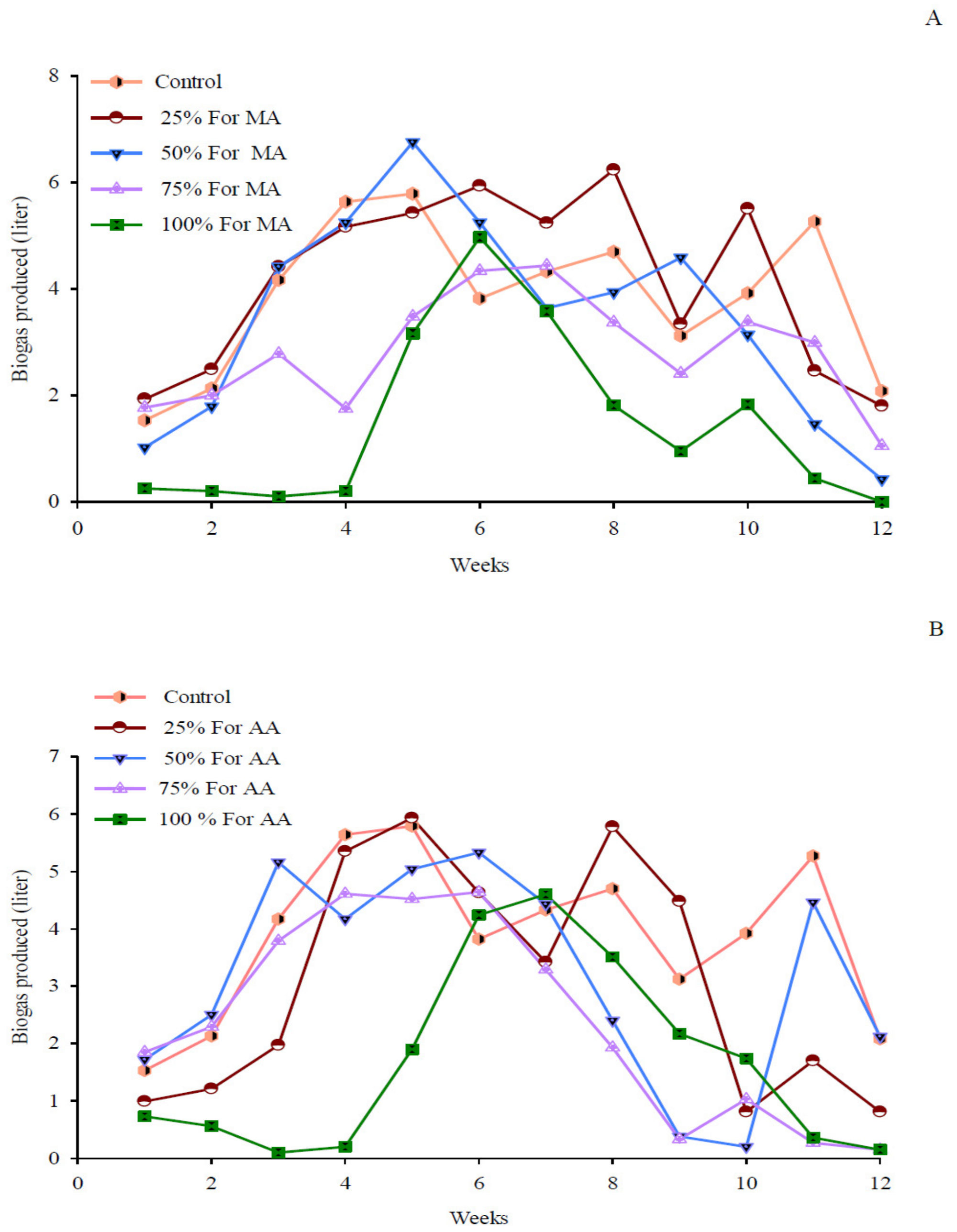

Figure 4 - Distribution of biogas produced during substrates anaerobic co-digestion containing swine manure and medium age (MA, A) and advanced age (AA, B) forage.

The anaerobic co-digestion of swine manure with grass inclusions presents itself as an alternative to increasing energy use in the farms. This collaborates with reductions of waste generation, whether from animal feeding or from the field. It promotes aggregation of value for properties. However, care must be taken with the quality and quantity of forage to be added in the substrates, since plants with more advanced maturation and in greater proportion, can result in a decline in biogas and methane production.

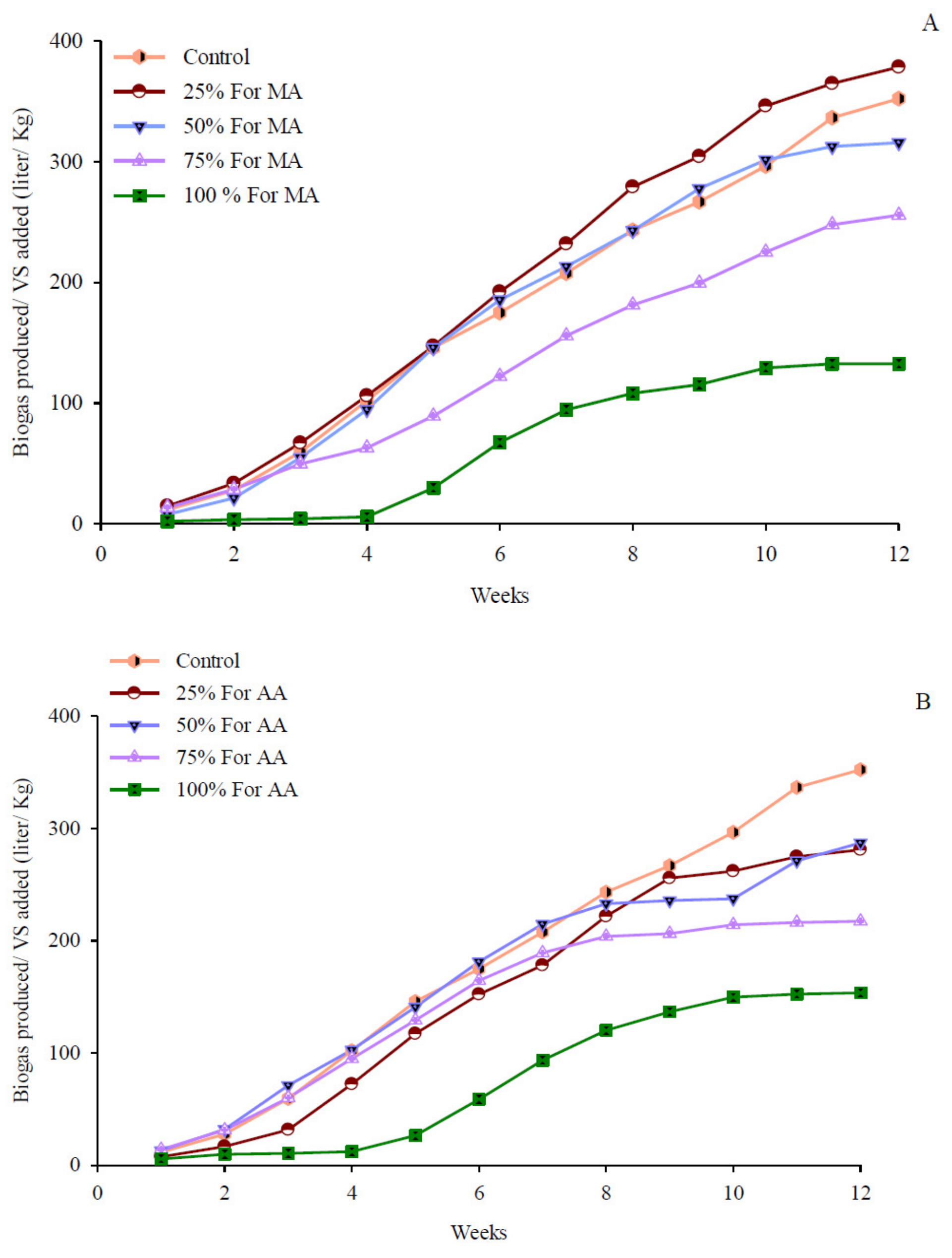

Figure 5 - Accumulated biogas potential production during substrates anaerobic co-digestion containing swine manure and medium age (MA, A) and advanced age (AA, B) forage.

CONCLUSION

Reductions in solids and fibrous constituents, as well as production of biogas and methane in substrates prepared with swine manure were influenced in different ways by the age of the harvest of the forages. For the medium age forage, the inclusion proportion should be up to 27.7% of the substrates, thus benefiting biogas and methane yields and the degradation of organic constituents compared to the isolated digestion of swine manure. For advanced age forage, co-digestion with swine manure is not recommended.

CONFLICTS OF INTEREST

The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

ACKNOWLEDGEMENTS

This study was carried out with the financial support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and, the Universidade Federal da Grande Dourados (UFGD).

AUTHORS´ CONTRIBUTIONS

The authors contributed equally to the manuscript.

REFERENCES

ABPA. **Associação Brasileira de Proteína Animal** – Anuário. 2021. Available from: <http://abpa-br.org/relatorios/>. Accessed: May. 18, 2021.

ABRAHAN, A. et al. Pretreatment strategies for enhanced biogas production from lignocellulosic biomass. **Bioresource Technology**, v. 301, p. 122725, 2020. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960852419319546>. Accessed: Jan. 19, 2021. doi: <https://doi.org/10.1016/j.biortech.2019.122725>.

APHA-AMERICAN PUBLIC HEALTH ASSOCIATION. **Standard methods for the examination of water and wastewater**. 22nd ed. Washington: American Public Health Association, American Water Works Association, Water Environment Federation, 2012. Available from: <https://www.standardmethods.org/doi/full/10.2105/SMWW.2882.011>. Accessed: Oct. 9, 2019.

CHANPLA, M. et al. Effect of harvesting age and performance evalution on biogasification from Napier grass in separeted stages process. **KSCE Journal of Civil Engineering**, v. 22, n. 1, p. 40-45, 2018. Available from: <https://www.researchgate.net/publication/315854964_Effect_of_harvesting_age_and_performance_evaluation_on_biogasification_from_Napier_grass_in_separated_stages_process>. Accessed: Oct. 25, 2019. doi: 10.1007/s12205-017-1164-y.

D´AQUINO, C.A. The effect of organic loading rate´s natural variation on the biogás yelds from swine manure digestion at different hydraulic retention time. **Engenharia Sanitária e Ambiental**, v. 24, n. 3, 2019. Available from: <https://www.scielo.br/scielo.php?pid=S1413-41522019000300613&script=sci_arttext>. Accessed: Jan. 23, 2021. doi: 10.1590/s1413-41522019124926.

DETMANN, E. et al. **Métodos para análise de alimentos**. Visconde do Rio Branco: INCT, 2012. 214 p. Available from: <https://scholar.google.com.br/citations?user=6_9scA0AAAAJ&hl=pt-BR>. Accessed: Oct. 9, 2019.

HARYANTO, A. et al. Biogas production from anaerobic codigestion of cowdung and elephant grass (Pennisetum purpureum) using batch digester. **IOP Conf. Ser. Earth Environ. Sci**, v. 141, 012011, 2017. Available from: <https://iopscience.iop.org/article/10.1088/1755-1315/141/1/012011>. Accessed: May. 18, 2020. doi: 10.1088/1755-1315/141/1/012011.

JIANG, B. et al. Comparison of the substrate enzymatic digestibility and lignin structure of wheat straw stems and leaves pretreated by green liquor. **Bioresource Technology**, v. 199, p. 181-187, 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960852415012109>. Accessed: Jan. 25, 2021. doi: 10.1016/j.biortech.2015.08.104.

KANG, X. et al. Enhanced methane production from anaerobic digestion of hybrid Pennisetum by selectively removing lignina with sodium chlorite. **Bioresource Technology**, v. 295, 122289, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852419315196>. Accessed: Jan. 7, 2020. doi: 10.1016/j.biortech.2019.122289.

KINYUA, M. N. et al. Effect of solids retention time on the bioavailability of organic carbon in anaerobically digested swine waste. **Bioresource Technology**, v. 162, p. 14-20, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852414004167>. Accessed: Jan. 20, 2021. doi: 10.1016/j.biortech.2014.03.111.

KUMAR, S. et al. Weak alkaline treatment of wheat and pearl millet straw for enhanced biogas production and its economic analysis. **Renewable Energy**, v. 139, p. 753-764, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960148119302988>. Accessed: Jan. 19, 2021. doi: 10.1016/j.renene.2019.02.133.

KUNZ. A. et al. **Fundamentos da digestão anaerobia, purificação do biogas, uso e tratamento do digestato**. Concórdia: Sbera: Embrapa Suínos e Aves, 2019. 209p.

KUPRYS-CARUK, M. et al. Influence of double-cut harvest system on biomass yield, quality and biogas production from C4 perennial grasses. **Biomass and Bioenergy**, v. 130, 105376, 2019. Available from: <https://www.sciencedirect.com/science/article/pii/S0961953419303253>. Accessed: Jul. 9, 2020. doi: 10.1016/j.biombioe.2019.105376.

LI, W. et al. Methane production through anaerobic digestion: Participation and digestion characteristics of cellulose, hemicellulose and lignin. **Applied Energy**, v. 226, p. 1219-1228, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0306261918307657>. Accessed: Feb. 7, 2020. doi: 10.1016/j.apenergy.2018.05.055.

LIANHUA, L. et al. Anaerobic co-digestion of pennisetum hybrid and pig manure: A comparative study of performance and microbial Community at different mixture ratio and organic loading rate. **Chemosphere**, v. 247, p. 125871, 2020. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S004565352030062X>. Accessed: May. 18, 2020. doi: 10.1016/j,chemosphere.2020.125871.

MAO, C. et al. Process performance and methane production optimizing of anaerobic co-digestion of swine manure and corn straw. **Scientific Reports** 7, 9379, 2017. Available from: <https://www.nature.com/articles/s41598-017-09977-6>. Accessed: Apr. 23, 2020. doi: 10.1038/s41598-017-09977-6.

McENIRY & O´KIELY. Anaerobic methane production from five commom grassland species at sequential stages of maturity.

Bioresource Technology, v. 127, p. 143-150, 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S0960852412014344>. Accessed: Jan. 22, 2021. doi: 10.1016/j.biortech.2012.09.084.

MOSET, V. et al. Co-digestion of cattle manure and grass harvested with different technologies. Effect on methane yield, digestate composition and energy balance. **Energy**, v. 141, p. 451-460, 2017. Available from: <https://www.sciencedirect.com/science/article/pii/S0360544217314445>. Accessed: Jan. 22, 2021. doi: 10.1016/j.energy.2017.08.068.

ORRICO JÚNIOR, M. A. P. et al. Avaliação de parâmetros da biodigestão anaeróbia de dejetos de suínos alimentados com dietas à base de milho e sorgo. **Engenharia Agrícola**, v. 30, n. 4, p. 600-607, 2010. Available from: <https://www.scielo.br/scielo.php?script=sci_abstract&pid=S0100-69162010000400004&lng=en&tlng=pt>. Accessed: Feb. 3, 2020. doi: 10.1590/S0100-69162010000400004.

PAHKALA & PIHALA. Different plants parts as raw material for fuel and pulp production. **Industrial Crops and Products**, v. 11, p. 119-128, 2000. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0926669099000503>. Accessed: Jan. 27, 2021. doi: 10.1016/S0926-6690(99)00050-3.

PINTO, L. P. et al. Levantamento de dados sobre os dejetos suínos e suas características. **Revista Brasileira de Energia Renováveis**, v. 3, n.3, 2014. Available from: <https://www.researchgate.net/publication/287689769_Levantamento_de_dados_sobre_os_dejetos_suinos_e_suas_caracteristicas>. Accessed: Feb. 7, 2020. doi: 10.5380/rber.v3i3.38467.

RODRIGUEZ, C. et al. Pretreatment techniques used in biogas production from grass. **Renewable and Sustainable Energy Reviews**, v. 68, p. 1193-1204, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1364032116002306>. Accessed: Nov. 5, 2019. doi: 10.1016/j.rser.2016.02.022.

SARITPONGTEERAKA, K. et al. Biomethanation efficiency of para-grass in piggery wastewater in single stage and temperature phased anaerobic systems. **Biocatalysis and Agricultural Biotechnology**, v.15, p.254-263, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1878818118303748>. Accessed: Jun. 18, 2020. doi: 10.1016/j.bcab.2018.06.021.

TOSI, P. et al. Avaliação do capm Elefante (Pennisetum purpureum Schum) cultivar Taiwan A-148 ensilado com diferentes técnicas de redução de umidade. **Revista Brasileira de Zootecnia**, v. 28, n. 5, p. 947-954, 1999. Available from: <https://www.rbz.org.br/pt-br/article/avaliacao-do-capim-elefante-pennisetum-purpureum-schum-cultivar-taiwan-a-148-ensilado-com-diferentes-tecnicas-de-reducao-de-umidade/>. Accessed: May. 29, 2020. doi: 10.1590/S1516-35981999000500006.

WALL, D. M. et al. Investigating two-phase digestion of grass silage for demand-driven biogas applications: Effect of particle size and rumen fluid addition. **Renewable Energy**, v. 86, p. 1215-1223, 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960148115303281>. Accessed: Jan. 11, 2020. doi: 10.1016/j.renene.2015.09.049**.**

WANG, Y. et al. CH_4, NH_3, N_2O and No emissions from stored biogas digester effluent of pig manure at different temperatures. **Agriculture, Ecosystems & Environment**, v. 217, p. 1-12, 2016. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0167880915301262>. Accessed: Jan. 08, 2021. doi: 10.1016/j.agee.2015.10.020.

WU, X. et al. Biogas and CH4 productivity by co-digesting swine manure with three crop residues as an external carbono sourc. **Bioresource Technology**, v. 101, n. 11 p. 4042-4047, 2010. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960852410001380>. Accessed: Nov. 18, 2019. doi: 10.1016/j.biortech.2010.01.052.

XIE-MING, X. et al. Dynamic changes of lignin contents of MT-1 elephant grass and its closely related cultivars. **Biomass and Bioenergy**, v. 35, n. 5, p. 1732-1738, 2011. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0961953411000195>. Accessed: Jan. 25, 2021. doi: 10.1016/j.biombioe.2011.01.018.

XIE, S. et al. A pilot scale study on synergistic effects of co-digestion of pig manure and grass silage. **International Biodeterioration & Biodegradation**, v. 123, p. 244-250, 2017b. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0964830517306200>. Accessed: Jul. 9, 2020. doi: 10.1016/j.ibiod.2017.07.005.

XIE, S.et al. Effect of pig manure to grass silage ratio on methane production in batch anaerobic co-digestion of concentrated pig manure and grass silage. **Bioresource Technology**, v. 102, n. 10, p. 5728-5733, 2011a. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0960852411003518>. Accessed: Nov. 18, 2019. doi: 10.1016/j.biortech.2011.03.009.