

WILLIAN RUFINO ANDRADE

**EFFECT OF BIOCHAR ADDITION ON ANAEROBIC DIGESTION OF
LAYING HEN MANURE**

Dissertation submitted to the Agricultural Engineering Graduate Program of the Universidade Federal de Viçosa as a partial fulfillment of the requirements for the degree of Magister Scientiae.

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Richard Stephen Gates
(Adviser)

I dedicate this work to my father and mother, the most supportive and amazing souls
God could have ever put in my path!

BIOGRAPHY

Willian Rufino Andrade, son of Januário Andrade and Sueli Aparecida Rufino Andrade, was born in Pompéia, São Paulo State - Brazil, on June 5th of 1993.

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He graduated in 2016 and become a bachelor in Animal Science. In March of 2017 he became a master student at the Graduate Program of Agricultural Engineering of the Universidade Federal de Viçosa at Viçosa, Minas Gerais State, Brazil.

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TABLE OF CONTENTS

ABSTRACT	vii
RESUMO	viii
1. GENERAL INTRODUCTION	1
CHAPTER 1 – LITERATURE REVIEW	4
2. LITERATURE REVIEW	4
2.1. Anaerobic Digestion	4
2.1.1. Factors that can affect the anaerobic digestion (AD) process	6
2.1.1.1. Temperature	6
2.1.1.2. Hydrogenionic potential (pH) and Alkalinity	7
2.1.1.3. Volatile fatty acids	8
2.1.1.4. Carbon: nitrogen ratio (C: N)	9
2.1.1.5. Total ammonia nitrogen concentration (TAN)	9
2.1.1.6. Substrate composition	11
2.1.1.7. Hydraulic Retention Time (HRT)	11
2.1.2. Anaerobic digestion feeding systems	13
2.1.3. Products from the anaerobic digestion process	15
2.1.3.1. Biogas	15
2.1.3.2. Digestate	15
3. Biochar and its use	16
CHAPTER 2 - Effect of biochar on monitoring parameters and biogas yield in the anaerobic digestion of laying hen manure	18
ABSTRACT	18
RESUMO	18
4. INTRODUCTION	19
5. MATERIAL AND METHODS	20
5.1. Location	20
5.2. Experimental design	20
5.3. Feedstock	21
5.4. Physical-chemical characterization	21
5.5. Assay of anaerobic digestion	22
5.6. Physical-chemical characterization of the digestate	24
5.7. Statistical Analysis	24
6. RESULTS AND DISCUSSION	24
6.1. Monitoring Parameters	24
6.2. Solids reduction and biogas yield	27
7. CONCLUSION	30
CHAPTER 3 - Anaerobic digestion of laying hen manure with biochar: monitoring parameters, biogas yield and digestate quality.	31
ABSTRACT	31
RESUMO	31
8. INTRODUCTION	32

9.	MATERIAL AND METHODS.....	33
9.1.	Location.....	33
9.2.	Experimental design	34
9.3.	Feedstock.....	34
9.4.	Physical-chemical characterization of residues and substrates	34
9.5.	Assay of anaerobic digestion	36
9.6.	Physical-chemical characterization of digestate.....	38
9.7.	Statistical Analysis	39
10.	RESULTS AND DISCUSSION	39
10.1.	Monitoring parameters.....	39
10.2.	Solids reduction and Biogas yield	44
10.3.	Digestate quality	49
11.	CONCLUSION.....	52
12.	FINAL CONSIDERATIONS AND SUGGESTIONS	53
13.	REFERENCES.....	54

ABSTRACT

ANDRADE, Willian Rufino, M.Sc., Universidade Federal de Viçosa, January, 2019. **Effect of biochar addition on anaerobic digestion of laying hen manure.** Adviser: Richard Stephen Gates. Co-advisers: Cecília de Fátima Souza Ferreira and Alisson Carraro Borges.

Anaerobic digestion is considered one of the most antique and suitable methods for waste treatment from urban and rural areas. It usually involves the action of a variety of fermentative microorganisms that metabolize and generates sub products used as precursors for biogas production. It is known that in spite of its appealing benefits, such as biogas and digestate generation, anaerobic plants require proper control of monitoring parameters that can directly affect the overall efficiency of the treatment system. Parameters such as pH, total ammonia nitrogen, alkalinity, volatile fatty acids and organic matter should always be well managed in order to maximize microorganism's activity and increase biogas production. Excess of total ammonia nitrogen for instance is one of the main reasons for anaerobic digestion failure since it can strongly affect fermentative microorganism's activity. Some wastes are known for its problematic regarding high concentration of total ammonia nitrogen, for instance, laying hen manure. Recent research has shown interesting improvement in terms of mitigating the excess total ammonia nitrogen throughout the anaerobic digestion process. Improvements could come from adoption of anaerobic codigestion, dilution of residues with water, addition of zeolite, charcoal, activated carbon and biochar. The use of an adsorbent substance such as biochar as a component in the anaerobic digestion process is new and may have a positive impact on the anaerobic digestion process allowing better stability and overall equilibrium of fermentation reactions. In order to provide new and concrete results to the literature, the present study proposed to assess the use of biochar in the anaerobic digestion of hen manure as a way to mitigate negative and inhibitory effects, expecting better equilibrium regarding monitoring parameters, and possible increase in the biogas yield. Two different assays were carried out to assess different inclusions (at 2.5, 5.0, and 7.5%) of biochar in substrates of batch anaerobic reactors formed by laying hen manure diluted in water at 5 and 7% of total solids, respectively. From both experiments, it is possible to understand that biochar has great potential to be used as buffering substance in anaerobic reactors, however, regarding biogas production its use did not display a positive effect on biogas yield.

RESUMO

ANDRADE, Willian Rufino, M.Sc., Universidade Federal de Viçosa, janeiro de 2019. **Efeito da adição de biochar na digestão anaeróbia de dejetos de aves poedeiras.** Orientador: Richard Stephen Gates. Coorientadores: Cecília de Fátima Souza Ferreira e Alisson Carraro Borges.

A digestão anaeróbia é considerada um dos métodos mais antigos e adequado para o tratamento de resíduos de zonas urbanas e rurais. Esse processo envolve a ação de uma gama de microrganismos fermentativos que metabolizam e geram subprodutos usados como precursores para a produção de biogás. Sabe-se que, apesar de seus benefícios, como a geração de biogás e digestato, plantas de biogás exigem o controle adequado dos parâmetros de monitoramento os quais podem afetar diretamente a eficiência geral do sistema de tratamento. Parâmetros como pH, nitrogênio amoniacal total, alcalinidade, ácidos graxos voláteis e matéria orgânica devem sempre ser bem gerenciados, a fim de maximizar a atividade dos microrganismos e aumentar a produção de biogás. O excesso de nitrogênio amoniacal total, por exemplo, é uma das principais razões para a ineficiência na digestão anaeróbia, uma vez que pode afetar fortemente a atividade de microrganismos fermentativos. Alguns resíduos são conhecidos por sua problemática em relação aos elevados valores e concentrações de nitrogênio amoniacal total a exemplo, dejetos de aves poedeiras. Recentes estudos mostraram melhorias na tocante mitigação do excesso de nitrogênio amoniacal total no processo de digestão anaeróbica. Tem-se sugerido a adoção de codigestão anaeróbia, diluição de resíduos em água, adição de zeolita, carvão comercial, carvão ativado e biochar. O uso de substâncias adsorventes como o biochar na digestão anaeróbia é novo, e é perceptível o seu impacto positivo no processo de digestão anaeróbia, permitindo maior estabilidade e equilíbrio global das reações. A fim de fornecer resultados novos e concretos à literatura, o presente estudo propôs avaliar o uso do biochar na digestão anaeróbia de dejetos de galinhas poedeiras como forma de mitigar efeitos inibitórios, esperando melhor equilíbrio em relação aos parâmetros de monitoramento, e possível aumento nos rendimentos de biogás. Foram realizados dois ensaios de digestão anaeróbia nos quais foram estudados a inclusão de diferentes concentrações (a 2,5; 5,0 e 7,5%) de biochar em substratos de biodigestores bateladas contendo dejetos de aves poedeiras diluídos em água a 5 e a 7% de sólidos totais. Respectivamente. De ambos os experimentos, foi possível entender que o biochar tem grande potencial para ser utilizado como substância

tamponante em reatores anaeróbios, entretanto, em relação ao rendimento de biogás, seu uso não apresentou efeito positivo.

1. GENERAL INTRODUCTION

Nowadays, the Brazilian egg industry predominantly uses conventional cage systems with a pyramidal configuration that allows the manure generated over time to be accumulated on the floor during the production cycle. The reutilization of the manure is considered of relevant importance in the production chain (Amaral et al., 2016).

Technological improvement and modernization of the egg industry has led to the intensification of production and due to that, intensive systems of production have been generating an extensive amount of manure (Bolan et al., 2010). Manure from laying hens is known for being a rich organic material especially in nitrogen, phosphorous and potassium and others macro and micronutrients (Bayrakdar, Sürmeli & Çalli, 2017).

According to Bolan et al., (2010) the amount of nitrogen, phosphorous and potassium in laying hens manure are respectively 32.8; 10.8 and 15.2 g/kg on dry weight basis. Moreover, ASAE (2005) give values of total nitrogen (TN) of laying chickens manure generated per day of 1.6 grams of TN/bird, which leads to a production of 784 grams of TN/bird over a 70-week egg production cycle. Along with this TN generated there is a reasonable amount of total ammonium nitrogen (TAN). Not just the presence of these components in the manure but also the presence of microorganism can lead to some problems when it comes to manure's utilization in agriculture under uncontrolled way, causing surface water eutrophication, air pollution, gas and odor emission to the atmosphere, and spread of pathogenic microorganism (Bolan et al., 2010; Surendra et al., 2013 e Čater, Zorec & Logar, 2014).

Composting and anaerobic digestion have been the most used systems to treat manure from livestock systems. However, the application of composting treatment for laying hens manure constrains the ability to harness the maximum energetic potential from the manure, and so anaerobic digestion might be exceptionally interesting for that reason.

The anaerobic digestion process is known to be the oldest method to treat residues and this process happens under specific conditions in which anaerobic microorganisms act on the substrate, breaking down the complex organic mater into simple components and transforming it into a mixture of gases that typically consist of 60-65% methane (CH₄), 35-40% carbon dioxide (CO₂), and traces of ammonia (NH₃), hydrogen sulfide (H₂S) and nitrogen (N₂) (Tauseef et al., 2013). Due to its combustion properties, methane produced during the anaerobic digestion process can be harvested for energy supply (Ennouri et al., 2016). The application of anaerobic digestion is not

only to treat residues and reduce its pollutant potential but also and especially for energy recovery (Holm-Nielsen, Seadi & Oleskowicz-Popiel, 2009; Fagbohunge et al., 2016).

Compared to other species, laying chickens manure has on average the highest volumetric biogas yield per kilogram of manure, and per kg of volatile solids (VS). This fact demonstrates the need to explore this organic residue in a more efficient way.

Primiano (2002), working with laying chicken manure, noticed volumetric biogas production of $0.10 \text{ m}^3 \text{ kg}^{-1}$ of manure and $0.560 \text{ m}^3 \text{ kg}^{-1}$ of VS_{in}. Sakar et al. (2009) have showed volumetric biogas yields of $0.627 \text{ m}^3 \text{ kg}^{-1}$ of VS_{in} working with laying chicken manure as a substrate of anaerobic digestion system. Carvallho (2015) reached mean values for volumetric biogas production of $1.52 \text{ m}^3 \text{ kg}^{-1}$ of manure and $0.57 \text{ m}^3 \text{ kg}^{-1}$ of VS_{in}. Similarly, Andrade et al. (2016) found volumetric biogas yields of $0.535 \text{ m}^3 \text{ kg}^{-1}$ of VS_{in}.

Even though the volumetric biogas yields for laying hen manure can be high, the high nitrogen concentration present in hen waste can be an issue to be overcome regarding the anaerobic digestion process. According to Nordell et al., (2013), organic matter that is rich in proteins (e.g. laying chicken manure) has high energetic potential, mainly for methane generation via anaerobic digestion. Nonetheless, considering that ammonia is generated when protein is broken down, using biomass rich in nitrogen in an anaerobic digestion system may lead a severe disturbance due to high ammonia concentration causing reduced activities of microorganism, and incomplete digestion of intermediate products such as volatile fatty acids, and as a result methanogenic activity is decreased (Zhang et al., 2011 and Rajagopal; Massé et al., 2014).

The literature has shown many projects that give some solutions to overcome high concentration of total ammonia nitrogen in the substrate of the anaerobic process such as: anaerobic codigestion (Wang et al. 2014), acclimation of microorganisms (Yenigün & Demirel, 2013), dilution (Yun et al. 2016), and use of adsorbent materials (Kumar et al., 1987; Montalvo et al., 2005 and Ho & Ho 2012).

Each of these methods have their advantages and disadvantages, relatively less work has been done on effect of adsorbent materials with respect to energetic performance and monitoring parameters of the fermentation process.

Biochar is an example of an adsorbent material that might be used in the AD process (Mumme et al., 2014). It is a solid material generated by pyrolysis under high temperature and in absence of oxygen (Guo et al., 2016).

Biochar used as an adsorbent component in anaerobic digestion is new and from the few works that have been carried out is noticeable its positive impact on the AD

process allowing better stability and overall equilibrium of reactions during the process (Kumar et al., 1987; Desai & Madamwar, 1994; Montalvo et al., 2005 and Ho & Ho 2012). Mumme et al. (2014) reported that the adsorption phenomena involving biochar with another substrate allows the sorption of inhibitory compounds through its pores and sites for binding, increase in the buffer capacity, and the formation of a biofilm to immobilize microorganisms. All these conditions previously mentioned are responsible to enhance microorganism's activity and consequently high volumetric biogas yields may be achieved.

Considering the exposed content, we hypothesize that the inclusion of biochar (at of 2.5, 5.0 and 7.5%) in the substrate composed by laying hen manure diluted in water used in the anaerobic digestion process will improve monitoring parameters of the anaerobic digestion and thus biogas yield will be improved as well.

CHAPTER 1 – LITERATURE REVIEW

2. LITERATURE REVIEW

2.1. Anaerobic Digestion

Anaerobic digestion (AD) is considered one of the oldest methods to treat residues and any kind of biomass. It occurs under action of a variety of microorganisms in absence of oxygen breaking down organic matter from its complex form making it simple to be used by other microorganisms responsible to use them as precursors to generate biogas and digestate as outcome (Bjpai, 2017). Biogas is a valuable resource that can be used to supply energy on-site and if its generation exceeds local energy needs it can be sold to electricity companies. There is also the generation of digestate which becomes useful to fertilization and as soil amendment.

The AD is described as a biochemical process whereby there is a complex consortium between different microorganisms in anaerobic environment degrading the original structure of the biomass in their respective essential components (Sawatdeenarenat et al., 2015). This process comprises four different steps of microorganism's activity until the generation of methane gas (CH_4), which are: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Chernicharo, 1997; Adekunle & Okolie, 2015).

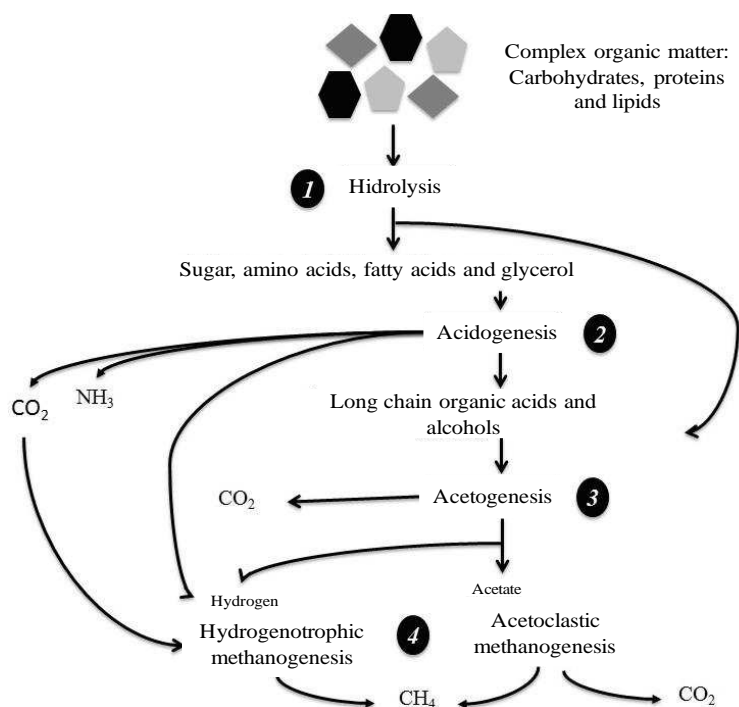


Figure 1. Representative diagram of the anaerobic digestion process of the organic matter up to the biogas production. Adapted from Angelidaki et al., (2011) and Toussaint (2013).

During hydrolysis (phase 1 in Figure 1) organic material of high molecular weight such as carbohydrates, proteins, lipids and fibers are converted into dissolved organic materials through action of exoenzymes (e.g. amylase, protease, lipase and cellulase) excreted by microorganisms (carbohydrates, protein, lipids and cellulose) generating more simplified components (monosaccharides, amino acids, glycerol, long chain fatty acids) which might then be assimilated by other microorganism (Bjpai, 2017). This step can limit the extent of organic material breakdown (Angelidaki et al., 2011), and can therefore influence the efficiency of the process during the following stages. According to Toussaint (2013) the hydrolysis of biomolecules such as carbohydrates can occur in a few hours and extend to a few days for proteins and lipids. Fractions that have a complex structure to be degraded, such as lignocellulosic content, are partially hydrolysable and require a longer period (Adekunle & Okolie, 2015).

After hydrolysis phase, substrate components assume simplified structures and are catabolized during acidogenic (phase 2) by acidogenic bacteria (fermentative microorganisms), generating volatile organic acids such as acetic, butyric and propionic acid (Bjpai, 2017). During the process of acidogenesis there is still the occurrence of ammonia (NH_3) and carbon dioxide (CO_2) release.

In a third step, acetogenesis takes place (phase 3) through metabolization of products generated by acidogenic bacteria. Volatile fatty acids of complex and longer carbon chains are oxidized generating a high amount of acetate and hydrogen ions (Yu & Shanbacher, 2010). Such molecules are precursors of methanogenesis, the final stage of the AD process for biogas production.

Last but not least, the methanogenesis might occur through two different pathways. The first one is through the acetoclastic path in which methanogenic microorganisms uses mainly acetate as a substrate for methane production; this route is known to be responsible for 70% of the methane generated. The second is through the hydrogenotrophic path in which methanogenic microorganisms use mainly hydrogen as electron donor and CO_2 as acceptor to produce methane; this route accounts for about 30% of overall CH_4 production (Gerardi, 2003; Al Seadi et al., 2008).

It is important to emphasize that the excess of hydrogen ions due to the oxidation reactions can cause acidification in the anaerobic medium, partially inhibiting and reducing acetogenic bacteria activity and consequently affecting methanogenic microorganisms' activity. According to Bjpai, (2017) the methanogenesis phase is extremely sensitive to changes of large magnitudes in the AD process since

methanogenic archaea are slow growing, sensitive to changes, and assimilate a reduced range of substrates. Therefore, monitoring control parameters (pH, alkalinity, ammonia nitrogen, volatile acidity, temperature, etc.) of the anaerobic digestion process must be done to keep the AD process in control.

2.1.1. Factors that can affect the anaerobic digestion (AD) process

2.1.1.1. Temperature

Temperature has been considered one of the most critical factors regarding performance and stability of the AD process, and so, adequate temperature control is essential to mitigate any oscillations during digester operation, which may, therefore, affect the microbial structure and its growth kinetics (Labatut et al., 2014).

It is known that the AD process may occur in different ranges of temperatures and the most common is the mesophilic, which correspond to variations from 15 to 45°C, followed by the thermophilic range from 50 to 65°C. Usually, anaerobic reactors tend to exhibit a stable and even better performance when operated in mesophilic temperature since diversity of microorganisms involved in the process under that temperature is greater compared to a few populations that are able to withstand thermophilic conditions (Khalid et al., 2011).

Despite energy cost for constant heating, operation of thermophilic anaerobic reactors has some advantages when compared to the mesophilic. Because activity of microorganisms that operate in this temperature range is high, greater reductions of volatile solids and pathogenic microorganisms can be reached, and, therefore, higher biogas yield might be expected (Mao et al., 2015). However, great attention to the process monitoring is indeed needed, since thermophilic microorganisms are sensitive to small temperature variations, and therefore the efficiency of the AD can be affected.

In general, high temperatures are beneficial to the rate and metabolic activity of AD microorganisms, but, this scenario can result in high concentrations of total ammonia nitrogen in the system, which has been cited by several authors as a toxic agent to the anaerobic process. Literature has reported that AD conducted in reactors at thermophilic temperatures are more susceptible to unexpected changes in the internal environment (Angelidaki & Ahring, 1993; Van Lier et al., 1996; Hansen et al., 1999; Kim et al. 2003), since high temperature accelerates hydrolysis, acidogenesis and acetogenesis leading the anaerobic system to an accumulation of long-chain volatile fatty acids of up to 40% (Labatut et al., 2014), causing a decrease in pH and alkalinity

(Kim et al., 2003) and increased ammonia concentration in the system (Hansen et al., 1998 & Chen et al., 2008).

Oscillations such as sharp rise or fall in temperature can affect the equilibrium of biochemical reactions and thus change stoichiometry of formed products, which might directly affect the methanogenic activity efficiency in the production of CH₄. According to Speece (1996) a reduction of 5°C in the internal temperature of a digester can reduce microorganism's activity by up to 34%, which might reduce methane production; nonetheless, in systems conducted with high ammonia concentration residues under thermophile temperature showed that a reduction in temperature from 50 to 35°C represented a relief to the AD microorganisms since there was a reduction in the rate of microbial activity. In that scenario ammonia levels are not reduced but temporarily controlled. It is also worth noting that this management of decrease in temperature influenced the recovery of the digestion process with a 34% increase in generation of methane.

2.1.1.2. Hydrogenionic potential (pH) and Alkalinity

Anaerobic microorganisms are relatively sensitive to variations that may occur in a reactor when not well managed. Due to physiological, metabolic, nutritional and growth kinetic differences (Panichnumsin et al., 2012) microorganisms tend to be tolerant to certain variations of pH, since they are symbiotic, which means they depend on one another for substrate metabolization and by-product utilization as the sole source of energy. However, the control of hydrogenionic potential (pH) in a narrow range is essential for the viability of the AD process at its maximum efficiency.

According to Aragaw et al. (2013) pH values for the anaerobic digestion process may vary from 6.0 to 8.0, nevertheless, in AD processing of animal manure, the ideal pH for the occurrence of the process and methane generation at high rates is 7.3. Despite this fact, it is known that anaerobic reactors might be operated in different ranges of pH due to the microorganism diversity and needs, for example, while methanogenic microorganism requires pH conditions between 6.8 and 7.3, acid-producing bacteria develops better activity at pH ranging from 5.0 to 6.0 (Sakar et al., 2009).

Alkalinity concentration in the AD process varies concomitantly with pH changes, since both parameters are related. Through different measuring scales it is possible to assess acidity, neutrality or basicity conditions in anaerobic reactors. The

alkalinity or buffering power of an anaerobic digestion system refers to the ability of the system to withstand drastic changes in pH under production conditions (due to high volatile solids breakdown) or addition of acids into the substrate.

All the alkalinity available in an anaerobic system may be characterized as partial, intermediate and total. Commonly, the amount of alkalinity consumed to neutralize the organic acids in the pH range of 5.75 to 7.3 refers to the partial alkalinity of the system, which is inherent to bicarbonate ions. Intermediate alkalinity refers to the amount of alkalinity responsible to the neutralization of anions and organic acids in a pH range varying from 5.75 to 4.3. The sum of both fractions accounts for the total alkalinity of a system. According to Ripley et al. (1986) and Jenkins et al., (1983) the minimum operational limit for the proper functioning of an anaerobic reactor should be 1200 mg CaCO₃ L⁻¹.

2.1.1.3. Volatile fatty acids

Despite the fact that some volatile fatty acids (VFA) are considered a substrate to methane-forming microorganisms, when VFA exceeds the optimum amount they might be more toxic than any other compound produced by microorganisms itself during the AD process. This compound when highly available in the substrate due to excess of easily degradable organic fraction, penetrates microorganism cell membranes and accumulates, which leads to the generation of toxic enzymes and proton imbalance (Siles et al., 2010).

To keep control of the AD process, the amount of VFA strongly affect the activity of microorganism, especially the methanogens. Despite VFA being directly related to pH, when it is controlled or at least kept near to the neutrality no major effect will be seen from VFA in the AD process, especially on methanogenic microorganisms (Burton & Turner 2003).

According to Niu et al., (2014) the beginning of inhibition might be noticed when VFA concentrations exceed 1800 mg/L, while Polprasert (2017) reported inhibitory edge for AD process due to VFA ranging from 6000 to 8000 mg L⁻¹. It is known that each anaerobic system will have a unique inhibitory range since organic matter, pre-treatment of substrate, organic load, reactor type and temperature range varies and due to that it is difficult to set a standard inhibitory edge.

It should be highlighted that VFA values do not indicate the amount of each acid (formate, acetate, butyrate and propionate) and which of them are more present,

nonetheless, it is known that about 85% of the volatile acid generated under the AD process is acetate (Gerardi, 2003).

2.1.1.4. Carbon: nitrogen ratio (C: N)

Carbon and nitrogen have relevant importance to the anaerobic digestion process and their ratio is considered a key factor, and it should be kept in the ratio of 20:1 to 30:1, which is considered to be optimal range for the anaerobic digestion process of livestock manure (Li et al., 2013), but Niu et al., (2014) have mentioned that might be acceptable a ratio ranging from 13:1 to 28:1.

High amount of rich organic material in nitrogen and poor concentration of carbon drives the AD process to run at a slower rate. It is usually accompanied with acidification and major reduction in biogas production. Low C: N ratio lead to the accumulation of TAN and VFA which automatically leads AD process to an inhibitory condition. The system might naturally recover from that, but it might take a longer period. On the other hand, a high C: N ration can cause a rapid uptake of nitrogen compounds by methanogenic microorganisms (meeting their requirement needs) leading the system to a lack in N and then there will be a high amount of available carbon due to difficulties to degrade it causing significant overall decrease in biogas production.

2.1.1.5. Total ammonia nitrogen concentration (TAN)

The generation of ammonia in an anaerobic reactor occurs due to the metabolization of protein compounds. Large and complex protein structures are hydrolyzed by bacteria which release exoenzymes breaking down peptide bonds (Figure 2), creating simple compounds (amino acids) which enter inside of bacterial cell where it is further degraded by endoenzymes generating various organic acids and also ammonia (NH_3) as show in the Figure 3. Its presence in the anaerobic reactor is relevant to improve the alkalinity of the system (Lahav and Morgan, 2004), however, when in high amount it might be inhibitory.

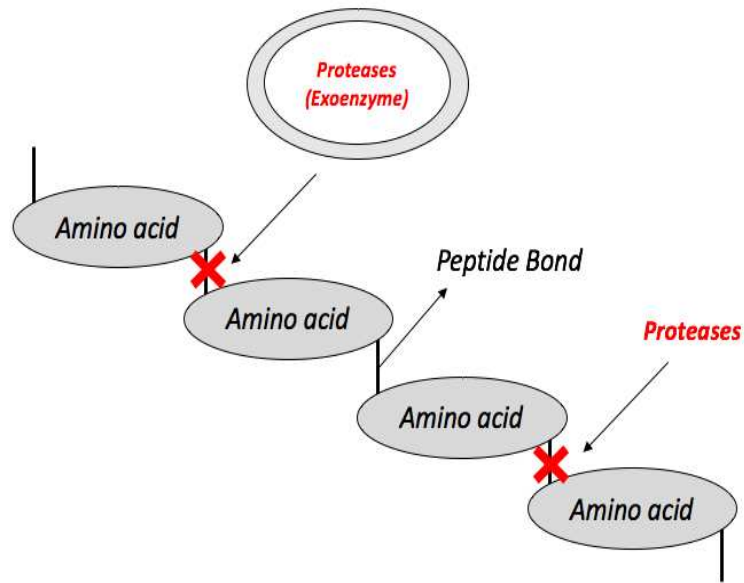


Figure 2. Protein structure showing amino acids linked by peptide bond and its structure being break down by exoenzymes (Protease). Adapted: Gerardi (2003).

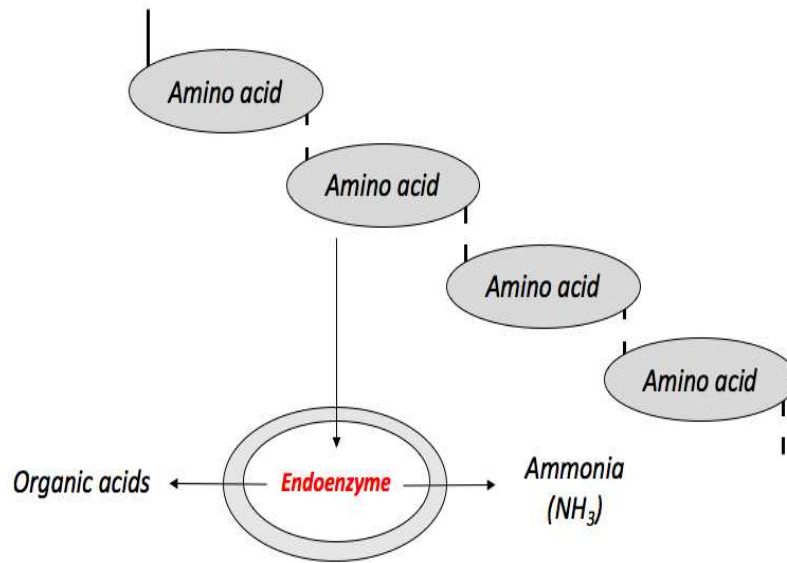


Figure 3. Ammonia (NH₃) and organic acid generation after amino acids be degraded by endoenzyme inside of the microorganism cell. Adapted: Gerardi (2003).

Nitrogen concentration has been on of the most studied topics when it comes to anaerobic digestion inhibition. Among the four types of anaerobic microorganisms, methanogens are the most likely to cease growth due to ammonia inhibition (Kovacs, 2015). Nonetheless, it is important to state that ammonia inhibition may occur under different circumstances since various factors (type of organic material, organic load, pre-treatment, reactor, temperature range and operational method) might influence

ammonia generation and inhibition edge. Previous acclimation of microorganism to the substrate can increase the inhibition edge.

When free ammonia concentrations increase on the range of 4051 - 5734 mg L⁻¹, acidogenic populations are hardly affected while methanogens lost 56.7% of its activity (Koster & Lettinga, 1988). Despite a wide range, Chen et al. (2016) also showed that methanogenic activity was reduced by 50% when total ammonia nitrogen (TAN) ranged from 1700 to 14000 mg L⁻¹. Niu et al., (2014) considers that the edge of anaerobic digestion inhibition by TAN is ranging from 3000 to 4000 mg L⁻¹, while Gerardi (2003) mentioned that it is possible to overcome troubles caused by ammonia toxicity if TAN is controlled in a range up to 1500 mg L⁻¹. According to Chernicharo (1997) TAN concentration in the anaerobic digestion process should not exceed 1000 mg L⁻¹ so that the environment does not become toxic to methanogenic microorganisms that are not acclimated to the high-TAN substrate.

Despite all the recommendations regarding TAN concentration, the literature has shown it is known that the optimum amount of TAN concentration which favors the bacteria and microorganism metabolic activity is 200 mg L⁻¹ (Liu & Sang, 2002).

2.1.1.6.Substrate composition

The composition of substrate has a great influence on the performance of the anaerobic digestion process, since it must provide certain nutrients for maintenance of vital processes of the microorganisms that actively act in the anaerobic digestion. These important nutrients are carbon (C), nitrogen (N) and phosphorus (P). Chernicharo (1997) cites a range of cations and anions that have key role for the occurrence of biosynthesis of cellular components such as Mg⁺², Ca⁺², Na⁺¹, K⁺¹, Fe⁺² and Cl⁻, SO₄⁻², respectively. Besides there are other trace elements cited to be important to the microbial grow such as Co, Cu, Mn, Mo, Zn, Ni and Se.

2.1.1.7.Hydraulic Retention Time (HRT)

HRT is the period in which digestion of added substrate in a digester is fully completed. Such a terminology is often defined by the ration between anaerobic digester total volume and volume of daily load. Factors such as type of substrate and digester can influence how efficient the AD process can be.

According to Meneses (2011) rural reactors require medium to longer retention time periods. On the other hand, industrial reactors, equipped with additional tools that speed up the digestion process, require short retention periods. The use of inoculum,

additives and pre-treatments (Xavier & Junior, 2010; Bruni, Jensen & Angelidaki, 2010; Zheng et al., 2014) has been cited as promising methods capable of accelerating substrate's digestion process, leading the AD system to higher reductions of volatile solids in short HRT, and consequently higher biogas production might be achieved (Toussaint, 2013).

Xavier & Junior (2010) verified that the use of 20% of inoculum in batch reactors fed with dairy cattle manure showed higher efficiency when compared to a system without inoculum inclusion. The authors observed that biogas start-up production was anticipated and the biogas yield of digesters supplied with 20% of inoculum in a 70-day TRH was similar to the yield of non-inoculated fed reactors, which were kept under operation for 150 days. Same behaviour noticed on Xavier and Junior's experiment was seen by Steil (2001) when laying hen manure was digested with or without inoculum material. She showed that anaerobic digester solely fed with hen manure diluted in water takes 133 days to generate about 1.45 m³ of biogas, while when 15% of inoculum is added the same amount of biogas (1.44 m³) production is attained in a shorter HRT of 56 days.

Shorter HRT can be efficiently used in specific cases adopted such as in the ones mentioned before, but, precautions should be taken since, as a consequence of short periods, there may be incomplete degradation of organic material (if period is too short and/or material is too recalcitrant), accumulation of volatile acids, inhibition of methane-producing microorganisms, low rates of volatile solids reduction and consequent undesirable biogas yield (Souza, 1984).

Medium to long HRT have been cited as being ideal, since in such conditions these factors may be achieved: 1) adequate buffering power for maintenance of biological activities in anaerobic environment; 2) adequate biogas production due to higher reductions in volatile solids 3) and higher reductions in pathogenic microorganisms.

The adoption of a suitable HRT is extremely important, especially when the production system aims digestate utilization as soil amendment and/or fertilization for crops. Usually longer HRT are required to achieve an adequate sanitation of digestate.

It is important to emphasize that to set up a HRT it should be taken into account not only the type of organic material, but also the type of digester that will be used. In the case of a continuous reactors, the HRT must be set up with caution, since there is a constant organic load and effluent outlet of processed material with high buffering power, thus, the equilibrium between the entrance and exit of material must be

maintained in order to allow proper anaerobic conditions for constant activity of anaerobic microorganisms (Braun, 2007).

2.1.2. Anaerobic digestion feeding systems

An anaerobic digester basically consists of a chamber, usually cylindrical, that stores and allows the fermentation of organic substrate, and also by a gas collector (gasometer) which store the gas up to its manipulation.

The use of anaerobic digestion is done mainly for conversion of organic biomass into methane (CH₄) as an energy source that can be burned, which further reduces methane to carbon dioxide. Methane can also be directly used in livestock facilities that requires heating system in certain developing critical phase, such as for young piglets and chicks. Another way to use CH₄ is through a convertor since methane can be transformed in electric energy and used by the own facility and the surplus energy might be stored in battery to be sell. Besides that, through anaerobic digester there is also the generation of a digestate, an effluent rich in nutrients.

Anaerobic digester in rural or urban areas is not only directly related to energy recovery but also to environmental safety, sustainability and valoration of a sub product that is usually undervalued.

Digesters might be classified by many ways, but the most usual is through the loading method which are: batch, semi-continuous and continuous method.

Batch feedstock loading is usually used in anaerobic digester that receives a single biomass load, and the organic material rests there up to its full degradation. The adopted hydraulic retention time for this system is defined as the duration between feedstock is loaded into and removed from the digester. In livestock production for example, there are some species that are raised in cycles, the most common are broiler chickens with a cycle of 42 to 45 days They are usually raised in barns with sawdust bed on the floor, which is removed only by the end of the cycle, with an exception, in many regions including Brazil, where bed is treated and reused for 4 to 6 cycles. There is also the laying hens' production system with full cycle of about 70 weeks, during this period dropping feces create a big amount of mixed wet and dry feces above the cages (in pyramidal cage systems, usually adopted in tropical countries). In a more modern laying hens' facilities, feces may be collected once or more per week through an automatic manure belt or a manure scraper machine but even in this cases, manure od stored in the farm until its transportation to a treatment system.

As mentioned before, the adoption of a batch AD system also depends on how manure is handled, for example, despite the fact that a pig factory production cycle (from growing phase up to slaughter) takes just 114 days to finish, manure generated in this system is usually flushed using a great amount of water, which totally changes the original characteristic of the raw swine manure from a dry condition to a swine wastewater. In this case, depending on how often facilities are cleaned there might be adopted semi-continuous or continuous load system for an anaerobic reactor.

Semi-continuous loading is based on an intermittent digester feeding which might be happening in intervals of 3 to 4 days or even more. Usually small dairy factory that process milk to cheese production and other dairy derivatives, small to medium pig farms and small-regional slaughter houses generate periodical amount of waste that might be stored in the feeding box for a couple of days before going to the anaerobic digester.

Last but not least, the continuous loading system relies on a constant flow of waste continuously directed to an anaerobic digester. As a load of new material goes to the anaerobic digester, a same amount goes out through an effluent outlet. Anaerobic digesters operated under continuous loading are usually common in medium to large facilities system such as swine farms, slaughter houses that constantly receive animals, dairy farms that constantly process milk and for large dairy cattle and beef cattle feedlots as well. In medium to large livestock systems there is a reasonable amount of waste generated daily and based on that data the anaerobic digester facility size and the hydraulic retention time are calculated. This method allows continuous production of biogas (Benicasa et al. 1991).

Although there are only three classifications regarding the loading method, there are different models of anaerobic digester available, such as: Chinese, Indian, tubular and UASB (Upflow Anaerobic Sludge Blanket) or also known as RAFA (Upflow Anaerobic Reactor).

It is important to understand that in order to adopt and build up an anaerobic digester an evaluation of several factors is required. This includes knowing what kind of residue is produced, the amount of solids in it, the periodicity at which it is generated, how the waste is waste handled, the energetic potential to generate biogas from the waste, the energy requirements of the facility, energy demand of nearby farms and facilities, availability of materials for the construction and maintenance of digesters and qualified technical staff for the digester operation. All these factors should be take into account to assess the technical and economic viability of this business.

2.1.3. Products from the anaerobic digestion process

2.1.3.1. Biogas

Biogas is a product of the conversion of volatile fatty acids into a mixture of methane gas (CH₄), carbon dioxide (CO₂), ammonia gas (NH₃), hydrogen sulfide (H₂S) and nitrogen (N₂) by anaerobic microorganisms. Its composition varies since factors such as type of organic matter, substrate's temperature, hydraulic retention period, agitation, use of additives, pre-treatment of the organic material can change biogas profile. But overall, when operated under stable conditions, the AD process yields between 50-75% CH₄ and 25-50% CO₂ by volume, while gases such as NH₃ (0 - 500 ppm v/v), H₂S (0 - 5000 ppm v/v) and N₂ (0-5% v/v) are present in small proportions (Surendra et al., 2013).

According to Nishimura et al., (2010) biogas has a high specific heat value, varying from 5000 to 7000 kcal/m³. If all CO₂ present in biogas is eliminated, specific heat increases and it can be efficiently used to supply unit's needs for heating, cooking and generating electricity (Simon & Bueno, 2006).

From a sustainable point of view, biogas is a promising renewable energy source that can be displace other energy sources. In the current scenario, reduction of non-renewable sources emission of polluting gases to the atmosphere becomes an important effort to mitigate negative impacts caused by agricultural activities, besides that, it may stimulate the transition of the current energy model that mostly relies on non-renewable fossil energy source.

2.1.3.2. Digestate

During anaerobic digestion, there is a biological transformation of the substrate through the fermentation process, leading to generation of an effluent called digestate, with physical-chemical properties of agronomic interest, being useful as soil amendment and fertilizer.

The action of anaerobic microorganisms on the substrate added in the digester allows the AD process to achieve a satisfactory rate of pathogenic microorganism destruction, stabilization of toxic compounds and odor reduction, which consequently does not attract flies, insects, rodents and other diseases vectors (Nkoa, 2014). As presented in a study developed by Farias et al., (2012) AD can obtain reductions of total coliforms close to 99.9%. In addition to obtaining a satisfactory sanitary profile, digestate preserves most of its chemical attributes from original organic matter (Provenzano et al., 2011).

Digestate can be applied to any type of crop (Silva et al., 2006), but one of the main problems related to its utilization in crops fertilization is the large volume of material needed to meet crop's nutrient needs. However, there are several ways and means to improve the physical-chemical quality of digestate, making it richer in nutrients, such as using additives, pre-treating the raw biomass and performing codigestion with other residues, the latter case, is a suitable alternative that aggregates different nutrient sources into a single substrate. This might then play an important role in reduction of the digestate needed to meet soil and crop's requirement.

3. Biochar and its use

Biochar is generated after a pyrolysis process of biomass from animal or biomass origin. The process is usually conducted under high temperature (400°C to 800°C) in the absence of oxygen (Nóbrega, 2011). It is basically composed of carbon and ash with a high specific surface area that is able to increase the capture of nutrients (Deem & Crow, 2017).

Due to its characteristics, biochar has been studied and vastly used on agricultural lands as a soil amendment, improving soil properties including bulk density, porosity, structure, texture, carbon content, pH and electric conductivity (Ding et al., 2016). Besides that, literature has some findings demonstrating positive effects of biochar as a mitigation tool in the reduction of greenhouse gas (GHG) emissions, such as CO₂, N₂O and CH₄ (Kammann et al., 2017). Despite that, some results have also shown that biochar has no effect on mitigating GHG emissions on soil.

Biochar has also been applied in the handling of manure from livestock systems. Composting processes have been one of the most studied and findings have shown that biochar has a role in accelerating composting, and in the reduction of GHG emissions and ammonia loss (He et al., 2018a; He et al., 2019b). Ravindran et al., (2019) found that biochar can increase porosity and water holding capacity of the composting bulk, which consequently has effect on a fast increase in temperature for the composting process. Other than that, the authors concluded biochar addition increases organic matter degradation and C/N ratio, while ammonia emission and pathogenic microorganisms are greatly reduced. He et al. (2018), and Jain et al. (2019) demonstrated a positive effect of biochar on the reduction of organic matter, bulk density and increase in the porosity.

Despite being covered in many literature review papers, very few studies have evaluated the role of biochar in anaerobic digestion. Since biochar is considered an exceptional adsorbent material, it has been understood by many researchers that biochar

may be a tool to mitigate inhibitory effects that usually happen during the anaerobic digestion process. Mumme et al., (2014) mention that adsorption phenomena that involves the biochar with another substrate allows the sorption of inhibitory components through its pores and sites for binding, increase in the buffering capacity and enhancing the formation of a biofilm to immobilize microorganisms. This scenario may allow better stability for an overall equilibrium of anaerobic reactions.

Hansen et al., (1999) demonstrated that activated carbon at the level of 5% improves the fermentation process of swine manure under high total ammonia nitrogen (TAN) concentrations. Kumar et al. (1987) demonstrated that the use of commercial charcoal as an additive for cattle manure digestion had positive effects, increasing biogas yield up to 16%. However, despite these positive findings, Mumme et al., (2014) demonstrated that addition of pyrochar into the anaerobic reactor fed only by inoculum of cattle slurry, maize, and maize silage reduced methane production by 16% after 63 days of fermentation. Nonetheless, they also showed that the use of hydrochar improved biogas yield in the extent of 46% as compared to control treatment, however, the biogas yield was still considered low compared to regular feedstock used for biogas production.

CHAPTER 2 - Effect of biochar on monitoring parameters and biogas yield in the anaerobic digestion of laying hen manure

ABSTRACT

This study aimed to assess the effect of biochar inclusion on monitoring parameters and biogas yield in batch reactors fed diluted laying hen manure at 5% of total solids. Three different levels (at 2.5, 5.0, and 7.5% by weight) of biochar inclusions were adopted with three replicates each, plus a control treatment, which had only hen manure diluted in water to 5% of total solids. Twelve 2-L batch anaerobic reactors were used in the fermentation process of 60 days' duration under controlled temperature at 35°C. Substrate and digestate material of each treatment were assessed for monitoring parameters of pH, total ammonia nitrogen, alkalinity, volatile fatty acids, total solids and volatile solids. Biogas production was recorded daily through the 60 days of fermentation. High pH and total ammonia nitrogen values were measured by the end of the experiment, ranging from 9.8 to 9.9 and 2354 to 2739 mg L⁻¹, respectively. Inclusion of biochar had a positive effect regarding the increase of buffering capacity of the substrate since partial alkalinity of substrates was greater for treatments with inclusion of biochar, with values ranging from 3164 to 3717 mg CaCO₃ L⁻¹. In addition to elevated total ammonia concentrations, volatile fatty acids were also higher than recommended values. Inclusion of 2.5, 5.0 and 7.5% of biochar did not promote better performance of anaerobic batch reactors fed laying hen manure in terms of pH values, total ammonia nitrogen, and volatile fatty acids concentration. Addition of the three rates of biochar did not enhance biogas yield per kilogram of waste, total solids in, volatile solids in and volatile solids removed as compared to control.

Key-words: anaerobic reactor, livestock waste management, methane

Efeito da inclusão de biochar quanto a parâmetros de monitoramento e rendimento de biogás na digestão anaeróbia de dejetos de galinhas poedeiras

RESUMO

O estudo teve como objetivo avaliar o efeito da inclusão de biochar quanto aos parâmetros de monitoramento e rendimento de biogás em reatores batelada alimentados com dejetos de galinhas poedeiras. Adotou-se três níveis (2,5; 5,0 e 7,5%) de inclusões de biochar com três repetições cada, além do tratamento controle, que continha apenas

dejetos de galinhas poedeiras diluído em água a 7% de sólidos totais. Foram utilizados doze reatores anaeróbios de dois litros de volume útil, submetidos a um tempo de retenção hidráulica de 60 dias sob temperatura controlada a 35°C. Substratos e digestatos de cada tratamento foram avaliados quanto aos parâmetros de monitoramento como pH, nitrogênio amoniacal total, alcalinidade, ácidos graxos voláteis, sólidos voláteis e totais. A produção de biogás foi registrada diariamente ao longo dos 60 dias. Elevados valores de pH e nitrogênio amoniacal total foram observados ao final do ensaio, os quais variaram de 9,84 a 9,89 e 2353,72 a 2739,24 mg L⁻¹, respectivamente. A inclusão do biochar apresentou efeito positivo quanto ao aumento da capacidade tamponante do substrato, uma vez que a alcalinidade parcial dos substratos apresentou maiores valores nos tratamentos com inclusões de biochar, com valores variando de 3164 a 371 mg CaCO₃ L⁻¹. Assim como para as concentrações totais de nitrogênio amoniacal, os ácidos graxos voláteis também foram superiores aos valores ideais recomendados na literatura. Inclusões de biochar a 2,5; 5,0 e 7,5% afetaram negativamente o pH e as concentrações de nitrogênio amoniacal e ácidos voláteis. A adição de biochar não promoveu melhoria no rendimento de biogás por quilograma de dejetos, sólidos totais adicionados, sólidos voláteis adicionados e sólidos voláteis reduzidos comparado ao controle.

Palavras-chaves: reator anaeróbio, manejo de dejetos animais, avicultura

4. INTRODUCTION

Anaerobic digestion has been adopted as one of the main treatment options for waste from urban and rural areas. This technique combines a range of benefits such as energy recovery, nutrient recycling, mitigation of GHG emissions and reduced sanitary risks of treated waste. This method, despite being undervalued in some countries, is efficiently used as a tool to harness biogas from a variety of wastes; nevertheless, in spite of its appealing benefits, biogas plants requires proper control of monitoring parameters that can directly affect the overall efficiency of the AD process.

As repeatedly mentioned in the literature, excess total ammonia nitrogen (TAN) levels in organic matter can strongly disturb the anaerobic digestion process, especially in anaerobic plants which treat organic matter that is rich in nitrogen, such as feces from laying hens that have high concentration of nitrogen and ammonia due to presence of uric acid (Massé et al., 2014; Farrow et al., 2016; Molaey et al., 2018).

Recently, some research has shown interesting improvements in terms of mitigating the excess TAN throughout the anaerobic digestion process through: a) acclimation of microorganisms to high total ammonia concentration substrates (Yenigün & Demirel, 2013); b) dilution of total solids (Yun et al. 2016); c) feeding anaerobic reactors with two or more residues as a way to dilute the negative effect of one of the feedstocks (Wang et al. 2014); and d) using adsorbent substances such as charcoal (Kumar et al., 1987), activated carbon (Hansen et al. 1999), zeolite (Ho & Ho 2012, Montalvo., et al 2015) and biochar (Mumme et al., 2014).

Few works have demonstrated the effect of biochar on the anaerobic digestion process. Mumme et al. (2014) revealed that the use of hydrochar and pyrochar did not contribute to the anaerobic digestion process when the anaerobic reactor was fed inoculum of cattle slurry, maize and maize silage. However, they demonstrated that pyrochar can be effective in terms of mitigating mild ammonia nitrogen risks. According to Mumme et al., (2014) the adsorption phenomena which involves biochar with other substrates improves anaerobic digestion by means of: a) sorption of inhibitory components through its pores and sites for binding, b) increase in the buffer capacity and c) formation of biofilm to immobilization of microorganism.

This study was designed to assess monitoring parameters and biogas yield in the anaerobic digestion of laying hen manure with different inclusion rates of biochar in batch anaerobic reactors.

5. MATERIAL AND METHODS

5.1. Location

The study was conducted in the Anaerobic Digestion Laboratory in the Agricultural Engineering Department at the Universidade Federal de Viçosa (UFV). The campus is located in latitude 20°45'45" south and longitude 42°52'04" east at an elevation of 649 m. The climate is Cwa according to Köppen's classification, with a hot rainy season, and a cool dry winter.

5.2. Experimental design

The experiment was conducted in two phases, the first phase comprised of physical-chemical characterization of laying hen feces, biochar and physical-chemical characterization of reactors feeding substrates. In the second phase, the effect of different levels of biochar inclusion on volumetric biogas production was conducted,

and the resultant physical-chemical digestate characteristics of the digestate were determined after the AD process.

To perform the assay, twelve anaerobic batch reactors of two-liter capacity were placed in a water bath under controlled temperature of 35°C. Three levels (2.5, 5.0, and 7.5% by weight) of biochar inclusion, plus a control level were used, with each treatment replicated three times. The experimental design was a completely randomized design with four treatments and three replicates.

5.3. Feedstock

The feces used in the experiment was collected from the laying hen barns from the Animal Science Department at Universidade Federal de Viçosa - UFV. Excreted feces were collected from the top portion of the pile as a way to collect fresh manure dropped under the cages. Manure was collected in a way to avoid any material other than feces. Collected samples were then placed in plastic bags and transported to the Anaerobic Digestion Laboratory in the Agricultural Engineering Department.

The biochar made of pyrolyzed broiler chicken litter was provided by a company from Sao Paulo State (SP Pesquisa e Tecnologia Ltda.).

5.4. Physical-chemical characterization

To perform the physic-chemical characterization of manure and substrate the following analyses were performed: pH of raw manure was done by placing the electrode in a substrate resulted from a mixture of 50 g of raw manure with 50 mL of distilled water in a 1:1 ratio. Biochar pH was recorded 30 min after dilution and constant stirring of a mixture of 20 g of biochar in 100 mL of distilled water. Regarding the feeding substrate, as it was already homogeneous, a pH electrode was used to record substrate data.

Total ammonia nitrogen (TAN) was done per the methodology suggested by APHA, AWWA, WPCF (2017) in which 10 mL sample was placed in a protein tube for distillation. To collect the distilled content extracted, an Erlenmeyer flask previously filled with 20 mL of boric acid at 4% was used to fix the ammonia as ammonia borate ($\text{BH}_{12}\text{N}_3\text{O}_3$). After that, 75 ml of the distilled extract was titrated using sulfuric acid (H_2SO_4) at 0.02 mol L⁻¹.

Solids analysis from feces, biochar and substrate was performed by placing previously weighed samples into an oven at 105°C for 24 h. After that, samples were weighed to attain the total solid (TS) value. Dried samples were then weighed and

submitted to an oven under 550°C for 4 hours to attain the volatile solids (VS), following methodology suggested by APHA, AWWA, WPCF (2017).

In Table 1 the mean values of characterized parameters from laying hen manure and biochar are provided.

Table 1. Mean values of characterization parameters from laying chicken manure and biochar made of broiler litter

Parameters	Manure	Biochar
pH	7.50	9.2
T-Ammonia nitrogen (mg L ⁻¹)	1171.3	240.8
Total Solids (%)	31.08	59.1
Volatile solids (% of TS)	70.38	53.4
Fixed solids (% of TS)	29.62	46.6

5.5. Assay of anaerobic digestion

In this phase, the study was carried out using twelve two-liter batch anaerobic reactors. Each reactor had a hose barb on top connected to a hose allowing the gas collection. In order to store the gas, cylindrical gas collectors built from PVC pipes were used. This apparatus was comprised by the inner (gas collector) and outer (supporter) PVC pipes as seen in the Figure 4.

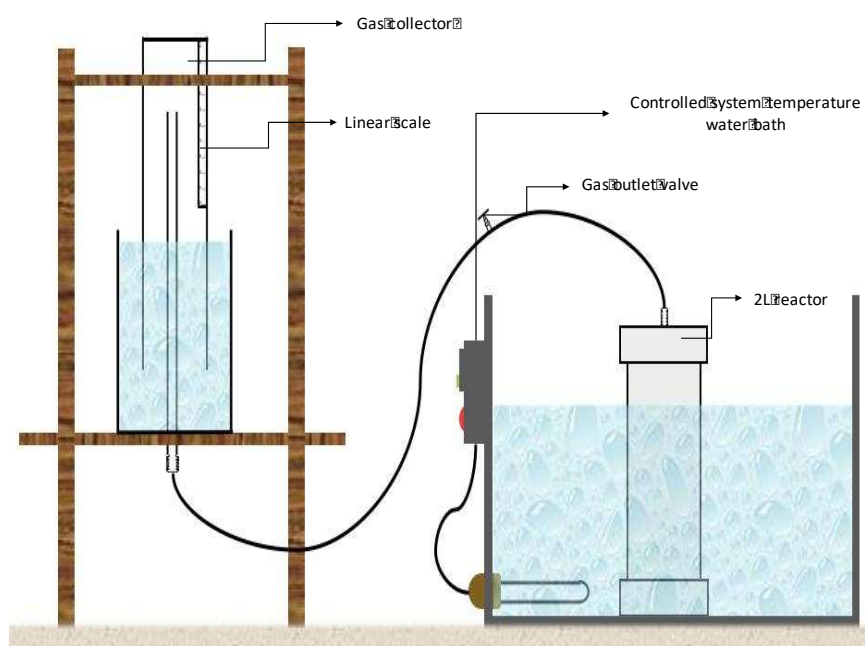


Figure 4. Schematic representation of the anaerobic reactors used in the experiment.

In order to feed the reactors, all substrates were formulated to attain 5% of total solids (TS) based on the concentrations of TS of the manure.

The adoption of 5% total solids was due to the fact that high concentrations are not beneficial to the efficiency of the fermentative process since it was used fresh manure with high TAN concentrations. Based on findings by Arruda et al., (2014) for fresh manure, dilution at 5% of TS concentration can be the most feasible in terms of TS concentration and less damaging in terms of TAN mg L⁻¹ that using higher concentrations of solids, which eventually increase TAN concentration.

It should be noticed that total solids from biochar was not considered in the 5% calculation, consequently, its addition increased the amount of TS according to its inclusion. This feeding method was adopted because reducing the organic matter from laying hens manure while biochar was increased could eventually affect the biogas production since from both substrates, the manure is the one that most contributes to the fermentative process generating precursors of biogas. Biochar is mainly composed by carbon and ashes, and its high inclusion associated with low amount of manure could influence in lower biogas yield.

Formulated substrate used to feed the reactors with its respective amount expressed in kilograms (kg) are displayed on Table 2.

Table 2. Composition of each component used to feed the batch anaerobic digester (kg)

Treatment	Manure	Water	Biochar	TS (%)	VS (%)	TS	VS
Control	0.412	2.048	-	4.62	70.83	0.060	0.042
Biochar 2.5%	0.412	2.048	64	5.76	66.50	0.074	0.049
Biochar 5.0%	0.412	2.048	128	7.19	65.08	0.093	0.060
Biochar 7.5%	0.412	2.048	192	7.77	64.12	0.101	0.064

Substrate prepared to feed the reactors were sampled and assessed for: pH, total ammonia nitrogen (TAN); alkalinity (partial, intermediate and total); volatile fatty acids (VFAs), total solids (TS) and volatile solids (VS) following methodology suggested by APHA, AWWA, WPCF (2017). All twelve anaerobic reactors were simultaneously sealed, placed in a bath filled with water by half and kept there under controlled temperature at 35°C through the entire experiment. The adopted hydraulic retention time was 60 days.

During the assay, biogas production was recorded twice daily at predefined times (9:00 a.m and 5:00 pm). To measure the volume of generated gas, a scale was

attached to the gasometer (as seen in the Figure 4) in order to record its displacement as pressure mounted the generated gas. The value was multiplied by the inner transversal section area of the gasometer and then corrected to experimental atmospheric pressure of 95.05 kPa, resulted from the combination of 93.89 kPa (Vicosa's atmospheric pressure) and 1.15 kPa (gasometer pressure).

To correct the biogas volume to 1 atm and 20°C, an equation resulted from the combination of Boyle's and Gay-Lussac's law as described by Caetano (1985). Biogas yield was calculated using data from daily biogas production and the amount of TS and VS_{in} and VS_{removed}. The results were expressed in m³ of biogas per kg of TS and VS_{in} and VS_{removed}.

5.6. Physical-chemical characterization of the digestate

To evaluate the quality of the digestate analyses of pH, total ammonia nitrogen (TAN); alkalinity (partial, intermediate and total); volatile fatty acids (VFAs) and total and volatile solids (VS) were performed following methodology suggested by APHA, AWWA, WPCF (2017).

5.7. Statistical Analysis

The design adopted in this experiment was a completely randomized design in which were considered four treatments replicated three times each. The mean values were assessed through an analysis of variance one-way ANOVA considering inclusion rate of biochar as the treatment. Mean values of all response variables by treatment were compared using Tukey test at 5% of probability using Speed Stat software (Carvalho & Mendes, 2017).

6. RESULTS AND DISCUSSION

6.1. Monitoring Parameters

Mean values for all pH and TAN substrate and digestate of substrate and digestate from batch anaerobic reactors fed laying hen manure different inclusion of biochar are presented on Table 3.

Table 3. Mean values for pH and total ammonia nitrogen (TAN mg L⁻¹) of substrate and digestate from batch anaerobic reactors fed with laying hen manure with different inclusion of biochar

Parameters	pH		TAN	
	Substrate	Digestate	Substrate	Digestate
Control	7.10 d	9.80 a	838 b	2734 a
Biochar 2.5%	7.37 c	9.84 a	971 a	2739 a
Biochar 5.0%	7.64 b	9.89 a	868 ab	2470 a
Biochar 7.5%	7.77 a	9.86 a	911 ab	2354 a
P value	<0.001	0.671	0.023	0.140
CV (%)	0.58	0.96	4.72	8.34

Means followed by different letters in the same row differ at 5% through Tukey test.

It was detected difference among treatments ($P < 0.05$) for pH of all substrates, which demonstrated a tendency of increase in the buffering capacity as inclusion of biochar was gradually increased. For digestates pH no significant difference was revealed. It is widely known an acceptable pH range to the anaerobic digestion varying from 6.0 to 8.0 (Aragaw et al. 2013), if taken into perspective the achieved values with recommended values on literature, digestate material, unlike substrate, showed high and inadequate values to the anaerobic digestion process.

As for pH, TAN also increased its values by the end of the experiment as compared to the initial. Higher TAN substrate concentration was detected on treatment that had 2.5% inclusion of biochar, which was statistically higher than control's treatment concentration but equal reactors fed biochar inclusions of 5.0 and 7.5%. No statistical difference was revealed for digestate of all treatments. The high increase in TAN was already expected, at end of the AD process TAN values were about 2.9 times higher than the initial. This increase is due to metabolization of protein compounds present on substrates which releases ammonia into the substrate. The ideal value to the fermentative process in anaerobic reactors is around 200 mg L⁻¹ of TAN as recommended by Liu & Sang (2002).

Although Niu et al., (2014) considers the edge of AD inhibition ranging from 3000 to 4000 mg L⁻¹, it should be mentioned that the efficiency of the fermentative process under high values of TAN depends on how well anaerobic microorganisms are acclimated to that harsh condition. In the present study, for instance, there was no

acclimation phase and, biogas production, which will be discussed latter, was strongly affected by that.

Hen feces usually contains high nitrogen (uric acid) concentration and when it undergoes through microorganisms metabolization generates organic acids and ammonia (NH₃). Gerardi (2003) mentions that while this process occurs there is also the production of ammonia bicarbonate, which might explain the high pH values for all digestate and alkalinity as well, discussed further on.

In the Table 4 are summarized mean values for partial (PA), total alkalinity (TA) (mg CaCO₃ L⁻¹), and for volatile fatty acids (mg L⁻¹) of substrates and digestates.

Table 4. Mean values for partial (PA), and total alkalinity (TA) (mg CaCO₃ L⁻¹), and volatile fatty acids (VFAs mg L⁻¹) of substrate and digestate from batch anaerobic reactors fed laying hen manure with different inclusion of biochar

Parameters	PA		TA		VFAs	
	*Sub	*Dig	Sub	Dig	Sub	Dig
Control	2424 c	10847 b	4886 b	14184 a	3727 a	3871 a
Biochar 2.5 %	3164 b	12600 b	6118 a	14367 a	3857 a	3671 ab
Biochar 5.0%	3422 ab	13367 a	5901 a	14417 a	3802 a	1437 b
Biochar 7.5%	3716 a	13833 a	6249 a	14967 a	3802 a	1870 ab
P value	0.001	0.012	0.008	0.643	0.135	0.001
CV (%)	5.87	6.68	6.41	5.29	12.7	33.86

*Sub: substrate; *Dig: digestate; Means followed by different letters in the same row differ at 5% through Tukey test.

Difference was revealed for partial alkalinity (PA) regarding substrate for all inclusions of biochar (P<0.05). Higher inclusions of biochar (7.5 and 5.0%) increased the buffering capacity of the substrate, while control's treatment displayed the lowest value for that parameter. Overall, all substrates showed a proper amount of ions bicarbonate as a buffer substance to the start-up of the AD process once the minimum operational limit should be 1200 mg CaCO₃ L⁻¹ as recommended by Jenkins et al. (1983).

Digestates had high concentrations in mg L⁻¹ of calcium carbonate as seen on Table 4. All reactors fed different inclusions of biochar was statistically similar and higher than that of control, however, no difference was revealed among control

treatment and 2.5 % of biochar inclusion. PA for all digestate increased in average about 4.3 times as compared to the initial values being in a range of 10847 to 13833 mg CaCO₃ L⁻¹. That excessive amount of ions bicarbonate at the end of the fermentative process can only be explained by the formation of ammonia bicarbonate while protein compounds are breaking down by anaerobic microorganisms which improves the alkalinity and also increase the pH.

For TA, it can be seen that control's treatment displayed the lowest value, while the others that had biochar inclusions were statistically similar. High values for TA were noticed to all digestate treatments and no statistical difference was revealed.

No statistical difference was observed for VFAs concentration for all substrates which showed values ranging from 3727 to 4720 mg L⁻¹. For digestate, treatment control displayed the highest values, equally similar to reactors fed 2.5 and 7.5% of biochar. Lower values were detected for treatment with inclusions of 5.0% of biochar (Table 4). The lower values for 5.0 and 7.5% of biochar might be explained by the fact that higher inclusion of biochar contributed to a high capacity of the overall buffering system condition, which allowed the VFAs not be accumulated in the system as it happened to the other treatments.

In overall, the scenario that can be draw in terms of monitoring parameters is of instability. It can be seen high concentration of alkalinity by the end of the anaerobic digestion process while VFAs concentrations which should be used as biogas precursors was accumulated in the reactor, as high values were detected. High PA concentrations are mainly due breakdown of protein compounds which generates at same time VFAs and ammonia. This lack in equilibrium might explain the high values for alkalinity, TAN and pH as well.

6.2. Solids reduction and biogas yield

Regarding to solids destruction it can be noticed that no statistic difference was revealed for total solids (TS), however, the volatile fraction of it did show statistical difference with higher reductions for control's treatment, similarly equal to reactors fed 2.5 and 5.0% of biochar, and higher that inclusions of 7.5% (Table 5). This last case, of the lowest reduction of volatile reduction can be explained by the fact that this treatment had the highest inclusion of biochar which in its composition has a high amount of un-degradable solids.

Table 5. Mean values for total solids (TS) and volatile solids (VS) reduction of substrate and digestate from batch anaerobic reactors fed laying hen manure with different inclusion of biochar

Treatments	Total solids	Volatile solids
	Reduction (%)	
Control	50.20 a	56.50 a
Biochar 2.5%	42.41 a	47.66 ab
Biochar 5.0%	44.37 a	47.33 ab
Biochar 7.5%	44.37 a	40.63 b
P value	0.146	0.050
CV (%)	12.97	11.63

Means followed by different letters in the same row differ at 5% through Tukey test.

Table 6 summarizes mean data for biogas yield stratifications which includes: biogas yield per kg of waste; biogas yield per kg of total solids _{in}; biogas yield per kg of volatile solids _{in} and biogas yield per kg of volatile solids _{removed}.

Table 6. Mean values for total biogas production, biogas yield per kg of waste ($\text{m}^3.\text{kg}^{-1}$), biogas yield per kg of TS _{in} ($\text{m}^3.\text{kg}^{-1}$), biogas yield per kg of VS _{in} ($\text{m}^3.\text{kg}^{-1}$) and biogas yield per kg of VS _{removed} ($\text{m}^3.\text{kg}^{-1}$) of batch anaerobic reactors fed laying hen manure with different inclusion of biochar

Treatments	Biogas yield	Biogas yield	Biogas yield	Biogas yield
	per kg of waste	per kg of TS _{in}	per kg of VS _{in}	per kg of VS _{re}
	$\text{m}^3.\text{kg}^{-1}$			
Control	0.007 a	0.049 a	0.070 a	0.125 ab
Biochar 2.5%	0.007 a	0.045 a	0.068 a	0.142 a
Biochar 5.0%	0.004 b	0.024 b	0.036 b	0.078 b
Biochar 7.5%	0.006 ab	0.035 ab	0.055 ab	0.137 a
P value	0.011	0.003	0.007	0.010
CV (%)	15.44	15.25	15.87	15.21

Means followed by different letters in the same row differ at 5% through Tukey test.

Higher biogas yield per kg of waste was detected for treatment control and for reactors fed 2.5% inclusion of biochar. The lowest biogas yield was obtained for

treatment that had 5.0% of biochar inclusion. Regarding biogas per kg of TS_{in} (m³.kg⁻¹) and VS_{in} (m³.kg⁻¹), the same behavior was detected as for the parameter previously discussed. As compared to findings on literature, it is seen that values attained on our assay are way lower than that attained by Farias et al., (2012) of 0.21 m³.kg VS_{in} and Fantozzi & Buratti of 0.22 m³.kg VS_{in}, and Zanato (2014) of 0.39 m³.kg VS_{in}.

In terms of biogas yield per kg of VS_{red} (m³.kg⁻¹) it can be seen that the lowest yield comes from reactors feed 5.0% of biochar while no statistical difference was detected among the other treatments, which had higher biogas yield.

As seen in the Figure 5, the behavior of the anaerobic digester that had 5.0% of biochar in its composition showed a very late responsiveness to the biogas production as compare to the other treatments. But in overall, the system worked under a very unstable condition in the first 30 days due to high amount of inhibitory condition such as TAN and pH which probably influenced the overall inefficiency in the conversion of volatile compounds into biogas.

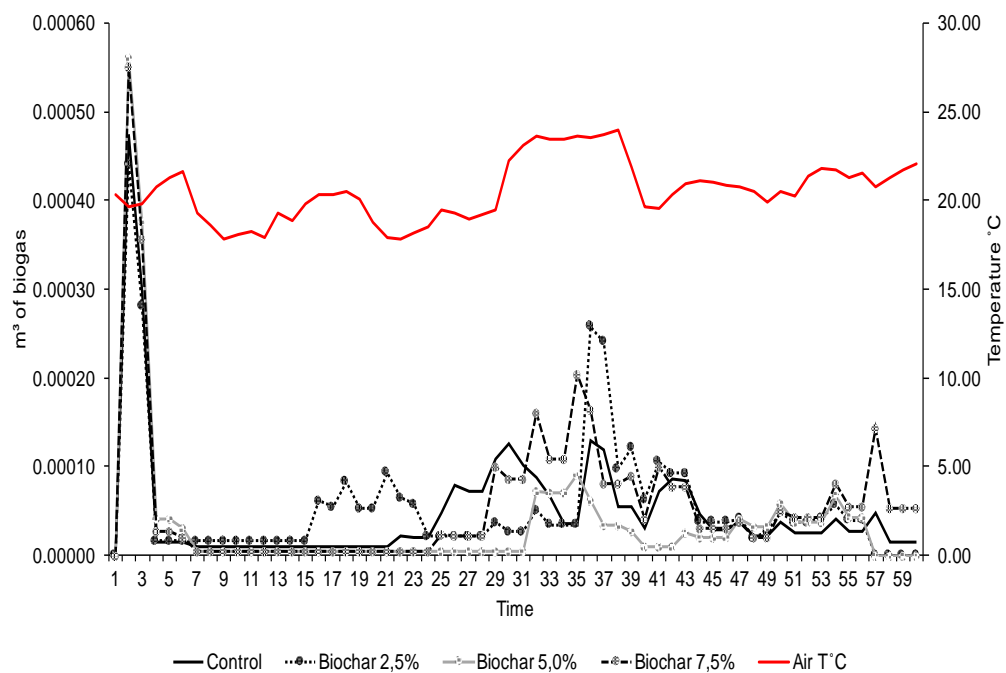


Figure 5. Daily biogas production from batch anaerobic reactors feed laying hen manure with different inclusion of biochar

The system showed a very long lag phase after the first peak of biogas production. This condition might be explained by a possible acclimation of fermentative microorganisms to a not-favorable conditions in terms of high TAN, pH and VFAs concentration. The following peak of biogas production was noticed in the 34th and 36th days by treatments 2.5 and 7.5%, respectively, but after a very long lag phase probably

used to microorganism acclimation, no expressive increase in biogas was noticed for any treatment, despite of its steady a continuous production up to the 60th day.

When reactors are running under stress due to some inhibitory conditions microorganisms they tend to reduce its activity keeping the system under a stable and steady biogas production but at a very low rate, which was what happened in the studied case. Perhaps a longer HRT would give the possibility to the system be fully recovered and increase the biogas production to another level as fermentative microorganisms were getting used to the adverse conditions.

Overall, the anaerobic digestion process was affected by the excess of TAN, high pH and high VFAs concentrations. The addition of biochar in this specific case did not have positive effect regarding biogas yield.

7. CONCLUSION

Inclusion of 2.5, 5.0 and 7.5% of biochar did not promote better performance of anaerobic batch reactors fed laying hen manure in terms of pH values, total ammonia nitrogen, and volatile fatty acids concentration.

Addition of the three rates of biochar did not enhance biogas yield per kilogram of waste, total solids in, volatile solids in and volatile solids as compared to control.

CHAPTER 3 - Anaerobic digestion of laying hen manure with biochar: monitoring parameters, biogas yield and digestate quality.

ABSTRACT

The use of an adsorbent substance such as biochar as a component in the AD process is new and it is noticeable its positive impact on the AD process allowing better stability and overall equilibrium of AD reactions. Thus, the present study proposed to assess anaerobic digestion process in batch reactors fed laying chicken manure with different inclusion of biochar regarding monitoring parameters, biogas yield and digestate quality. Three different levels (at 2.5, 5.0 and 7.5%) of biochar inclusions were adopted with 3 replicates each, plus control treatment, which had only hen manure diluted in water at 7% of total solids. Twelve, 2-L batch anaerobic reactors were used subjected to 86 days of hydraulic retention time under controlled temperature at 35°C. Substrate and digestate material of each treatment were assessed for evaluate monitoring parameters and its quality in terms of nitrogen, phosphorous and calcium concentration, while through of the 86 days, biogas production was daily recorded. Inclusions of biochar at the level of 2.5, 5.0 and 7.5% gradually reduces total ammonia concentration and electric conductivity on substrates and digestate. Despite of the positive effect on a faster start-up of biogas production detected on treatments that had inclusions of biochar on substrate, overall, biochar influenced in lower biogas yield when compared to control treatment. Biochar did not improve digestate quality in terms of nitrogen, phosphorous and calcium concentration. However, its inclusion influences in high pH values for digestate with low electric conductivity and total ammonia nitrogen concentrations.

Key-words: adsorption, anaerobic reactor, greenhouse gases, waste management

Digestão anaeróbia de dejetos de galinhas poedeiras com biochar: parâmetros de monitoramento, rendimento de biogás e qualidade do digestato

RESUMO

O uso de substâncias adsorventes como o biochar no processo de digestão anaeróbia é algo recente, contudo, a literatura tem citado impactos positivos no processo de digestão anaeróbia quanto a melhoria na estabilidade e equilíbrio geral das reações. Assim, o presente estudo propôs avaliar o processo de digestão anaeróbia em reatores em batelada alimentados com dejetos de galinhas poedeiras com diferentes percentuais de

adição de biochar quanto a parâmetros de monitoramento, rendimento de biogás e qualidade do digestato. Três níveis (2,5; 5,0 e 7,5%) de inclusão de biochar foram adotados com três repetições cada. O tratamento controle, também com três repetições, continha apenas dejetos diluídos em água, a 7% de sólidos totais. Foram utilizados 12 reatores anaeróbios de 2 litros de volume útil, cada, submetidos a um tempo de retenção hidráulica de 86 dias sob temperatura controlada de 35°C. Os substratos e digestatos de cada tratamento foram avaliados quanto aos parâmetros de monitoramento e qualidade em termos de valor nutritivo, e, durante os 86 dias a produção de biogás foi registrada diariamente. As inclusões de biochar ao nível de 2,5; 5,0 e 7,5% reduziram as concentrações de nitrogênio amoniacal total (mg L^{-1}) e condutividade elétrica (mS/cm) nos substratos e digestatos. Apesar do efeito positivo quanto ao rápido início na produção de biogás detectada em tratamentos que tiveram inclusões de biochar no substrato, em geral, o biochar influenciou em redução nos rendimentos de biogás quando comparado ao tratamento controle. O uso de biochar não promoveu melhorias na qualidade do digestato quanto as concentrações de nitrogênio. Fosforo e cálcio uma vez que não foram observadas diferenças estatísticas. No entanto, suas inclusões influenciaram no aumento dos valores de pH e redução de nitrogênio amoniacal total e condutividade elétrica.

Palavras-chaves: adsorção, reator anaeróbio, gases de efeito estufa, manejo de resíduo

8. INTRODUCTION

Energy recovery, nutrient recycling and mitigation of sanitary risks are the main reasons for adopting anaerobic digestion systems in industrial and agricultural plants. Anaerobic digestion (AD) has been vastly studied and used as a tool for harnessing the most (biogas and digestate) of valuable sub-products largely generated.

Despite of all knowledge literature has provided surrounding anaerobic digestion (AD), it still has some inconvenient problems in terms of monitoring parameters that may or may not strongly affect biogas production, for instance, total ammonia nitrogen concentrations (TAN). High levels of ammonia has been cited as one of the main problems regarding the fermentative process of laying hen manure (Massé & Singh, 2013; Farrow et al., 2016; Molaey et al., 2018) and it may suppress the real potential for biogas production.

Organic matter rich in proteins (i.g. laying chicken manure) has high energetic potential, mainly for biogas and methane generation via anaerobic digestion (Nordell et

al., 2013). Nonetheless, use of biomass rich in protein into an anaerobic reactor system may lead to a severe disturbance due to high ammonia concentration, causing reduced activity of microorganism, incomplete digestion of intermediate products such as volatile fatty acids and as a result methanogenic activity is decreased (Massé & Singh, 2013). A range of alternative ways to overcome high concentration of NH₃ nitrogen in the substrate of the AD process have been studied such as: anaerobic codigestion (Wang et al. 2014) acclimation of microorganism (Yenigün & Demirel, 2013) dilution (Yun et al. 2016) and use of adsorbent materials (Kumar et al., 1987; Montalvo et al., 2005 e Ho & Ho 2012).

Despite of that, very few studies have concentrated on the use of adsorbent material aiming to assess the energetic performance, monitoring parameters and digestate quality. Biochar is an example of an adsorbent material that might be elected to be used in anaerobic digestion (Mumme et al., 2014). It is a solid material generated after a pyrolysis process conducted under high temperature in absence of oxygen, and as a result, a material of high specific surface is generated (Guo et al., 2016).

Its use as adsorbent component in the AD process is new and from fewer works that have been carried out is noticeable its positive impact on the AD process allowing better stability and overall equilibrium of AD reactions (Kumar et al., 1987; Desai & Madamwar, 1994; Montalvo et al., 2005 and Ho & Ho 2012). Mumme et al., (2014) say that adsorption phenomena which involves biochar with other substrate improves AD process by means: a) sorption of inhibitory components through its pores and sites for biding, b) increase in the buffer capacity and c) formation of biofilm to immobilization of microorganism. All these conditions previously mentioned are responsible to enhance the AD process and consequently high volumetric biogas yields may be achieved (Montalvo et al., 2005).

The present study proposed to assess anaerobic digestion process in batch reactors fed laying hen manure diluted in water with different inclusion of biochar regarding monitoring parameters, biogas yield and digestate quality.

9. MATERIAL AND METHODS

9.1. Location

The study was conducted in the Anaerobic Digestion Laboratory in the Agricultural Engineering Department at the Universidade Federal de Viçosa (UFV). The campus is located in latitude 20°45'45" south and longitude 42°52'04" east at an elevation of 649 m. The climate is Cwa according to Köppen's classification, with a hot rainy season, and a cool dry winter.

9.2. Experimental design

The experiment was conducted in two phases, the first phase comprised by physical-chemical characterization of hen feces, biochar and physical-chemical characterization of reactors' feeding substrates. In the second phase it was carried out an assay to assess the effect of different inclusion of biochar in the volumetric biogas production and also to assess physical-chemical characterization of digestate after the AD process.

To perform the assay, twelve 2-L anaerobic batch reactors capacity were kept under controlled temperature at 35°C. Three levels (2.5, 5.0, and 7.5% by weight) of biochar inclusion plus the control, with each treatment replicated three times. A completely randomized design formed by 4 treatments and 3 replications was adopted.

9.3. Feedstock

Feces used in the experiment were collected from beneath cages in the laying chicken barns from the Animal Science Department at Universidade Federal de Viçosa. At the time of collection, manure was homogenized and was composed of excreted feces, fed waste, cracked eggs, bugs, larva and dust. This mixed altogether, placed in plastic bags and transported to the Anaerobic Digestion Laboratory in the Agricultural Engineering Department. The biochar was made of pyrolyzed broiler chicken litter provided by a company from Sao Paulo State (SP Pesquisa e Tecnologia Ltda.).

9.4. Physical-chemical characterization of residues and substrates

To perform the physic-chemical characterization of manure and substrate the following analyses were performed: pH of raw manure was done by placing the electrode in a substrate resulted from a mixture of 50 g of raw manure with 50 mL of distilled water in a 1:1 ratio. Biochar pH was recorded 30 min after dilution and constant stirring of a mixture of 20 g of biochar in 100 mL of distilled water. Regarding the feeding substrate, as it was already homogeneous, a pH electrode was used to record substrate data.

Total ammonia nitrogen (TAN) was done per the methodology suggested by APHA, AWWA, WPCF (2017) in which 10 mL sample was placed in a protein tube for distillation. To collect the distilled content extracted, an Erlenmeyer flask previously filled with 20 mL of boric acid at 4% was used to fix the ammonia as ammonia borate

($\text{BH}_{12}\text{N}_3\text{O}_3$). After that, 75 ml of the distilled extract was titrated using sulfuric acid (H_2SO_4) at 0.02 mol L^{-1} .

Solids analysis from feces, biochar and substrate was performed by placing previously weighed samples into an oven at 105°C for 24 h. After that, samples were weighed to attain the total solid (TS) value. Dried samples were then weighed and submitted to an oven under 550°C for 4 hours to attain the volatile solids (VS), following methodology suggested by APHA, AWWA, WPCF (2017).

Total carbon (TC) was determined by gravimetric method which consists on submitting samples to a furnace for ignition under 550°C for four hours. After that, with the weight difference of mass taken before and after organic matter is determined and total C can be estimated using 1.8 conversion factor as suggested by Jiménez & Garcia (1992).

Samples used to assess total nitrogen (N), phosphorous (P) and potassium (K) were dried in an oven under 65°C for 72 hours in order to remove all the water content. For total nitrogen determination about 0.2 grams of dried sample was subjected to digestion under 350°C for about 3 hours using 5 ml of H_2SO_4 (P.A) and a digestion mixture of copper sulfate (CuSO_4) and sodium sulfate (Na_2SO_3) in the ratio of 1:10. Digestion procedure was consummate when samples were displaying a translucent green color. Digested samples were then subjected to distillation procedure in which for each sample 25 ml of 10 mol L^{-1} NaOH was added in to it. Distilled material was fixed on boric acid and then titration procedure was done to determinate the amount of N in each sample.

For phosphorous, potassium and calcium same digestion procedure was adopted. About 0.2 grams of dried sample was placed in an Erlenmeyer flask where was also added 10 mL of nitric-perchloric acid. The material was then subjected to digestion for about 2 hours under temperature of 300°C . After that an extract of mineral content is attained and the Erlenmeyer flask of each sample was washed down using 50 ml of distilled water. Washed material was rapidly filtrated and placed in 50 mL vessels for further analysis. Obtained material from nitric-perchloric digestion was used to determination of total phosphorous through spectrophotometric procedure. Calcium determination as done by atomic absorption spectrophotometry procedure.

In Table 7 are shown the mean values of characterized parameters from laying hen manure and biochar.

Table 7. Mean values of characterization parameters from laying hen manure and biochar made of broiler litter

Parameters	Manure	Biochar
pH	9.47	10.29
T-ammonia nitrogen (mg.L ⁻¹)	997.50	52.50
Total Solids (%)	55.71	62.98
Volatile solids (% of DM)	70.38	53.40
Fixed solids (%)	29.62	46.60
T- Carbon (% of DM)	30.95	29.66
T-Nitrogen (% of DM)	2.83	2.17
T-Phosphorous (% of DM)	4.76	3.12
Calcium (% of DM)	2.71	0.81

In addition to chemical characterization, biochar was also subjected to an analysis of particle size determination using an electromagnetic sieve. In order to do that, 400 grams of biochar was assessed by using an electromagnetic sieve with size range of 2.36 mm to 75 μ m. Particle size in the range of 590 μ m accounted for about 59% of all material. The biochar was considered fine, which is theoretically an advantage since small particles size has greater superficial area.

9.5. Assay of anaerobic digestion

In this phase, the study was carried out using twelve two-liter's batch anaerobic reactors. Each reactor had a hose barb on top connected to a hose allowing the gas collection. In order to store the gas, cylindrical gas collectors build of PVC pipes were used. This apparatus was comprised by the inner (gas collector) and outer (supporter) PVC pipes as seen in the Figure 6.

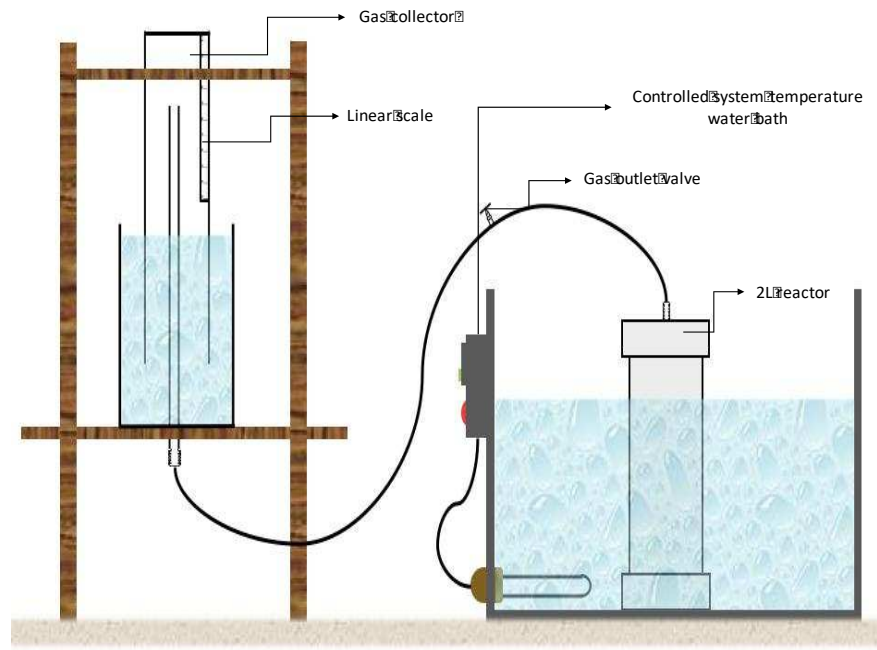


Figure 6. Schematic representation of the anaerobic reactors used in the experiment.

In order to feed the reactors, all substrates were formulated to attain 7% of total solids (Lucas Junior 1994) based on the concentrations of TS of the manure and biochar. Since it was used an old and dry manure, which consequently has its ammonia concentration reduced due to continuous volatilization to the atmosphere, total solids at 7% was the most suitable option since higher amount of organic matter (with lower TAN concentrations) could be added into the reactor.

Formulated substrate used to feed reactors with its respective components amount expressed in mass percent are displayed in Table 8.

Table 8. Mass percent (%) of each component used to feed batch anaerobic digesters and their respective amount of total and volatile solids

Treatment	Manure	Water	Biochar	Total (kg)	TS	VS
Control	15.16	84.84	0	2.5	6.71	65.16
Biochar 2.5%	13.24	85.08	1.68	2.5	7.06	65.46
Biochar 5.0%	11.36	85.24	3.36	2.5	6.87	66.09
Biochar 7.5%	9.48	85.48	5.04	2.5	6.68	61.09

All twelve anaerobic reactors were simultaneously sealed, placed in a bath filled with water by half and kept there under controlled temperature at 35°C through the entire experiment. The adopted hydraulic retention time was 86 days.

During the assay, biogas production was recorded periodically at predefined times (9:00 a.m and 5:00 pm). To measure the volume of generated gas, a scale was attached to the gasometer (as seen in the Figure 6) in order to record its displacement as pressure mounted the generated gas. The value was multiplied by the inner transversal section area of the gasometer and then corrected to experimental atmospheric pressure of 95.05 kPa, resulted from the combination of 93.89 kPa (Vicosa's atmospheric pressure) and 1.15 kPa (gasometer pressure).

To correct the biogas volume to 1 atm and 20°C, an equation resulted from the combination of Boyle's and Gay-Lussac's law as described by Caetano (1985) was used in which:

V_0 = corrected biogas volume, m³;

P_0 = corrected pressure of biogas (101.32 kPa);

T_0 = Corrected temperature of biogas (274.99 K);

V_1 = volume of biogas in the gasometer;

P_1 = biogas pressure at the reading (93.89 + 1.15 kPa);

T_1 = biogas temperature at reading K as follows:

$$\text{Corrected biogas volume: } \frac{V_0 \times P_0}{T_0} = \frac{V_1 \times P_1}{T_1}$$

Biogas yield was calculated using data from daily biogas production and the amount of waste, substrate, total solids_{in}, volatile solids_{in} and volatile solids_{removed}. The results were expressed in m³ of biogas per kg of waste, substrate, TS and VS_{in} and VS_{removed}.

9.6. Physical-chemical characterization of digestate

Substrate prepared to feed reactors and the obtained digestate after 86 days of fermentative process were sampled and assessed for: pH, total ammonia nitrogen (TAN); electric conductivity (EC), alkalinity (partial, intermediate and total); volatile fatty acids (VFAs), total solids (TS) and volatile solids (VS), total nitrogen (TN), phosphorous (P), potassium (K) and calcium (Ca) were determined using methodology suggested by APHA, AWWA, WPCF (2017). Total carbon (TC) was done as methodology suggested by Jiménez & Garcia (1992).

9.7. Statistical Analysis

The design adopted in this experiment was a completely randomized design in which were considered four treatments replicated three times each. The mean values were assessed through an analysis of variance one-way ANOVA considering inclusion rate of biochar as the treatment. Mean values of all response variables by treatment were compared using Tukey test at 5% of probability using Speed Stat software (Carvalho & Mendes, 2017).

10. RESULTS AND DISCUSSION

10.1. Monitoring parameters

Data regarding pH, total ammonia nitrogen (TAN) and electric conductivity (EC) for substrates and digestate of batch reactors fed laying hen manure with biochar are presented on Table 9.

Table 9. Mean values for pH, Total Ammonia Nitrogen (TAN mg L⁻¹) and EC (mS/cm) of substrate and digestate from batch anaerobic reactors fed laying hen manure different inclusion of biochar

Treatments	pH		TAN		EC	
	Substrate	Digestate	Substrate	Digestate	Substrate	Digestate
Control	8.87 d	8.32 a	548 a	1794 a	10.57 a	20.73 a
Biochar 2.5%	9.17 c	8.44 a	568 a	1401 b	11.30 a	18.14 b
Biochar 5.0%	9.37 b	8.48 a	469 ab	951 c	10.52 a	16.31 b
Biochar 7.5%	9.73 a	8.53 a	324 b	509 d	9.10 a	13.80 c
P value	0.001	0.105	0.006	0.001	0.146	0.001
CV (%)	0.38	1.08	14.64	6.87	9.97	4.21

Means followed by different letters in the same row differ at 5% through Tukey test.

Mean values for all pH of substrates used to feed the reactors were statistically different as compared to one another ($P < 0.05$) while pH digestate values did not revealed significant difference as seen in Table 9. Substrate pH values were not in an acceptable range to the anaerobic digestion process just like digestate values, which showed high values ranging from 8.32 to 8.53. According to Aragaw et al. (2013) anaerobic digestion process can occurs under a pH that may vary from 6.0 to 8.0, with a high efficiency at pH of 6.8 to 7.3 (Sakar et al., 2009).

Despite of substrate and digestate's inadequate pH values for the overall anaerobic digestion process, Demirer & Chen (2004) mentions that acidogenic bacteria

has high growing and thus better activity rate under pH of 5.2 to 6.5 while archaea methanogenic has ideal growth and high activity rate under pH range of 7.5 to 8.5. In the context, it is possible to infer that at end of the process all assessed pH were close to the ideal range for methanogenic microorganism activity, nonetheless, it was not necessarily a positive aspect once for methane production, acidogenic and acetogenic microorganism have to have besides other parameters, ideal substrate pH conditions to synthesize complex substrates that will then generate precursors for archaea microorganism activity to produce methane.

As higher the inclusion of biochar, higher was the pH values observed since biochar contributes to buffer capacity with some ions such as calcium and magnesium. Despite of pH above ideal values, it was not corrected to the ideal range.

As noticed on Table 9, total ammonia nitrogen (TAN) increased its values by the end of the experiment as compared to the initial. No statistical difference was detected for substrate of control, and for inclusions of 2.5 and 5.0% of biochar. Higher inclusions of biochar (at 7.5%) has led lower TAN concentration in substrate. This happens because that treatment had the lower inclusion of rich organic matter material in protein (laying hen manure), which contributes to the increase of TAN, while biochar is a carbonaceous material with low concentration of TAN.

Increase in TAN concentrations by the end of the trial was already expected, at end of the 86th day of fermentative process TAN value for control treatment was in average 3.2 times higher than the initial. Inclusion of biochar in the substrate has led lower increase in TAN concentrations since lower load of rich organic matter material in protein is added eliciting less degradation and conversion of it. Despite of Liu & Sang (2002) recommendations of TAN ideal values around 200 mg L⁻¹, literature has shown that anaerobic digestion process can successfully occurs under high concentrations of TAN.

Niu et al., (2014) considers the edge of anaerobic digestion inhibition ranging from 3000 to 4000 mg L⁻¹, but should be mentioned that the efficiency of the fermentative process under high values of TAN depends on how well anaerobic microorganisms are acclimated to that harsh condition. The higher values of TAN concentration for treatments control and 2.5% biochar may have led to certain instability along the process but as biogas was generated and methane was detected through burning tests, an acclimation of microorganisms to the adverse conditions probably happened.

Regarding electric conductivity, it was not revealed statistical difference for all substrates ($P>0.05$), however, higher values for EC were detected in the digestate for treatment control ($P<0.05$).

EC is a parameter that indicates presence nutrients on a substrate (Carmo & Silva, 2012), therefore, higher the EC, richer in nutrients digestate is. Higher values for EC for treatment control might be by the fact that control's treatment had the highest inclusion of hen manure that due to hen's diet may have reasonable amounts of dicalcium phosphate (CaHPO_4), sodium chloride (NaCl), limestone, and calcium (Ca), which has in its composition calcium carbonate (CaCO_3) that might contributes to the increase of EC values. The other treatments had a linear decrease of manure inclusion, which lowered EC.

Besides other components that are not well digested and absorbed while going through hen's digestive tract and thus eliminated in the feces, hen feces usually have great amount of protein compounds that when degrade by microorganisms generates organic acids and ammonia (NH_3). Gerardi (2003) mentions that while this process occurs there is also the production of ammonia bicarbonate, which might explain the high pH values for all digestate (Table 9) and for alkalinity as well, as seen on Table 10.

Table 10. Mean values for partial (PA), intermediate (IA) and total alkalinity (TA) ($\text{mg CaCO}_3 \text{ L}^{-1}$) and AI: AP ratio of substrate and digestate from batch anaerobic reactors feed laying hen manure different inclusion of biochar

Treatments	PA		IA		TA		IA:PA	
	*Sub	*Dig	Sub	Dig	Sub	Dig	Sub	Dig
Control	1595 a	7021 a	500 a	1153 a	2095 a	8507 a	0.31 a	0.15 a
Biochar 2.5 %	1547 a	6664 a	480 a	695 b	2027 a	7360 a	0.31 a	0.10 b
Biochar 5.0%	1488 a	4564 b	452 a	488 bc	1933 a	5052 b	0.31 a	0.11 b
Biochar 7.5%	1438 a	3497 b	430 a	283 c	1867 a	3780 b	0.30 a	0.08 b
P value	0.760	0.001	0.622	0.001	0.624	0.001	0.993	0.001
CV (%)	12.66	10.93	14.59	19.32	11.18	12.07	16.64	8.38

*Sub: substrate; *Dig: digestate; Means followed by different letters in the same row differ at 5% through Tukey test.

Values for substrates were in the range of 1438 to 1595 $\text{mg CaCO}_3 \text{ L}^{-1}$, which showed reasonable amount of ions bicarbonate as a buffer substance to the start-up of

AD process once the minimum operational limit should be $1200 \text{ mg CaCO}_3 \text{ L}^{-1}$ (Jenkins et al., 1983). Partial alkalinity plays important role in the equilibrium of the AD process because it enables substrate under fermentation do not get acidified due to rapid fermentation and production of metabolites such as organic acids by microorganism. As compared to substrate material, digestate of all treatments increased alkalinity values, indicating a high buffering capacity. High levels of partial alkalinity for all treatments, specially for treatment control and for reactors feed 2.5% of biochar are due to formation of ammonia bicarbonate.

Intermediate alkalinity (IA) did not show statistical difference for substrate of all treatments, nonetheless, digestate material revealed statistical difference, in which control treatment displayed the highest values while reactors fed 7.5% of biochar the lowers (Table 10). IA has been mentioned to be useless since 80% of the buffer capacity comes from PA. Regarding total alkalinity (TA) in its sense it accounts all possible buffering substances (PA+IA) that may help the AD system to be kept under control. High values for TA were noticed to all substrate in spite of no statistical difference, while digestate displayed higher values for treatments control and 2.5% of biochar ($P < 0.05$).

For IA:PA ratio, no statistical difference was noticed for all substrates which had values around 0.30, close to the optimum range values recommended by Ripley et al., (1986) of 0.10 to 0.35. Despite of statistical difference for digestate material ($P < 0.05$), with treatment control being higher than the others, digestate from all treatments were fairly around the optimum recommended values. The IA:PA ration might be used as a key parameter to better understand whether AD process is running under ideal conditions.

In Table 11 are presented mean values for volatile fatty acids concentration and volatile fatty acids: partial alkalinity ration of substrate and digestate.

Table 11. Mean values for volatile fatty acids (VFAs mg L⁻¹) and volatile fatty acids: partial alkalinity ratio (VFA: PA mg L⁻¹) of substrate and digestate from batch anaerobic reactors fed laying hen manure with different inclusion of biochar

Treatments	VFAs mg L ⁻¹		VFA: PA mg L ⁻¹	
	Substrate	Digestate	Substrate	Digestate
Control	801 a	1028 a	0.51 a	0.15 ab
Biochar 2.5%	969 a	458 b	0.63 a	0.07 b
Biochar 5.0%	768 a	580 b	0.52a	0.13 ab
Biochar 7.5%	686 a	610 b	0.48 a	0.18 a
P value	0.113	0.001	0.288	0.019
CV (%)	15.46	15.12	18.81	23.87

Means followed by different letters in the same row differ at 5% through Tukey test.

No statistical difference was observed for volatile fatty acids (VFAs) concentration for substrate material which showed values ranging from 686 to 969 mg L⁻¹, but for digestate material significant difference was detected in which control treatment had higher values as compared to the other treatments as seen on Table 11.

Treatments that had inclusions of biochar displayed a reduction of volatile fatty acids concentration by the end of the trial, while control treatment increased. The lower values for 2.5, 5.0 and 7.5% of biochar might be explained by the fact that higher inclusion of biochar contributed to a high capacity of the overall buffering system condition which allowed the VFAs not be accumulated in the system and be rapid converted into biogas.

Same statistical behaviour was noticed to VFA:PA ratio regarding substrate with no significant difference (Table 11), but for digestate, higher values was observed in reactors fed 7.5% of biochar, which was similar to treatment control and 5.0 % of biochar. This parameter is very important since it indicates how well-balanced are the metabolization and uptake of intermediates compounds generated during the AD degradation. All treatments regarding substrate material showed VFA:PA (mg L⁻¹) values higher than what is recommended for AD process of up to 0.4 VFA:PA (mg L⁻¹). However, after 86 days of fermentative process digestate values were in the range of 0.07 to 0.18 VFA: PA (mg L⁻¹), which is an indicative of process stability. According to Zhang et al., (2014), VFA: alkalinity ratios is a suitable tool to estimate the stability of the fermentation system.

10.2. Solids reduction and Biogas yield

Table 12 displays summarized mean values for total solids (TS), volatile solids (VS) and carbon: nitrogen (C: N) ratio of substrates and digestates.

Table 12. Mean values for total solids (TS) and volatile solids (VS) reduction, and carbon: nitrogen (C: N) ratio of substrate and digestate from batch anaerobic reactors feed laying hen manure different inclusion of biochar

Treatments	TS	VS	C: N ratio	
	Reduction (%)		Substrate	Digestate
Control	32.88 b	55.50 a	12.20 b	5.29 a
Biochar 2.5%	43.86 a	57.40 a	15.70 a	5.62 a
Biochar 5.0%	38.77 ab	54.07 a	15.53 a	6.07 a
Biochar 7.5%	45.20 a	55.02 a	14.70 a	5.68 a
P value	0.002	0.576	0.001	0.231
CV (%)	6.61	5.20	5.01	7.30

Means followed by different letters in the same row differ at 5% through Tukey test.

Statistical difference was detected for TS reduction, as seen on Table 12, while no significant difference was revealed for volatile solids reductions, which had values ranging from 54.07 to 57.40%, respectively. In terms of TS, high reduction values occurred on treatment that had 7.5% inclusion of biochar being higher than control treatment and statistically similar to treatments that had inclusions of 2.5 and 5.0%.

The volatile fraction is all components present on substrate that are efficiently used by fermentative microorganism to be converted into biogas. Higher the reductions values, higher are expected the biogas production, while pathogenic microorganisms are greatly reduced as demonstrated by Farias et al., (2012). The same authors achieved total and volatile solids reduction of 46.88 and 59.88%, respectively, in the fermentative process of old hen manure digested in anaerobic batch reactor with TS load of about 4%. Usually, lower amounts of TS for feeding the reactor are recommended since it may mitigate toxic effect by total ammonia nitrogen, which emphatically reduces biogas production.

Carbon: nitrogen ratio of substrate revealed effect of treatment in which lower values for C: N ratio was observed on treatment control as seen in Table 12. Despite of differences in the amount of biochar added for the other three treatments, they revealed to be statistically similar with values in the range of 14.70 to 15.70.

Only substrates of treatments which had biochar inclusion had values for C: N ratio in the range of 13 to 28: 1, recommended by Niu et al., (2014). According to these authors, despite of ideal C: N range be 20 to 30: 1, AD process can successfully be conducted in C: N ration varying from 13 to 28: 1.

No statistical difference was observed for C: N ratio on digestate which was extremely low with values ranging from 5.29 to 6.07. Substrate of control treatment and for all digestate material, C: N ratio was low as compared to optimum values for the AD process. However, according to Sgorlon et al., (2011) after fermentative process, digestate should have around 10: 1 ration to be considered a stabilized material, which usually has a minimum microbial activity.

Table 13 summarizes biogas yield per kg of waste, total solids $_{in}$, volatile solids $_{in}$ and of volatile solids $_{re}$ ($m^3.kg^{-1}$) of batch anaerobic reactors fed laying hen manure with different inclusions of biochar.

Table 13. Mean values for biogas yield per kg of waste ($m^3.kg^{-1}$), biogas yield per kg of TS $_{in}$ ($m^3.kg^{-1}$), biogas yield per kg of VS $_{in}$ ($m^3.kg^{-1}$) and biogas yield per kg of VS $_{re}$ ($m^3.kg^{-1}$) of substrate and digestate from batch anaerobic reactors feed laying hen manure with different inclusions of biochar

Treatments	Biogas yield	Biogas yield	Biogas yield	Biogas yield
	per kg of waste	per kg of TS $_{in}$	per kg of VS $_{in}$	per kg of VS $_{re}$
	$m^3.kg^{-1}$			
Control	0.025 a	0.134 a	0.203 a	0.366 a
Biochar 2.5%	0.015 b	0.064 b	0.101 b	0.177 b
Biochar 5.0%	0.009 c	0.035 c	0.052 c	0.096 c
Biochar 7.5%	0.006 c	0.022 c	0.035 c	0.064 c
P value	0.001	0.001	0.001	0.001
CV (%)	13.42	13.58	14.16	16.33

Means followed by different letters in the same row differ at 5% through Tukey test.

Higher values for biogas yield per kg of waste, TS $_{in}$, VS $_{in}$ and VS $_{re}$ ($m^3.kg^{-1}$) were attained by control treatment. Inclusions of 2.5% of biochar displayed better performance in terms of biogas yield as compared to the other inclusions, despite of being significantly lower than that of control.

Inclusions of 2.5, 5.0 and 7.5% of biochar in reactors fed diluted hen manure do not seem to be suitable since it lowered biogas yield in terms of TS and VS $_{in}$ ($m^3.kg^{-1}$).

It is clear that as biochar inclusion increases, biogas production decreases and this effect might be due to the proportions of each component used to feed the reactor.

In terms of biogas yield per kg of VS added, the achieved values on this (table 13) trial was quite similar as compared to some equal experiment conditions results found on literature by; Farias et al., (2012) of 0.21 m³/kg VS_{in} and Fantozzi & Buratti of 0.22 m³/kg VS_{in}, but lower than Zanato (2014) of 0.39 m³/kg VS_{in}. High pH of all digestate and improper C: N ratio, may have lead to kinetic instabilities, affecting the biogas yield throughout the assay.

Regarding biogas yield per kg of VS removed, inclusions of 2.5% of biochar decreased biogas yield to about 51.6% while for the other treatments the magnitude of decrease in biogas yield were 73.8 and 82.5% when compared to control treatment. Literature reviews papers reports that biochar may be an alternative to increase biogas production since its absorption sites can provide proper environment conditions in terms of monitoring parameters for anaerobic microorganisms' growth, however, the findings on this research do not corroborate with that.

Figure 7 illustrates the daily biogas production of the batch anaerobic reactors fed laying hen manure with different inclusions of biochar.

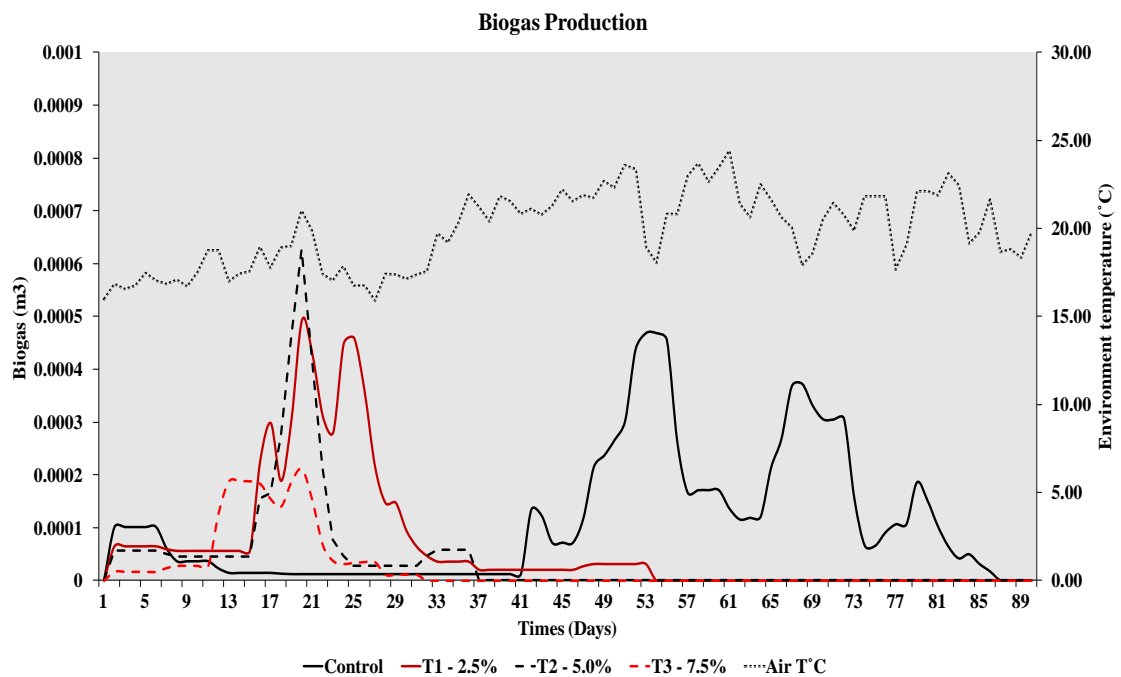


Figure 7. Daily biogas production from batch anaerobic reactors feed laying hen manure different inclusion of biochar

As seen in Figure 7, initial peak in the first days was not detected as it was expected, and that probably happened due to the high pH of all substrates used to feed

the reactors. It is known that in the beginning of fermentative process for biogas production there is higher activity of acetogenic microorganisms that converts complex molecules into precursors for biogas production. This class of microorganisms are known for better development and activity under a more acidified pH, and, since pH substrates were not corrected to neutral condition or below it, certain instability may have happened and lower fermentative action of this microorganisms' group took place at that time, which certainly, influenced the kinetics of overall metabolization of substrate, lowering the conversion rate of precursors into biogas.

In spite of adversities, reactors fed hen manure with different inclusions of biochar displayed an unexpected and interesting behaviour along the experimental period. As seen in the Figure 7, inclusions of biochar in substrate comprised by hen manure diluted in water anticipated the biogas production, which slowly started on the first week, peaked around the 20 to 25th days, ceasing its production around the 36th day. On the other hand, control treatment, which displayed a discreet first peak of biogas in the first week, only started to produce expressive amounts of biogas on the 41th day, with a peak of biogas production around the 53th day and another in the 66th day, lowering then its production up to the end.

In the Figure 8 it is illustrated the development and cumulative biogas production of batch anaerobic reactors feed laying hen manure different inclusion of biochar.

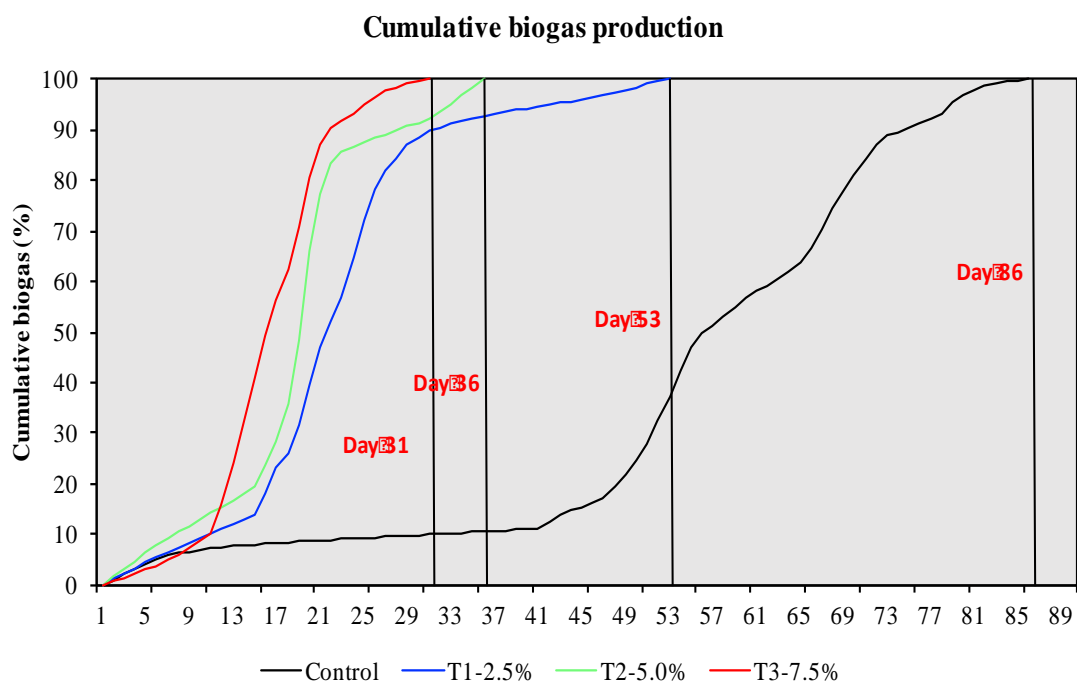


Figure 8. Cumulative biogas production from batch anaerobic reactors feed laying hen manure different inclusion of biochar

The fast start-up on biogas production noticed on digesters fed with inclusions of biochar as seen in Figure 7 and 8, might be due to the fact that a better C: N ratio at the beginning of the process meet the minimum metabolic requirements of fermentative microorganisms contributing to an early metabolization of organic matter and generation of volatile fatty acids for biogas production.

It is seen on Figure 7 that when biochar is not added into the substrate for the fermentative process in anaerobic reactors, biogas production displays a very slow start with low cumulative biogas in the first 41 days, after which, its productions peaks in up to the 86th.

C: N ratio has great influence in overall biogas production since it influences directly how organic matter is used by microorganism. Despite of lower biogas production as compared to control the use of 2.5% of biochar may be an option in systems to achieve early biogas production. However, besides an early start of biogas production, biogas plants look for a longer perpetuation of its production at high level, and taking it into account further research may be done using inoculum, since it not only improves biogas production as demonstrated by Steil (2001) and Xavier & Junior (2010) but also offers better conditions regarding monitoring parameters for microorganism development and therefore AD process can be carried under conditions that favors biogas production at it high level.

The findings on this research indicates that inclusions (at 2.5, 5.0, and 7.0% by weight) of biochar in substrate formed by laying hen manure diluted in water at 7% TS under a hydraulic retention time of 86 days did not contribute to the overall biogas yield.

10.3. Digestate quality

Figure 9 illustrates digestate mean values for pH, total ammonia nitrogen expressed as mg L^{-1} and electric conductivity in $\mu\text{S/cm}$.

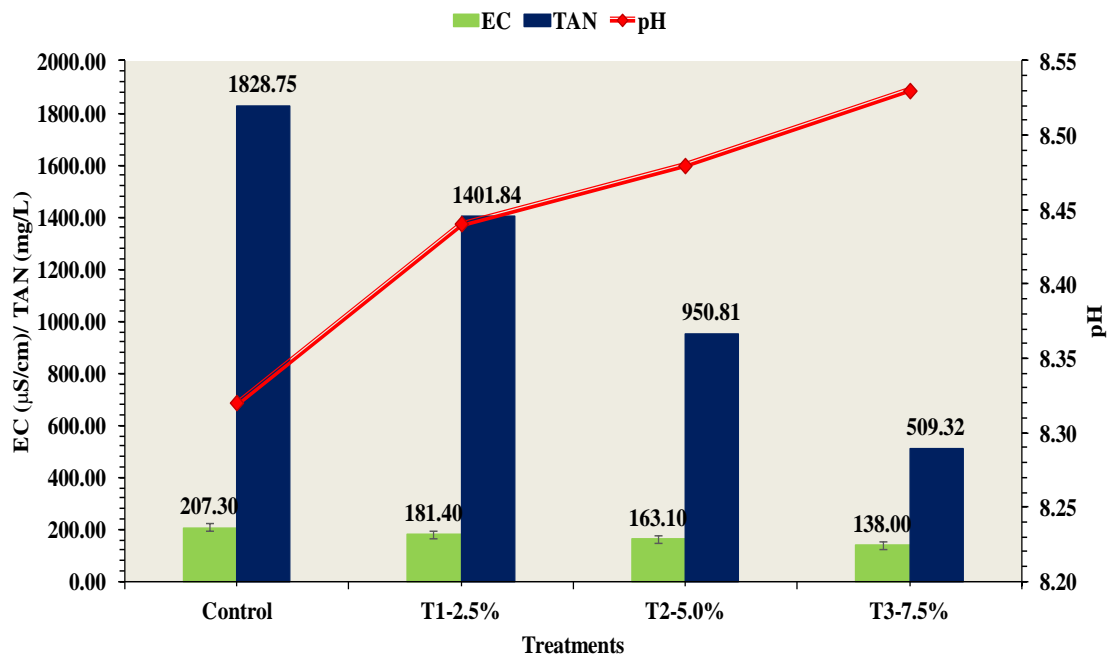


Figure 9. pH, electric conductivity (EC) and total ammonia nitrogen (TAN) values for digestate material from batch anaerobic reactor fed laying hen manure diluted in water with different inclusions of biochar.

Digestate's pH values were in the range of 8.3 to 8.5 which are relatively alkaline. High values detected in digestate might be due to high formation of ammonia bicarbonate, naturally produced while rich protein substrate is broken down by fermentative microorganisms.

In most of Brazil, with very few exceptions, soil is highly acidic due to great aluminum' soil concentration (Meda et al., 2001; Caires et al., 2008; Buol et al., 2009; Pértile et al., 2012), which is a toxic component for plants growth. Taking into account that most vegetal cultures requires ideal pH around neutral condition, digestate with high pH may improve soil's alkalinity increasing soil's pH, and thus, important nutrient may become available in reasonable amount for plants growth.

Regarding electric conductivity, it was not revealed statistical difference for all substrates ($P>0.05$), however, higher values for EC was detected in the digestate for control treatment ($P<0.05$).

EC is a parameter that indicates the presence of cations and anions, usually, higher the EC, richer in nutrients digestate is (USDA, 2011). However, despite of high

EC be associate to high levels of nutrients such as phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), and cooper (Cu) (USDA, 2011), it does not indicate the predominance of a nutrient or which is more available in digestate, but EC may be indeed used as an important indicator of nutrients for plants in the digestate.

It should be noticed that high EC values may jeopardize some plants' physiological process since some vegetal cultures are very sensitive to high conductivity values. Corn, for example has moderate tolerance to salinity, and EC higher than 4 ds/m⁻¹ can strongly affect corn's crops yield (USDA, 2011). High amount of salinity might lead ionic toxicity due to its accumulation on vegetal tissue (Nivas et al., 2011).

Besides that, high EC may affect kinetic of ammonia nitrogen conversion into nitrite, which is the main path for plants' nitrogen absorption (Maçãs, 2008). Other than that, improper osmotic balance due high salinity can affect not only the nitrogen's uptake by plants but also, phosphorous and others macro and micro-nutrients essentially important for many plants' metabolic functions.

Higher values of EC for treatment control might be by the fact that control's treatment had the highest percentage of hen manure diluted into water, while the other treatments had a linear decrease of manure inclusion, which lowered EC. In formulated laying hens diet there is a reasonable amount of components such as dicalcium phosphate (CaHPO₄), sodium chloride (NaCl), limestone, which has calcium carbonate (CaCO₃), and calcium (Ca), that may justify high values for EC detected, specially in control's treatment. Since manure is a junction of feces, feathers, peeled skin, yolk, egg shell, ration, water and others, manure can get very rich in elements that increases its salinity.

As observed in Figure 9, higher values for TAN were achieved in the control, and a linear decrease was seen as biochar inclusion was increased. High values of TAN observed in the control and also for 2.5% of biochar inclusion indicate greater amount of N source for soil and crops.

Table 15 summarizes mean values of Total nitrogen, phosphorous and calcium concentration of substrate and digestate from batch anaerobic reactors fed laying hen manure with different inclusions of biochar.

Table 15. Mean values for Total nitrogen (TN), phosphorous (TP), calcium and total carbon concentration of digestate material from Anaerobic reactor feed laying hen manure diluted in water with different inclusions of biochar (values expressed in g/100g)

Treatments	Total nitrogen		Total phosphorous		Calcium	
	Substrate	Digestate	Substrate	Digestate	Substrate	Digestate
Control	3.01a	4.60 a	4.71b	7.11 a	1.19 a	1.72 a
Biochar 2.5%	2.24b	4.76 a	5.82a	7.22 a	1.39 a	1.50 a
Biochar 5.0%	2.38b	4.57 a	4.48 b	6.98 a	1.30 a	1.35 a
Biochar 7.5%	2.31b	4.93 a	4.87 b	5.77 b	1.47 a	1.52 a
P value	0.001	0.613	0.006	0.011	0.579	0.246
CV (%)	5.65	7.69	6.52	6.41	18.60	13.25

Means followed by different letters in the same row differ at 5% through Tukey test.

In terms of total nitrogen, statistical difference was revealed among substrates ($P < 0.05$) with high values of TN for control. The high TN observed in the control is probably due to high percentage of manure on that treatment, which contributed to a high Total-N. No statistical difference was detected for digestate material which had values in the range of 4.57 to 4.93% on DM basis, as seen in table 15.

The average amount of TN on this assay for substrate of 2.48% was lower than nitrogen concentrations found by Farias et al., (2012) of 2.58%. Achieved values on this assay for digestate TN was higher as compared to findings from Carvalho (2015) whose TN average was 2.11% on digestate material in the anaerobic digestion of laying hen manure using continuous anaerobic reactors.

Average Total-N for digestate is close to mean values of TN on laying hens' manure attained by Amanullah et al., (2010) in the range of 5.8 to 7.6% (DM basis). It should be noticed that the use of fresh raw manure in crop fields may lead to caustic effect due to rapid availability of nitrogen, and thus previous treatment are indeed needed to attain a more stable material. According to Sedyama et al., (2008) non-stable organic fertilizer can inhibit seeds germination and root growth, besides of offering sanitary risk to the vegetal culture and soil due to pathogenic microorganisms.

Higher concentration of TP regarding substrate was detected for treatment that had 2.5% of biochar inclusions while no difference was revealed among the other treatments, with values ranging from 4.48 to 4.87% on DM basis. Regarding digestate,

lower concentration of Total-P was seen for treatment that had 7.5% on biochar inclusion as seen on Table 9. When expressed in P₂O₅ substrate and digestate had global average values of 11.38 and 15.50% on DM basis, respectively.

No statistical difference was seen for substrate and digestate for all treatments regarding calcium concentrations that had values in the range of 1.19 to 1.47 and 1.35 to 1.72%, respectively. High values of Ca concentration on substrate is due to its inclusion on hens' diet which is indeed important for bone calcification, egg shell formation and other equally important metabolic functions. As previously mentioned, manure is a junction of feces, feathers, concentrate ration, egg shell and others, all these components can increase the quantity of different macro and micro-nutrients on manure, including calcium.

Increased concentrations on nitrogen, phosphorous, potassium and calcium detected on digested as compared to substrate material are due to the concentration of them along the 86 days of hydraulic retention time while organic fraction was broken down by fermentative microorganisms. Since the AD process using hen laying manure diluted in water with addition of biochar did not display a persistent biogas along the 86th days, lower fermentative activity on organic matter probably influenced a less accumulative content of nitrogen, phosphorous, potassium and calcium on final digestate material. Longer hydraulic retention time and even lower inclusions of biochar, up to 2.5% may improve the performance of the AD process and therefore better results in terms of macronutrients might be achieved.

11. CONCLUSION

Inclusions of biochar at 2.5, 5.0 and 7.5% by weight in substrate formed by laying hen manure diluted in water at 7% total solids with a hydraulic retention time of 86 days contributed to ideal concentrations of total ammonia nitrogen, volatile fatty acids and electric conductivity.

Biogas yield from anaerobic batch reactors fed different inclusions of biochar displayed a faster start-up of biogas production; however, its inclusions at 2.5, 5.0 and 7.5% decreased overall biogas yield.

Regarding digestate quality, biochar did not improve digestate quality in terms of total nitrogen, phosphorous and calcium concentration.

12. FINAL CONSIDERATIONS AND SUGGESTIONS

Based on findings on this research we can provide some suggestions for further research to improve the anaerobic digestion process of hen manure diluted in water with inclusions of biochar:

1. Inclusions of biochar in anaerobic reactors operated with hen manure should be done at a maximum level of 2.5%, as higher levels greatly decrease biogas yield.
2. When detected high pH of manure and biochar is detected, and also in the reactor's feeding substrate, we suggest an acidification in order to bring down pH to values around 6.8 to 7.3.
3. To allow a better start-up in terms of biogas production and probably a higher biogas yield by the end of the fermentative process, we suggest utilization of inoculum. It not only promotes a fast start of biogas production but also provided to the substrate a population of fermentative and methanogenic microorganisms already established, thus, it can improve the AD process along the entire fermentative process.
4. For better understanding of the daily effect of biochar on the anaerobic digestion process of laying hen manure, it is suggested to conduct experiments under semi-continuous and continuous feeding systems. In that way, it is possible to better understand the dynamics of each monitoring parameter and its influence on the anaerobic digestion process.
5. An economical analysis considering total biogas production, the amount of biogas yield per kilogram of volatile solids added and removed, and the current methane and biochar's commercial prices should be taken into account in order to determine the suitability of using biochar as an additive in anaerobic digestion.

13. REFERENCES

- ADEKUNLE, K. F. and OKOLIE, J. A. A review of biochemical process of anaerobic digestion. **Advances in Bioscience and Biotechnology**, v.6, p.205- 222, 2015
- AL SEADI T, RUTZ D, PRASSL H, KÖTTNER M, FINSTERWALDER T, VOLK S. More about anaerobic digestion (AD). **In: Biogas handbook**. University of Southern Denmark, Esbjerg, 2008.
- AMANULLAH, M. M., SEKAR, S., & MUTHUKRISHNAN, P. Prospects and potential of hen manure. **Asian Journal of Plant Sciences**, v.9, n.4, p.172-182, 2010.
- AMARAL, G. F., GUIMARÃES, D. D., NASCIMENTO, J. C. D. O. F., & CUSTODIO, S. **Avicultura de postura: estrutura da cadeia produtiva, panorama do setor no Brasil e no mundo e o apoio do BNDES**. BNDES Setorial, Rio de Janeiro. 2016. 43p.
- ANGELIDAKI, I.; AHRING, B. K. Thermophilic anaerobic digestion of livestock waste: the effect of ammonia **Applied Microbiology Biotechnology**, v. 38, n. 3, p.560-564, 1993.
- ANGELIDAKI, I.; KARAKASHEV, D.; BATSTONE, D. J.; PLUGGE, C. M.; & STAMS, A. J. M. Biomethanation and Its Potential. **Methods in Enzymology**, p.327–351, 2011.
- APHA. AWWA. WPCF. 2017. Standard methods for the examination of water and wastewater. 23rd ed. Washington. 1504p.
- ARAGAW, T; ANDARGIE, M; GESSESSE, A. Co-digestion of cattle manure with organic kitchen waste to increase biogas production using rumen fluid as inoculums. **International Journal of Physical Science**, v.8, n.11, p.443-450, 2013.
- ARRUDA, L. D. O.; XAVIER, C. A. N.; TREVIZAN, P. S. F.; SOUZA, J. E. Nitrogênio amoniacal em substratos de biodigestores contendo dejetos de aves poedeiras em diferentes diluições. **In: X Congresso Latinoamericano y del Caribe de Ingenieria Agricola – CLIA**, julho de 2012 - londrina - PR, Brasil.
- BAYRAKDAR, A., SÜRMELE, R. Ö., & ÇALLI, B. Dry anaerobic digestion of chicken manure coupled with membrane separation of ammonia. **Bioresource Technology**, v.244, p.816-823, 2017.
- BENINCASA, M.; ORTOLANI, A.F.; JUNIOR, J.L. **Biodigestores convencionais?** Jaboticabal: FCAV, UNESP, 1991. 25p.

- BJPAI P., Anaerobic Technology in Pulp and Paper Industry, Basics of Anaerobic Digestion Process Springer Briefs in **Applied Sciences and Technology**, 2017. 7p.
- BOLAN, N. S.; SZOGI, A. A.; CHUASAVATHI, T.; SESHADRI, B.; ROTHROCK, M.J.; PANNEERSELVAM, P. Uses and management of hen litter. **Hen Science Journal**, v.66, p.673–698, 2010.
- BRAUN, R. **Anaerobic digestion: a multi-faceted process for energy, environmental management and rural development**. In P. Ranalli (ed.), Improvement of Crop Plants for Industrial End Uses, p.335–416. 2007.
- BRUNI, E.; JENSEN, A. P.; ANGELIDAKI, I. Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. **Bioresource technology**, v.101, n.22. p.8713-8720, 2010.
- BUOL, S. W. Soils and agriculture in central-west and north Brazil. **Scientia Agricola**, n.66, n.5, p.697-707, 2009
- BURTON, C. H., & TURNER, C. **Manure management: Treatment strategies for sustainable agriculture**. Editions Quae, 2003.
- CAIRES, E. F., BARTH, G., GARBUIO, F. J., & CHURKA, S. Soil acidity, liming and soybean performance under no-till. **Scientia Agricola**, v.65, n.5, p-532-540, 2008.
- CARMO, D. L & SILVA, C. A. Métodos de quantificação de carbono e matéria orgânica em resíduos orgânicos. **Revista Brasileira de Ciência do Solo**, v. 36, n.4, p.1211-1220, 2012.
- CARVALHO, A. M. X.; MENDES, F.Q. SPEED Stat: a minimalist and intuitive spreadsheet program for classical experimental statistics. Lavras, MG: UFLA: 2017, 333p.
- CARVALHO, K. C. N. **Caracterização quali-quantitativa e biodigestão anaeróbia de dejetos de poedeiras comerciais alimentadas com rações contendo duas fontes de minerais e níveis de óleo de alecrim**. 58f. Dissertação (Mestrado em Zootecnia). Universidade Estadual de Mato Grosso do Sul, Aquidauana, - MS, 2015.
- ČATER, M., ZOREC, M., & LOGAR, R. M. Methods for Improving Anaerobic Lignocellulosic Substrates Degradation for Enhanced Biogas Production. **Springer Science Reviews**, v.2, n.1-2, p.51-61, 2014.
- CHEN, Y., CHENG, J.J., CREAMER, K.S. Inhibition of anaerobic digestion process: a review. **Bioresource technology**, n. 99, v.10, p.4044-4064, 2008.

- CHEN, H., WANG, W., XUE, L., CHEN, C., LIU, G., & ZHANG, R. Effects of ammonia on anaerobic digestion of food waste: process performance and microbial community. **Energy & Fuels**, v.30, n.7, p.5749-5757, 2016.
- CHERNICHARO, C.A.L. **Reatores anaeróbios: princípios do tratamento biológico de águas residuárias**. 2. Ed. Belo Horizonte, UFMG, 1997. p.379
- DEEM, L. M.; CROW, S. E. **Biochar**. Reference Module in Earth Systems and Environmental Sciences, 2017.5p.
- DEMIRER, G. N.; CHEN, S. Effect of retention time and organic loading rate on anaerobic acidification and biogasification of dairy manure. **Journal of Chemical Technology and Biotechnology**, Oxford, v. 79, p 1381-1387, 2004.
- DESAI, M., MADAMWAR, D. Anaerobic digestion of a mixture of cheese whey, hen waste and cattle dung: a study of the use of adsorbents to improve digester performance. **Environmental Pollution**, v.86, p. 337–340, 1994.
- DING, Y., LIU, Y., LIU, S., LI, Z., TAN, X., HUANG, X & ZHENG, B. Biochar to improve soil fertility. A review. **Agronomy for sustainable development**, v.36, n.2, p.36, 2016.
- ENNOURI, H., MILADI, B., DIAZ, S. Z., GÜELFO, L. A. F., SOLERA, R., HAMDI, M., & BOUALLAGUI, H. Effect of thermal pre-treatment on the biogas production and microbial communities balance during anaerobic digestion of urban and industrial waste activated sludge. **Bioresource technology**, 214, 184-191, 2016.
- FAGBOHUNGBE, M. O., HERBERT, B. M., HURST, L., IBETO, C. N., LI, H., USMANI, S. Q., & SEMPLE, K. T. The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. **Waste Management**. 2016.
- FANTOZZI, F., & BURATTI, C. Biogas production from different substrates in an experimental continuously stirred tank reactor anaerobic digester. **Bioresource technology**, v.100, n.23, p-5783-5789, 2009.
- FARIAS, R. M.; ORRICO JUNIOR, A. P.; ORRICO, A. C. A.; GARCIA, R. G.; CENTURION, S. R.; FERNANDES, A. R. M. Biodigestão anaeróbia de dejetos de poedeiras coletados após diferentes períodos de acúmulo. **Ciência Rural**, Santa Maria, v.42, n.6, p.1089-1094, 2012.
- FARROW, C., CROLLA, A., KINSLEY, C., & MCBEAN, E. Anaerobic digestion of hen manure: Process optimization employing struvite precipitation and novel digestion technologies. **Environmental Progress & Sustainable Energy**, v.36, n.1, p-73-82, 2017.

- GERARDI, M. H. The microbiology of anaerobic digesters. first ed., Wiley-Interscience. 2003.
- GUO, M., UCHIMIYA, S. M., & HE, Z. **Agricultural and environmental applications of biochar: Advances and barriers**. Soil Science Society of America, Inc., 2016. 504p.
- HANSEN, K. H., ANGELIDAKI, I., AND AHRING, B. K. Anaerobic digestion of swine manure: inhibition by ammonia. **Water research**, v. 32, n.1, p.5-12, 1998.
- HANSEN, K.H., ANGELIDAKI, I., AHRING, B. K. Improving thermophilic anaerobic digestion of swine manure. **Water research**, v. 33, n.8, p.1805-1810, 1999.
- HE, X., YIN, H., SUN, X., HAN, L., & HUANG, G. Effect of different particle-size biochar on methane emissions during pig manure/wheat straw aerobic composting: Insights into pore characterization and microbial mechanisms. **Bioresource technology**, v.268, p.633-637, 2018.
- HE, X., YIN, H., HAN, L., CUI, R., FANG, C., & HUANG, G. Effects of biochar size and type on gaseous emissions during pig manure/wheat straw aerobic composting: Insights into multivariate-microscale characterization and microbial mechanism. **Bioresource technology**, v.271, p.375-382, 2019.
- HOLM-NIELSEN, J. B., AL SEADI, T. AND OLESKOWICZ-POPIEL, P. The future of anaerobic digestion and biogas utilization. **Bioresource Technology**, v.100, p.5478- 5484, 2009.
- HO, L., HO, G. Mitigating ammonia inhibition of thermophilic anaerobic treatment of digested piggery wastewater: use of pH reduction, zeolite, biomass and humic acid. **Water Resource**, v.46, p.4339-4350, 2012.
- JAIN, M. S., PAUL, S., & KALAMDHAD, A. S. Utilization of Biochar as an amendment during lignocellulose waste composting: Impact on composting physics and Realization (probability) amongst physical properties. **Process Safety and Environmental Protection**, v.121, p.229-238, 2019.
- JENKINS, S. R.; MORGAN, J. M.; SAWYER, C. L. Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration. **Journal Water Pollution Control Federation**, v.55, n.5, p.448-453, 1983.
- JIMÉNEZ, E.I. & GARCIA, V.P. Relationships between organic carbon and total organic matter in municipal solid wastes and city refuse composts. **Bioresource Technology**, v.41, p-265-272, 1992.
- KAMMANN, C., IPPOLITO, J., HAGEMANN, N., BORCHARD, N., CAYUELA, M. L., ESTAVILLO, J. M., & RASSE, D. Biochar as a tool to reduce the agricultural

- greenhouse-gas burden—knowns, unknowns and future research needs. **Journal of Environmental Engineering and Landscape Management**, v.25, n.2, p.114-139, 2017.
- KHALID A., ARSHAD, M., ANJUM, M., MAHMOOD, T., AND DAWSON, L. The anaerobic digestion of solid organic waste. **Waste Management**, v. 31, n. 8, p. 1717-1744, 2011.
- KIM, M., GOMEZ, C. Y., AHN, Y., & SPEECE, R. E. Hydrolysis and acidogenesis of particulate organic material in mesophilic and thermophilic anaerobic digestion. **Environmental technology**, v.24, n. 9, p. 1183-1190, 2003.
- KOSTER, I. W., & LETTINGA, G. Anaerobic digestion at extreme ammonia concentrations. **Biological Wastes**, v.25, n.1, p.51-59, 1988.
- KOVÁCS, E., WIRTH, R., MARÓTI, G., BAGI, Z., NAGY, K., MINÁROVITS, J., & KOVÁCS, K. L. Augmented biogas production from protein-rich substrates and associated metagenomic changes. **Bioresource technology**, v.178, p.254-261, 2015.
- KUMAR, S., JAIN, M.C., CHHONKAR, P.K. A note on stimulation of biogas production from cattle dung by addition of charcoal. **Biological Wastes**, v.20, p.209–215, 1987.
- LABATUT, R. A., ANGENENT, L. T., & SCOTT, N. R. Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance and stability. **Water research** v. 53, p.249-258, 2014.
- LAHAV, O., & MORGAN, B. E. Titration methodologies for monitoring of anaerobic digestion in developing countries - a review. **Journal of Chemical Technology & Biotechnology**, v.79, n.12, p.1331-1341, 2004.
- LI, Y., ZHANG, R., CHEN, C., LIU, G., HE, Y., LIU, X. Biogas production from co-digestion of corn stover and chicken manure under anaerobic wet, hemi-solid, and solid state conditions. **Bioresouce. Technology** v.149, p.406–412, 2013
- LIU, T., SANG, S. Ammonia inhibition on thermophilic acetoclastic methanogens. **Water Science Technology**, v.45, p.113–120, 2002
- MAO, C., FENG, Y., WANG, X., & REN, G. Review on research achievements of biogas from anaerobic digestion. **Renewable and Sustainable Energy Reviews**, v.45, p.540-555, 2015.
- MAÇÃS, J. Nitrogênio nítrico e amoniacal no desenvolvimento da parte aérea de milho cultivado em Argissolo. 2008. 59 f (Doctoral dissertation, Dissertação

- (Mestrado em Ciência do Solo) Universidade Federal do Rio Grande do Sul. Porto Alegre, RS, 2008.
- MASSÉ, D. I., RAJAGOPAL, R., & SINGH, G. Technical and operational feasibility of psychrophilic anaerobic digestion biotechnology for processing ammonia-rich waste. **Applied Energy**, v.120, p.49-55, 2014.
- MEDA, A. R., CASSIOLATO, M. E., PAVAN, M. A., & MIYAZAWA, M. Alleviating soil acidity through plant organic compounds. **Brazilian Archives of Biology and Technology**, v.44, n.2, p.185-189, 2001.
- MENESES, S. L. D. **Cana-de-açúcar e silagem de cana em codigestão com esterco bovino na produção de biogás**.101f. Tese (Doutorado em Zootecnia), Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal - SP, 2011.
- MOLAEY, R., BAYRAKDAR, A., SÜRMELE, R. Ö., & ÇALLI, B. Anaerobic digestion of chicken manure: Mitigating process inhibition at high ammonia concentrations by selenium supplementation. **Biomass and Bioenergy**, v.108, p.439-446, 2018.
- MONTALVO, S., DÍAZ, F., GUERRERO, L., SÁNCHEZ, E., BORJA, R. Effect of particle size and doses of zeolite addition on anaerobic digestion processes of synthetic and piggery wastes. **Process Biochemistry**, v.40, n.3, p.1475–1481, 2005.
- MUMME J, SROCKE F, HEEG K, WERNER M. Use of biochars in anaerobic digestion. **Bioresource Technology**, v.164, p.189–197, 2014.
- NÓBREGA, Í. P. C. **Efeitos do biochar nas propriedades físicas e químicas do solo: sequestro de carbono no solo**. 38p. Dissertação de Mestrado (Mestrado em Engenharia Ambiental), Universidade Técnica de Lisboa, 2011.
- NORDELL, E., HANSSON, A.B., AND KARLSSON, M. Zeolites relieves inhibitory stress from high concentrations of long chain fatty acids. **Waste Management**, v.33, p.2659–2663, 2013.
- NISHIMURA, R., KOLTERMANN, P. I., PEREIRA, V. M., ORTEGA, J. M., SOUZA, K. C. G., & DOTTO, L. F. Avaliação da qualidade da energia elétrica produzida por grupo gerador movido a biogás. In: **Industry Applications (INDUSCON), 2010 9th IEEE/IAS International Conference on**. IEEE, 2010. p. 1-6.
- NKOA, R. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. **Agronomy for Sustainable Development**, v. 34, n. 2, p.473-492, 2014.

- NIU, Q., HOJO, T., QIAO, W., QIANG, H., & LI, Y. Y. Characterization of methanogenesis, acidogenesis and hydrolysis in thermophilic methane fermentation of chicken manure. **Chemical Engineering Journal**, v. 244, p.587-596, 2014.
- NIVAS, D., GAIKWAD, D. K., & CHAVAN, P. D. Physiological responses of two *Morinda* species under saline conditions. **American Journal of Plant Physiology**, n.6, v.3, p.157-166, 2011.
- PANICHNUMSIN, P.; NOPHARATANA, A.; AHRING, B.; CHAIPRASERT, P. Enhanced biomethanation in co-digestion of Cassava pulp and pig manure using a two-phase anaerobic system. **Journal of Sustainable Energy & Environment**, v.3, n. 2, p.73-79, 2012.
- PÉRTILE, P., ALBUQUERQUE, J. A., GATIBONI, L. C., COSTA, A. D., & WARMLING, M. I. Application of alkaline waste from pulp industry to acid soil with pine. **Revista Brasileira de Ciência do Solo**, v.36, n.3, p.939-950, 2012.
- POLPRASERT, C. Organic waste recycling: technology and management. IWA publishing, 2017.509p.
- PRIMIANO, I. P. **Biodigestão anaeróbia de dejetos da avicultura de postura: uso de inóculo em biodigestores batelada**. 2002. 51f. Trabalho de Conclusão de Curso (Graduação em Agronomia) - Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista. Jaboticabal, 2002.
- PROVENZANO, M. R.; IANNUZI, G.; FABBRI, C.; SENESI, N. Qualitative characterization and differentiation of digestates from different biowastes using FTIR and fluorescence spectroscopies. **Journal of Environmental Protection**, v. 2, p.83–89, 2011.
- RAJAGOPAL, R., MASSÉ, D. I., & SINGH, G. A critical review on inhibition of anaerobic digestion process by excess ammonia. **Bioresource Technology**, n.143, p.632-641, 2013.
- RAVINDRAN, B., NGUYEN, D. D., CHAUDHARY, D. K., CHANG, S. W., KIM, J., LEE, S. R., ... & LEE, J. Influence of biochar on physico-chemical and microbial community during swine manure composting process. **Journal of environmental management**, v.232, p.592-599, 2019.
- RIPLEY, L. E.; BOYLE, W. C.; CONVERSE, J. C. Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. **Journal Water Pollution Control Federation**, v.58, n.5, p.406-411, 1986.

- SAKAR, S., YETILMEZSOY, K., & KOCAK, E. Anaerobic digestion technology in hen and livestock waste treatment—a literature review. **Waste Management and Research**, v.27, n.1, p.3-18, 2009.
- SAWATDEENARUNAT, C., SURENDRA, K. C., TAKARA, D., OECHSNER, H., & KHANAL, S. K. Anaerobic digestion of lingo cellulosic biomass: Challenges and opportunities. **Bioresource technology**, v.178, p.178-186, 2015.
- SEDIYAMA, M. A., VIDIGAL, S. M., PEDROSA, M. W., PINTO, C. L., & SALGADO, L. T. Fermentação de esterco de suínos para uso como adubo orgânico. Revista **Brasileira de Engenharia Agrícola e Ambiental**, v.12, n.6, p.638-645, 2008.
- SGORLON, J., RIZK, M. C., BERGAMASCO, R., & GRANHEN TAVARES, C. R. Avaliação da DQO e da relação C/N obtidas no tratamento anaeróbio de resíduos fruti-hortícolas. **Acta Scientiarum. Technology**, v.33, n.4, 2011.
- SILVA, A. F., PINTO, J. M., FRANÇA, C. R. R. S., FERNANDES, S. C., GOMES, T. D. A., DA SILVA, M. S. L., & ANB, M. Preparo e uso de biofertilizantes líquidos. Embrapa Semi-Árido. Comunicado técnico (2007). 4p.
- SILES, J. A., BREKELMANS, J., MARTIN, M. A., CHICA, A. F., & MARTIN, A. Impact of ammonia and sulphate concentration on thermophilic anaerobic digestion. **Bioresource technology**, v.101, n.23, p.9040-9048, 2010.
- SIMON, E. J.; BUENO, O. C. **Viabilidade da implantação de biodigestores como fonte alternativa de energia e de produção de compostos orgânicos em áreas de assentamentos rurais do estado de São Paulo**. 21 ed. Botucatu: UNESP, 2006. 50p.
- SOUZA, M. E. D. Fatores que influenciam a digestão anaeróbia. **Revista DAE**, v. 44, n. 137, p.88- 94, 1984.
- SPEECE R.E. Anaerobic biotechnology for industrial wastewater. Archaea Press, Nashville, TN, 1996.
- STEIL, L. **Avaliação do uso de inóculos na biodigestão anaeróbia de resíduos de aves de postura, frangos de corte e suínos**. 2001. 109f. Dissertação (Mestrado em Biotecnologia) - Instituto de Química, Universidade Estadual Paulista, Araraquara, 2001.
- SURENDRA, K. C.; TAKARA, D.; JASINSKI, J.; KUMAR KHANAL, S. Household anaerobic digester for bioenergy production in developing countries: opportunities and challenges. **Environmental technology**, v.34, p.1671-1689, 2013.

- TAUSEEF, S. M., PREMALATHA, M., ABBASI, T., & ABBASI, S. A. Methane capture from livestock manure. *Journal of environmental management*, v.117, p. 187-207. 2013.
- TOUSSAINT, R. **Anaerobic Digestion: Bioenergy and Biofertilizer Production for Haiti**. 135f. Doctoral dissertation (Master of Science), University of Florida. 2013.
- VAN LIER, J.B., MARTIN, J.L.S., LETTINGA, G. Effect of temperature on the anaerobic thermophilic conversion of volatile fatty acids by dispersed and granular sludge. **Water research**, n. 30, v.1, p.199-207, 1996.
- USDA – United States Department of Agriculture. Natural Resources Conservation Service Soils. **In: Soil Quality indicators**, 2011. Find in: <https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/health/assessment/?cid=steprdb1237387>. Accessed in: 30 December of 2018.
- XAVIER, C. A. N.; LUCAS JUNIOR, J. Parâmetros de dimensionamento para biodigestores batelada operados com dejetos de vacas leiteiras com e sem uso de inoculo. **Engenharia Agrícola**, v.30, n.2, p.212-223, 2010.
- WANG, X., LU, X., LI, F., & YANG, G. Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. **PloS one**, v.9, n. 5, e97265, 2014.
- YENIGÜN, O., DEMIREL, B. Ammonia inhibition in anaerobic digestion: a review. **Process Biochemistry**, n.48, p.901 - 911, 2013.
- YUN, Y. M., KIM, D. H., CHO, S. K., SHIN, H. S., JUNG, K. W., & KIM, H. W. Mitigation of ammonia inhibition by internal dilution in high-rate anaerobic digestion of food waste leachate and evidences of microbial community response. **Biotechnology and bioengineering**, v.113, n.9, p.1892-1901, 2016.
- ZANATO, J. A. F. **Produção e qualidade do biogás gerado com os dejetos de diferentes espécies animais**. 112f. Tese (Doutorado em Zootecnia), Universidade Estadual Paulista, Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal - SP, 2014.
- ZHENG, Y., ZHAO, J., XU, F., & LI, Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. **Progress in Energy and Combustion Science**, v.42, p.35-53, 2014.