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PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA CIVIL
(SANEAMENTO AMBIENTAL)

AMANDA DE SOUSA E SILVA

# STRATEGIES FOR ENHANCING THE SEMI-DRY AND DRY ANAEROBIC BIOMETANIZATION OF SWINE MANURE

FORTALEZA
2024

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Thesis presented to the Postgraduate Program in Civil Engineering at the Federal University of Ceará, as a partial requirement for obtaining the degree of Doctor in Civil Engineering. Concentration Area: Environmental Sanitation.

Advisor: Prof. André Bezerra dos Santos (Ph.D.)

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To my parents, Neide and Mário, to my husband Flávio and professor Erlon *in memory*.

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# RESUMO

A suinocultura é uma das atividades agrícolas mais importantes do mundo, sendo o Brasil o quarto maior produtor e exportador de suínos. Entretanto, esta atividade gera grandes volumes de resíduos, como os dejetos suínos (DS), com alta carga de matéria orgânica, nitrogênio, fósforo, metais pesados, patógenos e antibióticos, o que acarreta diversos problemas ambientais. Aliado a isso, a crise climática e energética requer a busca por fontes alternativas de energia. Neste contexto, a digestão anaeróbia (DA) surge como uma alternativa sustentável para converter resíduos em energia (biogás e metano). Portanto, este trabalho buscou avaliar estratégias para potencializar a biometanização anaeróbia de dejetos suínos, como pré-tratamento termoalcalino (3% NaOH a 121 ºC por 30 min), teor de sólidos totais - ST (10 e 15%), razão substrato/inóculo (SI) (1, 3 e 5 $g.SV_{substrato} / g.SV_{inóculo}$) e adição de carvão ativado granular – CAG (10, 20 e 30 g/L) como material condutor. Foram utilizadas análises de modelagem cinética, para melhor compreender a influência dos parâmetros avaliados e gerar coeficientes cinéticos importantes para otimização e escalonamento do processo; e ferramentas de biologia molecular, para compreender as diferentes populações microbianas envolvidas e as mudanças na dinâmica ecológica. Para isso, experimentos de Potencial Bioquímico de Metano (PBM) foram conduzidos em regime de batelada sob temperatura mesofílica e agitação orbital de 150 rpm durante 90 dias. Na primeira fase da pesquisa, o pré-tratamento aumentou a biodegradabilidade do dejeto, aumentando assim o rendimento acumulado de metano para as duas condições testadas (10% ST: de 30 para 205 mL $CH_4$/gSV e 15% ST: de 0 para 136 mL $CH_4$/gSV). Por outro lado, o aumento da razão SI para o dejeto pré-tratado mostrou-se viável apenas na condição semisseca (205, 268 e 187 mL $CH_4$/gSV para SI 1, 3 e 5, respectivamente), pois na seca só houve produção significativa de metano em SI 1 (136 mL $CH_4$/gSV). Na segunda fase, a adição de 20 g CAG/L promoveu o aumento do rendimento de metano de 3 para 154 e 155 mL $CH_4$/gSV com dejeto bruto e pré-tratado, respectivamente, na condição semisseca (10% ST). Portanto não houve diferença significativa ao realizar o pré-tratamento. Com relação ao estudo da dosagem de CAG para o dejeto pré-tratado na DA semisseca, a adição de 10 g CAG/L apresentou rendimento de 190 mL $CH_4$/gSV, valor 21% maior do que com 20 e 30 g CAG/L, embora a cinética tenha sido mais favorável para a concentração de 20 g/L, de acordo com o modelo Gompertz Modificado, que foi o que melhor descreveu o processo. Na DA seca (15% ST), a concentração ideal de CAG foi de 30 g CAG/L (157 mL $CH_4$/gSV).

**Palavras-Chave:** Digestão anaeróbia; biometanização; pré-tratamento; razão substrato inóculo; digestão semisseca; digestão seca; materiais condutores; carvão ativado granular.

# ABSTRACT

Pig farming is one of the most important agricultural activities in the world, with Brazil being the fourth largest producer and exporter of pigs. However, this activity generates large volumes of organic waste, such as swine manure (DS), with a high load of organic matter, nitrogen, phosphorus, heavy metals, pathogens, and antibiotics, which causes several environmental problems. In addition, a climate and energy crisis requires the search for alternative energy sources. In this context, anaerobic digestion (AD) emerges as a sustainable alternative to convert waste into energy (biogas and methane). Therefore, this work sought to evaluate strategies to enhance the anaerobic biometanization of swine manure, such as thermos-alkaline pretreatment (3% NaOH at 121 ºC for 30 min), total solids content – TS (10 and 15% TS), substrate/inoculum (SI) ratio (1, 3 and 5 $g.VS_{substrate}$ / $g.VS_{inoculum}$) and addition of granular activated carbon - GAC (10, 20 and 30 g/L) as conductive material, applying kinetic modeling analysis to understand the influence of the evaluated parameters better and generate important kinetic coefficients for optimization and process scaling, and molecular biology tools for a better comprehension of the different microbial population involved and ecology dynamics changes. For this, Biochemical Methane Potential (BMP) experiments were conducted in batch mode under mesophilic temperature and orbital shaking at 150 rpm for 90 days. In the first phase of the research, the pretreatment increased the biodegradability of the manure, thus increasing the accumulated methane yield for the two conditions tested (10% TS: from 30 to 205 mL $CH_4$/gVS and 15% TS: from 0 to 136 mL $CH_4$/gVS). On the other hand, increasing the SI ratio for the pretreated manure proved to be viable only in the semi-dry condition (205, 268, and 187 mL $CH_4$/gVS for SI 1, 3, and 5, respectively), as in the dry condition there was only significant methane production in SI 1 (136 mL $CH_4$/gVS). In the second phase, the addition of 20 g GAC/L promoted an increase in methane yield from 3 to 154 and 155 mL $CH_4$/gVS with raw and pretreated manure, respectively, in semi-dry condition (10% TS). Therefore, there was no significant difference when carrying out pretreatment. Regarding the study of GAC dosage for pretreated manure in semi-dry AD, the addition of 10 g GAC/L yielded 190 mL $CH_4$/gVS, a value 21% higher than with 20 and 30 g GAC/L, although the kinetics were more favorable for the concentration of 20 g/L, according to the Modified Gompertz model, which best described the process. In dry AD (15% TS), the optimal GAC concentration was 30 g/L (157 mL $CH_4$/gVS).

**Keywords**: Anaerobic digestion; biometanization; pretreatment; substrate inoculum ratio; semi-dry digestion; dry digestion; conductive materials; granular activated carbon.

# LIST OF FIGURES

# LIST OF TABLES

# SUMMARY

# 1 INTRODUCTION

Pig farming is an important agricultural activity because pork is the second most consumed animal protein worldwide. Global pig meat output is forecasted to be 124.6 million tons in 2022, up 1.8 percent from 2021 (FAO, 2022). Brazil is the fourth largest producer and exporter of pigs worldwide and has all the conditions to advance in this ranking, as it shows growth in this sector year after year. In 2022, there were 56.15 million slaughtered pigs in Brazil, an increase of almost 6% compared to 2021 (STATISTA, 2023).

This activity generates a large amount of waste containing a variety of pollutants, such as organic matter, nitrogen, phosphorus, heavy metals, antimicrobials, and pathogens (Hu et al., 2019a; Zhang et al., 2022). These pollutants can contaminate the environment if this waste is not managed properly (Jongbloed, 2008; Zhu et al., 2013). Then, correctly managing organic waste is a major challenge for the livestock sector, which must meet the Sustainable Development Goals (SDGs) established by the United Nations Framework Convention on Climate Change to recover waste more sustainably (UNITED NATIONS, 2015). In addition, the challenges of climate change and energy security are increasing, and sustainable solutions to meet energy needs are of utmost importance. Renewable energy sources, such as biogas and biomethane from biomass, have been emphasized as the most important contributors to minimizing greenhouse gas (GHG) emissions and ensuring energy supply by replacing natural gas (Feng et al., 2023a).

In this context, swine manure is an excellent substrate to produce biogas from anaerobic digestion (AD). This biochemical process converts organic matter into bioproducts, mainly methane, in the absence of oxygen (Afotey & Sarpong, 2023). Furthermore, when using this waste to produce renewable energy, besides the economic and environmental gains, there is also social gain, as communities close to animal farms also benefit by alleviating odor and disease transmission problems linked to animal manure, which contributes to the SDGs (Afotey & Sarpong, 2023).

The AD has three classifications based on the total solids content of the system: wet ($TS < 10\%$), semi-dry ($10\% \leq TS < 15\%$), and dry ($TS \geq 15\%$). This content influences the design and operation of reactors (LI et al., 2021b; WANG et al., 2023). The possibility of using smaller reactors, reduced water used, and energy savings for heating are advantages of dry and semi-dry conditions (Angelonidi; Smith, 2015; Chen et al., 2015; Hu et al., 2019a). On the other hand, it presents biological and technological disadvantages due to the excess of solids in the reactor, such as the difficulty of mixing and homogenizing the medium, consequently affecting methane production due to the deficiency in the diffusive transport of soluble and intermediate compounds (Rocamora et al., 2020).

It should also be noted that AD has several factors that limit the process and need to be optimized, such as substrate bioavailability, the presence of inhibitors such as volatile fatty acids (VFAs) and ammonia, reactor design, and other operational conditions such as pH, temperature, hydraulic retention time, organic load, substrate inoculum (SI) ratio, and total solids content (Meegoda et al., 2018). Bottlenecks such as low waste-to-energy conversion efficiency and low substrate digestibility must also be considered to make AD plants adapt to the industrial scale, as well as research gaps regarding concerns scaling effects on methane production on specific substrates (Holliger; De Laclos; Hack, 2017; Zheng et al., 2022).

The AD process can be divided into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is usually the limiting stage for complex substrates such as swine manure (Silva et al., 2020). So, optimizing this phase is one of the first steps to making anaerobic digestion more effective, which can be done, for example, by pretreating the substrate. The pretreatment techniques are classified as mechanical, physical, and chemical (Nguyen et al., 2021). Thermo-alkaline pretreatment, which combines the advantages of two types of treatments, is very promising for improving the production of methane-rich biogas from AD (Zou et al., 2020).

Therefore, it is important to investigate the operational parameters of AD, such as organic loading rate — OLR, hydraulic retention time — HRT, and substrate/inoculum ratio — SI (De Groof et al., 2019). Furthermore, there are some studies about the influence of the SI ratio on maximum biogas formation by using different substrates, such as food waste and a mixture of food waste and green waste. However, the same evaluation for swine manure is scarce in the literature.

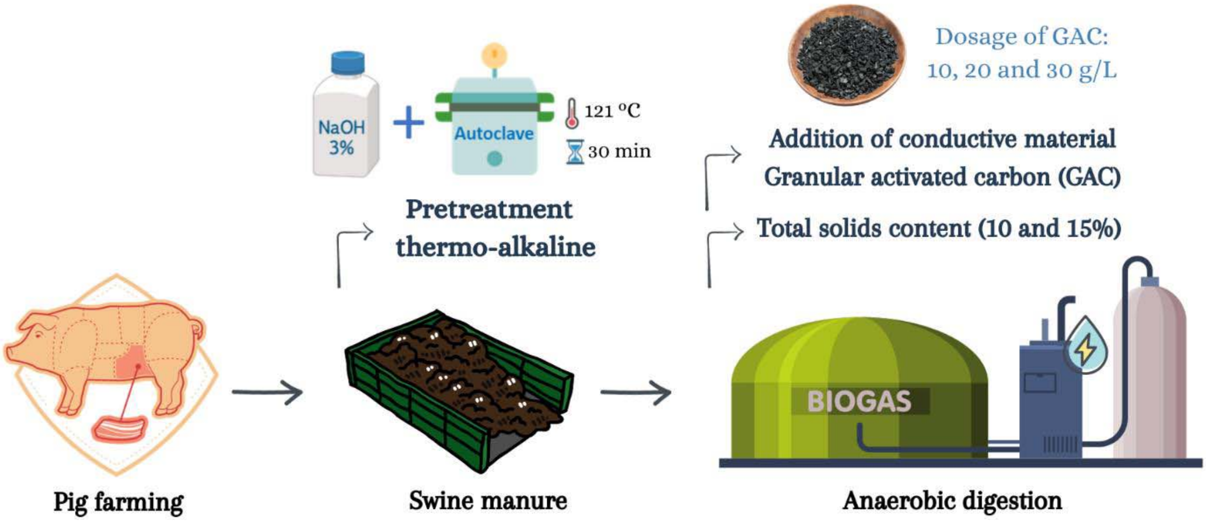
Another important technique to optimize anaerobic digestion is the addition of conductive materials, whose main role is to facilitate the syntrophy of microorganisms, which usually exhibit slow growth in oxygen-deprived systems due to a limited metabolic energy gain. The objective is to enable collaborative reactions throughout the anaerobic digestion stages for the conversion and degradation of compounds (Martins et al., 2018). Essentially, fermentative bacteria and methanogenic archaea possess complementary metabolisms, necessitating syntrophy for both the direct exchange of interspecies electrons (*Direct Interspecies Electron Transfer* - DIET) and chemical compounds (hydrogen or formate) that indirectly transport interspecies electrons (*Indirect Interspecies Electron Transfer* – IIET) (Mostafa et al., 2020).

The DIET is considerably faster and more energy efficient than IIET (Baek et al., 2018) and can occur through biotic (cytochromes and conductive pili) and abiotic (mediated by conductive additives) pathways (Feng et al., 2023b). Conductive materials can be metal or carbon-based. They differ from metal-based conductive materials because they are generally larger than the microorganisms involved in the process. It provides a large surface for fixation and growth, favoring the direct transfer of electrons and enabling interaction without physical contact between the bacteria that donate electrons and the archaea that receive electrons (Kang et al., 2021; Lovley, 2017). Granular Activated Carbon (GAC) is an economical, lightweight, chemically stable carbon-based material with high biocompatibility, can act as an adsorbent for toxic compounds, facilitates the immobilization of microorganisms without forming aggregates, and can substitute conductive *pili* during DIET due to its superior electrical conductivity. In other words, GAC enables the efficient transfer of electrons from cell to cell (Gahlot et al., 2020).

To enhance comprehension of the various stages in this biochemical process, employing kinetic analysis through mathematical models becomes a crucial tool. It aids in predicting methane production and in the design and optimization of reactor performance (Hu et al., 2019c). This analysis's outcome enables quantifying the influence of variables and inhibitions encountered throughout the bioprocess on both the biogas production rate and yield (Bedoić et al., 2020).

Therefore, this work sought to evaluate strategies such as pretreatment, substrate/inoculum ratio (S/I) and granular activated carbon addition for enhancing the semi-dry and dry anaerobic biometanization of swine manure using kinetic analysis and molecular biology tools for process optimization and scale up (Figure 1.1).

Figure 1.1 – Schematic of strategies applied for enhancing the anaerobic biometanization of swine manure.



Source: Author (2023).

# 2 OBJECTIVE

## 2.1 General objective

To evaluate strategies such as pretreatment, substrate/inoculum ratio (S/I) and granular activated carbon addition for enhancing the semi-dry and dry anaerobic biometanization of swine manure using kinetic analysis and molecular biology tools for process optimization and scale up.

## 2.2 Specific objectives

1. To study the effect of thermal-alkaline pretreatment on the biodegradability of swine manure;
2. To evaluate the influence of the parameters substrate/inoculum ratio (S/I) and total solids content (TS) on methane production from swine manure;
3. To optimize anaerobic digestion by adding granular activated carbon (GAC) as a conductive material;
4. To determine the optimal dosage of GAC to maximize methane production from swine manure;
5. To investigate the influence of TS content on the anaerobic digestion of swine manure with the addition of GAC;
6. To evaluate the need for pretreatment of swine manure for anaerobic digestion with GAC addition;
7. To determine the kinetic model that best describes methane production from swine manure;
8. To obtain kinetic parameters that govern dry and semi-dry anaerobic digestion of swine manure based on the models evaluated;
9. To evaluate the microbiological changes in the different strategies investigated by using molecular biology techniques such as metagenomic sequencing.

# 3 EFFECT OF THERMO-ALKALINE PRETREATMENT AND SUBSTRATE INOCULUM RATIO ON METHANE PRODUCTION FROM DRY AND SEMI-DRY ANAEROBIC DIGESTION OF SWINE MANURE

## ABSTRACT

Alternative energy sources are increasingly being sought in the face of climate problems and the finite nature of oil resources. Thus, biogas rich in methane has gained much attention in research. This can be obtained from agro-industrial waste sources, such as swine manure (SM), through anaerobic digestion (AD). However, it is necessary to optimize the process to add economic value. For that, pretreatment techniques are often used to improve the accessibility of microorganisms to the substrate. In this context, this work aimed to analyze the influence of thermo-alkaline pretreatment (3% NaOH at 121 ºC for 30 minutes) and the substrate/inoculum ratio (SI) on AD in semi-dry and dry conditions (10 and 15% total solids – TS), besides to using mathematical modeling to obtain kinetic parameters that assist in the optimization and scaling of the process. Molecular biology tools were also applied for a better comprehension of the different microbial population involved and ecology dynamics changes. The experiments were conducted in batch mode, in mesophilic conditions with orbital shaking at 150 rpm for 90 days. Swine manure pretreatment promoted an increase of 680% (from 30 to 205 mL/gVS) in cumulative methane yield in semi-dry conditions. It was also observed that increasing the SI ratio from 1 to 3 $\text{g.VS}_{\text{substrate}}/\text{g.VS}_{\text{inoculum}}$ increased the accumulated methane production from 205 to 268 mL/gVS, although the production of SI 3 only became greater than that of SI1 from the 50th day. When increasing the TS content to 15% with SI 1, the accumulated methane yield was 136 mL/gVS, 34% lower than the 10% TS condition. The main microorganisms involved in the process were the genera *Firmicutes*, *Aminivibrio*, *Candidatus Caldatribacterium*, *Methanobacterium*, and *Methanosaeta*. Furthermore, Modified Gompertz was the model that better described the experimental data.

**Keywords:** anaerobic digestion; biogas; swine manure; pretreatment; substrate/inoculum ratio; mathematical modeling.

## 3.1 Introduction

The challenges of climate change and energy security are increasing, and sustainable solutions to meet energy needs are of utmost importance. There are reports that fossil fuel sources will soon run out, and therefore, there is a need for alternative sources such as renewable energy (Singh et al., 2023). Biomass is a potential renewable energy source that can be converted into biogas using anaerobic digestion (AD). Recently, biogas and biomethane have been emphasized as the most important contributors to minimizing greenhouse gas (GHG) emissions and ensuring energy supply by replacing natural gas. The commercial development of biogas industries has grown since the mid-1970s due to the energy crisis in Europe, and agricultural waste has been heavily utilized (Feng et al., 2023a).

Such agricultural waste causes several environmental problems if not managed properly. Manure is the most abundant source of organic matter in the livestock sector. Generally, it does not receive adequate treatment, releasing carbon dioxide, methane, and ammonia and contributing to GHG emissions (Salamat et al., 2020). The production of pig manure stands out, as pork is the 2nd most consumed animal protein in the world, behind only poultry protein. Global pig meat output is forecasted to be 124.6 million tons in 2022, up 1.8 percent from 2021 (Fao, 2022).

So, when using this waste to produce renewable energy, in addition to economic and environmental gains, there is also social gain, as communities close to animal farms also benefit by alleviating odor and disease transmission problems linked to animal manure, which contributes to the sustainable development goals (SDGs) (Afotey & Sarpong, 2023). Since swine manure is rich in proteins, lipids, and carbohydrates, it is an excellent substrate for the production of biogas from AD, the most effective method for converting organic waste into energy, which consists of a biochemical process that occurs in the absence of oxygen (Afotey & Sarpong, 2023). AD is divided into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

Hydrolysis is usually the limiting step in the process in complex substrates, such as swine manure. Therefore, the substrate's bioavailability is the first key point to optimize this process, and pretreatment techniques are fundamental for this (Coarita Fernandez et al., 2020). Pretreatment may involve physical/mechanical, thermal, chemical, or biological processes to break the substrate into smaller particles, increasing its surface area and modifying its composition, which can facilitate microbial activity and increase biogas production, and decrease the hydraulic retention time (Atelge et al., 2020; Jang et al., 2014; Nguyen et al., 2021).

Thermal pretreatments are already applied on a large scale to various substrates (animal by-products, sludge, and lignocellulosic biomass). However, their high heat or electricity requirements restrict their benefits (Carrere et al., 2016). On the other hand, chemical pretreatments are currently limited on a laboratory scale due to their cost and environmental consequences. However, some alkaline treatments (mainly lime) have shown promising results, especially with lignocellulosic substrates and animal by-products (Brémond et al., 2018).

Therefore, alkaline processes have often been combined with heat treatment to reduce the alkaline dose and the process temperature (Chiappero et al., 2019). So, thermal-alkaline pretreatment combines the advantages of the two methods and provides synergistic effects by solubilizing macromolecules and reducing medium viscosity, increasing biogas yield and favoring AD kinetics (Guo et al., 2017; Toutian et al., 2021).

In addition to substrate biodegradability, another very important factor in AD is the ratio between substrate and microorganisms (SI ratio) due to the requirement of nutrient balance by anaerobic microbes for their growth as well as to maintain a stable environment (Cao et al., 2020). A high SI ratio can cause toxicity, destroy syntropy between microbes, and irreversibly acidify the reactor. In contrast, a low SI ratio can impair the induction of enzymes required for AD and maximum methane production cannot be achieved (Li et al., 2022). Furthermore, other disadvantages are associated with low SI, such as high sludge concentration and low substrate flow rate, requiring a larger reactor scale to maintain the balance between the substrate and microorganisms (Liang et al., 2023).

Therefore, the identification of an ideal SI ratio in AD has been widely studied, but the ideal SI ratio differs in numerous studies due to the substrate nutritional composition and the physicochemical properties and microbial characteristics of the inoculum that vary widely, which can directly affect the results of studies to explore the SI ratio (Cao et al., 2020; Hobbs et al., 2018; Shahbaz et al., 2019; Xiao et al., 2022). Furthermore, different substrates can react differently with a given inoculum, affecting the main microorganisms and intermediate metabolites in AD differently, resulting in variations in functional metabolic pathways. Therefore, the mechanism of microbial interactions and functional metabolism under different SI ratios during the AD process remains unclear and needs further investigation(Juanga-Labayen; Yanac; Yuan, 2020; Li et al., 2022).

In addition, AD can be classified based on total solids (TS) content into wet (TS < 10%), semi-dry (10% ≤ TS < 15%), and dry (TS ≥ 15%) (WANG et al., 2023). The problems inherent in AD can be aggravated in dry conditions due to lower mass transfer efficiency (Xiao et al., 2019). Therefore, improving the efficiency of AD in methane production is always an important research niche. (Zhang et al., 2019a). Furthermore, mathematical modeling methods are an alternative that can be used to predict the performance of AD systems, making it possible to estimate important kinetic parameters to design and operate AD plants more efficiently (Silva et al., 2021).

In this work, the influence of the thermo-alkaline pretreatment and the SI ratio parameter on the production of biogas rich in methane was evaluated from semi-dry (10% TS) and dry (15% TS) anaerobic digestion of swine manure in mesophilic conditions and batch regime, aiming to optimize and scale the process through the determination of kinetic parameters obtained from mathematical modeling. Molecular biology tools were also applied for a better comprehension of the different microbial population involved and ecology dynamics changes.

## 3.2 Materials and methods

### *3.2.1 Substrate and inoculum*

Swine manure (SM) was collected at the Department of Animal Husbandry at the Federal University of Ceará from the dry cleaning of pig pens. After collection, the manure was subjected to physical-chemical characterization and stored at 4ºC until use. The sludge used as inoculum was collected from a brewery wastewater anaerobic treatment plant (Pacatuba - CE). Subsequently, the sludge was characterized following the same parameters as swine manure.

The thermal-alkaline pretreatment of swine manure was carried out with the addition of sodium hydroxide (NaOH 3% w/v) in a proportion of 60% mass/volume and incubation in an autoclave (Autoclave Vertical, Marconi LTDA, Brazil) at 121ºC for 30 minutes.

The physicochemical characterization of the raw and pretreated swine manure and sludge are shown in Table 3.1, which was carried out through the following analyses: pH, solid series, soluble chemical oxygen demand (CODs), and soluble ammoniacal nitrogen (TANs), according to APHA (2017).

Table 3.1 – Physicochemical characterization of fresh and pretreated swine manure and inoculum.

| Parameter | Raw swine manure | Pretreated swine manure | Inoculum |
|---|---|---|---|
| **Total solids - TS (%)** | 24.4 | 22.7 | 6.6 |
| **Volatile solids - VS (%)** | 20.0 | 17.5 | 5.2 |
| **VS/TS (%)** | 82.2 | 76.8 | 78.4 |
| **pH** | 5.7 | 7.0 | 6.8 |
| **$COD_S$* (mg/L)** | 16,700 | 39,200 | 1,500 |
| **$TAN_S$* (mg/L)** | 795 | 680 | 212 |

* $COD_S$ - soluble Chemical Oxygen Demand; $TAN_S$ - soluble ammoniacal nitrogen.
Source: Author (2023).

### *3.2.2 Biochemical Methane Potential (BMP)*

The experiments were conducted in 300 mL borosilicate reactors with 70 mL working volume, operated in batch mode. The reaction medium comprised pretreated raw or swine manure, sludge, and macro and micronutrient solutions (Table 3.2).

Table 3.2 – Concentration of macro and micronutrient solutions.

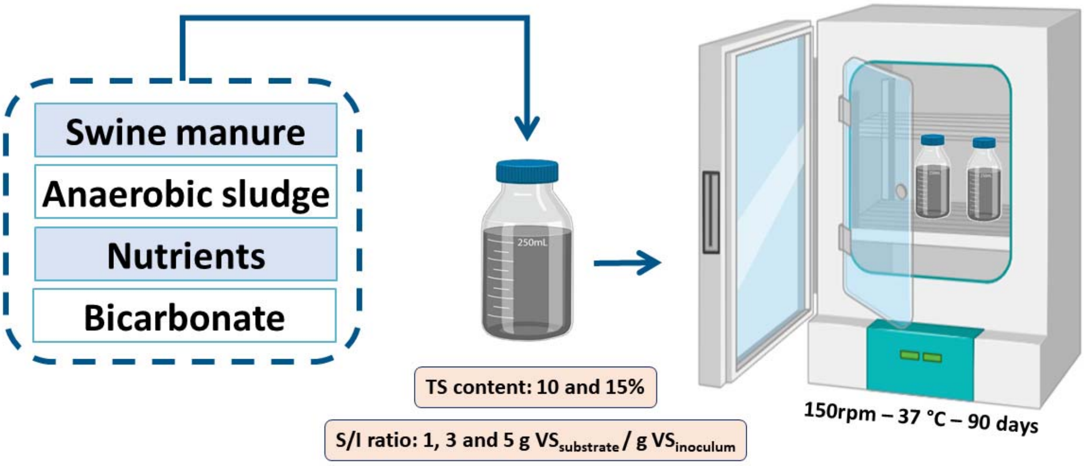
| Nutrients | Concentration (mg/L) |
|---|---|
| $NH_4Cl$ | 28,000 |
| $K_2HPO_4$ | 25,000 |
| $MgSO_4.7H_2O$ | 10,000 |
| $CaCl_2.2H_2O$ | 670 |
| $H_3BO_3$ | 50 |
| $FeCl_2.4H_2O$ | 2,000 |
| ZnCl | 50 |
| $MnCl_2.4H_2O$ | 500 |
| $CuCl_2.2H_2O$ | 38 |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ | 50 |
| $AlCl_3$ | 90 |
| $CoCl_2.6H_2O$ | 2,000 |
| $NiCl_2.6H_2O$ | 92 |
| $Na_2SeO_3.5H_2O$ | 162 |
| EDTA | 1,000 |
| HCl 36% | 1 |

Source: Author (2023).

First, the effect of pretreatment using the substrate/inoculum ratio of 1 $gVS_{substrate}$ /$gVS_{inoculum}$ at 10 and 15% TS was studied ($10RSM_{SI\text{-}1}$: sludge + raw swine manure at 10% TS; $10PSM_{SI\text{-}1}$: sludge + pretreated swine manure at 10% TS; $15RSM_{SI\text{-}1}$: sludge + raw swine manure at 15% TS; $15PSM_{SI\text{-}1}$: sludge + pretreated swine manure at 15% TS). Second, the other SI ratios studied in the test for pretreated swine manure was 3 and 5 $gVS_{substrate}$ /$gVS_{inoculum}$ at 10 and 15% ($10PSM_{SI\text{-}3}$: SI ratio 3 at 10% TS; $10PSM_{SI\text{-}5}$: SI ratio 5 at 10% TS; $15PSM_{SI\text{-}3}$: SI ratio 3 at 15% TS; $15PSM_{SI-5}$: SI ratio 5 at 15% TS). Furthermore, the following control groups were adopted: sludge ($CT_S$), raw swine manure ($CT_{RSM}$), and pretreated swine manure ($CT_{PSM}$).

Afterward, the pH was corrected to 7.0 and buffered with sodium bicarbonate. So, the reactors were sealed with butyl rubber stoppers and purged with $N_2$ for 1 minute to create an anaerobic environment. The flasks were kept in a shaker incubator (MA-420, Marconi LTDA, Brazil) under orbital shaking at 150 rpm at 37 °C until methane production stabilized (90 days) (Figure 3.1).

Figure 3.1 – Schematic of Biochemical Methane Potential (BMP) test with swine manure as substrate in dry and semi-dry anaerobic digestion.



Source: Author (2023).

Biogas production was quantified indirectly by measuring the manometric pressure in the reactors. Biogas composition was analyzed by gas chromatography with dielectric barrier ionization discharge (GC BID-2010 Plus, Shimadzu Corporation, Japan) equipped with a GS-GASPRO column (60 m x 0.32 mm) (Agilent Technologies Inc., USA). The oven, injector, and detector temperatures were 250, 50, and 100 °C, respectively. Helium gas (White Martins LTDA, Brazil) was used as a carrier gas in a 2 mL/min flow, and the run time of the method was 9 min. Biogas quantification was performed by recording the accumulated pressure in each batch reactor using a gauge pressure transmitter (Warme LTDA, Brazil). Analyzes of pH, $COD_S$, and $TAN_S$ were also carried out at the end of the experiment.

### *3.2.3 Kinetic models*

Kinetic models presented in Table 3.3 were used to facilitate the understanding of the evolution of biogas production.

Table 3.3 – Kinetic models selected to describe methane production.

| **Kinetic model** | **Kinetic model equation** |
|---|---|
| **First-order** | $P_t = P[1 - exp(-kt)]$ |
| **Second-order** | $P_t = \frac{k''(P)^2 t}{1 + k''(P)t}$ |
| **Monomolecular** | $P_t = P\left[1 - \exp\left(-k(t - \lambda)\right)\right]$ |
| **Modified Gompertz** | $P_t = Pexp\left\{-exp\left[\frac{\mu_m e}{P}(\lambda - t) + 1\right]\right\}$ |
| **Logistic** | $P_t = \frac{P}{1 + exp\left[\frac{4\,\mu_m(\lambda - t)}{P} + 2\right]}$ |
| **Transfer** | $P_t = P\left\{1 - exp\left[-\frac{\mu_m(t - \lambda)}{P}\right]\right\}$ |

Legend: $P_t$: methane accumulated during the incubation period (mL/gVS). P: volume of methane generated during the experiment (mL/gVS). k: methane production rate first-order constant (1/d). K'': methane production rate second-order constant (1/d) t: digestion time (d). e: Euler number (dimensionless). λ: time of the lag phase (d). $\mu_m$: maximum rate of methane production (mL/gVS.d).
Source: Author (2023).

In order to estimate the parameters of the chosen kinetic models, a non-linear least squares analysis was carried out with the Solver tool in Microsoft Excel 2021. The values predicted by this model were correlated with the experimental ones by calculating the coefficient of determination ($R^2$), according to Eq. (3.1):

$$R^2 = 1 - \frac{\Sigma_i (Y_{i,exp} - Y_{i,est})^2}{\Sigma_i (Y_{i,exp} - \bar{Y})^2} \tag{3.1}$$

Where:

$Y_{i,exp}$ is the experimental data value; $Y_{i,est}$ is the value estimated by the model; Ȳ is the mean of the experimental data.

To select the model that best describes the bioprocess, the following error functions were performed: root mean square error (RMSE) and Akaike Information Criterion (AIC)

(AKAIKE, 1998; JAHEDSARAVANI; MARHABAN; MASSINAEI, 2014; LIMA; MAMEDE; LIMA NETO, 2018). The greater the adequacy of the data estimated by the kinetic model to the experimental data, the smaller the error value. The error functions were calculated according to Eq. (3.2) and (3.3):

$$RMSE = \sqrt{\frac{\Sigma_i (Y_{i,exp} - Y_{i,est})^2}{n}} \tag{3.2}$$

Where:
$n$ is the number of experimental data points (observations).

$$AIC = n \cdot ln(\frac{SS}{n}) + 2np \tag{3.3}$$

Where:
AIC is the Akaike Information Criterion (dimensionless); SS is the squared sum of the residuals; *n* is the number of experimental data observations; *np* is the number of model parameters.

### *3.2.4 DNA extraction, 16S rRNA gene sequencing, and data processing*

Genetic sequencing analyses and data processing were carried out in the Microbial and Molecular Ecology Laboratory of the UFC Biology Department. DNA was extracted from samples collected from the reactors using PowerSoil® (MoBio Laboratories Inc., USA) according to the manufacturer's instructions. The amplicon library for the V4 region of the 16S rRNA gene was prepared as described by Illumina (2013) using region-specific primers (515F/806R). After indexing, PCR products were cleaned with Agencourt AMPure XP - PCR purification beads (Beckman Coulter, Brea, CA, USA) based on the manufacturer's instructions and quantified with the dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Libraries were sequenced using the MiSeq 300-Cycle Reagent Kit v2 (Illumina, 2013) with a MiSeq Desktop Sequencer (ILLUMINA).

The data obtained by sequencing were analyzed with bioinformatics tools as follows. Sequencing reads were trimmed, assembled, and denoised using DADA2 v1.28.0 (Callahan et al., 2016). The resulting amplicon sequencing variants (ASVs) were taxonomically classified using the IDTAXA classifier (Murali; Bhargava; Wright, 2018) from DECIPHER package v2.28.0 (Wright, 2016) based on release 138 of SILVA rRNA database (Quast et al., 2013).

### *3.2.5 Preliminary energy balance analysis*

The preliminary energy balance analysis was carried out considering the extra energy expense with thermo-alkaline pretreatment (heat requirement) and the extra energy gain obtained with increased methane production for pretreated SM. The heat (H) necessary to pretreat swine manure was estimated using equation 3.4 (Sambusiti et al., 2013):

$$H = \frac{C.(T_{final} - T_{initial})}{TS} \times \frac{1000}{3600} \tag{3.4}$$

Where:

C is the specific heat capacity of the substrate, assumed equal to the specific heat capacity of water (4.187 kJ/kg.°C ); $T_{\text{initial}}$ (°C) is the initial temperature of the substrate, assumed as 25 °C; $T_{\text{final}}$ (°C) is the final temperature of the substrate (121 °C); TS (kgTS/$m^3$) is the total solid content of the substrate (244 kgTS/$m^3$); 3600 is the conversion factor between kJ and kWh.

The extra energy gain with the increase in methane production ($E_{increment}$) was estimated according to equation 3.5 (Liu et al., 2020):

$$E_{increment} = E_{CH_4} \times \left(V_{CH_4\,PSM} - V_{CH_4\,RSM}\right) \times \eta \tag{3.5}$$

Where:

$E_{CH_4}$is the energy content of methane (6.5 kWh/$m^3$), $V_{CH_4\,Pt}$ is the volume of generated methane ($m^3$) with pretreated swine manure, $V_{CH_4 R}$ is the volume of generated methane ($m^3$) with raw swine manure, and η is a conversion factor (0.85 for thermal energy).

### *3.2.6 Statistical analyzes*

The data obtained experimentally were analyzed statistically using Microcal Origin 8.1 software (Microcal Software Inc., Northampton, MA, USA) through analysis of variance (ANOVA) with a 95% confidence level and 5% probability ($p < 0.05$). Tukey's tests were used to compare the different treatments. Thus, the data were presented using the average value followed by the statistical treatment letter, where equal letters mean no significant difference for $p < 0.05$.

## 3.3 Results and Discussion

### *3.3.1 Organic matter conversion and methane-rich biogas production*

Regarding organic matter metabolization (Table 3.4), control $CT_S$ showed an increase in $COD_S$ due to the hydrolysis of possible organic matter remaining in the sludge and cell lysis processes during substrate absence. So, not all hydrolyzed organic matter was converted into methane, but it is noted that the sludge used presented satisfactory methanogenic activity with 48 % of $CH_4$ even in the absence of substrate (Table 3.5).

The $COD_S$ of $CT_{RSM}$ and $CT_{PSM}$ controls increased significantly (Table 3.4), probably due to the hydrolysis of the complex swine manure material, converting the particulate organic matter into soluble ones. It is also observed that the initial $COD_S$ of $CT_{PSM}$ is higher than that of $CT_{RSM}$, as the pretreatment has already solubilized part of the organic matter. However, the increase in $COD_S$ at the end of the process was smaller, possibly because part of the hydrolytic microorganisms was inactivated in the pretreatment. It is also observed that controls of raw swine manure ($CT_{RSM}$) and pretreated swine manure ($CT_{PSM}$) showed some biogas production (54-64 mL/gVS), but this was composed only of $CO_2$, without methane (Table 3.5).

On the other hand, the reactors with sludge and swine manure achieved better results. For semi-dry AD with raw swine manure ($10RSM_{SI\text{-}1}$), biogas production was 71 mL/gVS, but the $CH_4$ composition was below expectations for AD (43%), while with pretreated manure ($10PSM_{SI\text{-}1}$) was 302 mL/gVS with 68% of methane. Thus, in semi-dry conditions, swine manure pretreatment promoted an increase of 580% in cumulative methane yield. This is because pretreatment can increase manure digestibility, solubilizing complex organic compounds that can be metabolized by the microorganisms present in the anaerobic sludge, leading to increased production of biogas rich in methane (Nguyen et al., 2021; Zhu et al., 2021)

Table 3.4 – Organic matter based on soluble chemical oxygen demand.

| **Reactor** | **COD$_{\text{S}}$ (g/L)** | |
|---|---|---|
| | **Initial** | **Final** |
| **CT$_{\text{S}}$** | 3.303 | 6.888 |
| **CT$_{\text{RSM}}$** | 6.872 | 49.033 |
| **CT$_{\text{PSM}}$** | 17.274 | 28.514 |
| **10RSM$_{\text{SI-1}}$** | 6.961 | 37.646 |
| **10PSM$_{\text{SI-1}}$** | 11.406 | 10.138 |
| **10PSM$_{\text{SI-3}}$** | 11.940 | 7.714 |
| **10PSM$_{\text{SI-5}}$** | 12.562 | 27.246 |
| **15RSM$_{\text{SI-1}}$** | 18.105 | 53.021 |
| **15PSM$_{\text{SI-1}}$** | 18.874 | 25.468 |
| **15PSM$_{\text{SI-3}}$** | 17.972 | 49.042 |
| **15PSM$_{\text{SI-5}}$** | 22.728 | 46.717 |

Legend: COD$_{\text{S}}$ - soluble Chemical Oxygen Demand; CT$_{\text{S}}$ – sludge; CT$_{\text{RSM}}$ – raw swine manure; CT$_{\text{PSM}}$ – pretreated swine manure; 10RSM$_{\text{SI-1}}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{\text{SI-1}}$ – pretreated swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{\text{SI-3}}$ – pretreated swine manure + sludge at SI ratio 3 and 10% TS; 10PSM$_{\text{SI-5}}$ – pretreated swine manure + sludge at SI ratio 5 and 10% TS; 15RSM$_{\text{SI-1}}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 15PSM$_{\text{SI-1}}$ – pretreated swine manure + sludge at SI ratio 1 and 15% TS; 15PSM$_{\text{SI-3}}$ – pretreated swine manure + sludge at SI ratio 3 and 15% TS; 15PSM$_{\text{SI-5}}$ – pretreated swine manure + sludge at SI ratio 5 and 15% TS).

Source: Author (2023).

Table 3.5 – Final cumulative biogas and methane yield and methane composition.

| **Reactor** | **Biogas (mL/gVS)** | **Methane (mL/gVS)** | **Percentage of methane (%)** |
|---|---|---|---|
| **CT$_{\text{S}}$** | 114$^{a}$ | 55$^{a}$ | 48$^{a}$ |
| **CT$_{\text{RSM}}$** | 64$^{b}$ | 0$^{b}$ | 0$^{b}$ |
| **CT$_{\text{PSM}}$** | 54$^{b}$ | 0$^{b}$ | 0$^{b}$ |
| **10RSM$_{\text{SI-1}}$** | 71$^{b}$ | 30$^{a}$ | 43$^{a}$ |
| **10PSM$_{\text{SI-1}}$** | 302$^{c}$ | 205$^{c}$ | 68$^{c}$ |
| **10PSM$_{\text{SI-3}}$** | 407$^{d}$ | 268$^{d}$ | 66$^{c}$ |
| **10PSM$_{\text{SI-5}}$** | 360$^{e}$ | 187$^{c}$ | 52$^{a}$ |
| **15RSM$_{\text{SI-1}}$** | 43$^{b}$ | 0$^{b}$ | 0$^{c}$ |
| **15PSM$_{\text{SI-1}}$** | 244$^{f}$ | 136$^{e}$ | 56$^{a,c}$ |
| **15PSM$_{\text{SI-3}}$** | 90$^{a,b}$ | 7$^{b}$ | 1$^{b}$ |
| **15PSM$_{\text{SI-5}}$** | 96$^{a,b}$ | 11$^{b}$ | 1$^{b}$ |

Legend: CT$_{\text{RSM}}$ – raw swine manure; CT$_{\text{PSM}}$ – pretreated swine manure; 10RSM$_{\text{SI-1}}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{\text{SI-1}}$ – pretreated swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{\text{SI-3}}$ – pretreated swine manure + sludge at SI ratio 3 and 10% TS; 10PSM$_{\text{SI-5}}$ – pretreated swine manure + sludge at SI ratio 5 and 10% TS; 15RSM$_{\text{SI-1}}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 15PSM$_{\text{SI-1}}$ – pretreated swine manure + sludge at SI ratio 1 and 15% TS; 15PSM$_{\text{SI-3}}$ – pretreated swine manure + sludge at SI ratio 3 and 15% TS; 15PSM$_{\text{SI-5}}$ – pretreated swine manure + sludge at SI ratio 5 and 15% TS). Equal letters mean no significant difference ($p < 0.05$).

Source: Author (2023).

For dry AD, there was not even any significant methane production when using raw substrate ($15RSM_{SI-1}$), due to mass transfer problems linked to the increase in total solids content, which impairs biomethanization (Liang et al., 2022; Xiao et al., 2019). In contrast, with pretreated manure ($15PSM_{SI-1}$), biogas production was 244 mL/gVS with 56% methane (136 mL $CH_4$/gVS), showing that the pretreatment is efficient in improving dry anaerobic digestion too. However, this value was lower than that obtained in semi-dry AD (205 mL $CH_4$/gVS), representing a 34% reduction in $CH_4$ production when increasing the content of TS from 10 to 15%. So, the pretreatment improved the methane-rich biogas production from swine manure in both conditions (semi-dry and dry), but increasing the total solids content in the medium is a challenge.

Concerning organic matter conversion, $10PSM_{SI-1}$ showed an 11% reduction in $COD_S$ and significant methane production in semi-dry AD. However, in $10RSM_{SI-1}$, there was a large increase in $COD_S$ without such a significant methane production, suggesting effective hydrolysis in the process but inefficient methanogenesis. It is, therefore, understood that pretreatment favors methanogenesis in semi-dry anaerobic digestion. In dry AD, there was an increase in $COD_S$ also when using pretreated swine manure, although it was a smaller increase when compared to raw swine manure. This occurs because although pretreatment improves substrate solubilization, in a medium with a higher total solids content and consequent lower water concentration, the diffusion of the compounds is impaired, thus reducing the efficiency of the methane formation process, which promotes the effective organic matter removal from the environment (Sayara; Sánchez, 2019).

By increasing the SI ratio when using pretreated substrate in semi-dry AD, the $10PSM_{SI-3}$ group was the most efficient, with a 35% $COD_S$ reduction justified by the greater conversion to methane. However, in the condition of a higher SI ratio ($10PSM_{SI-5}$), there was an increase in $COD_S$, possibly due to its methanogenic stage being unable to keep up with the velocity of the initial stages of anaerobic digestion in the expected time. There was the solubilization of part of the organic matter with hydrolysis, but not its effective consumption (conversion in methane). In dry AD, there was an increase in $COD_S$ even for the SI ratio of 1 $gVS_{substrate}$ /$gVS_{inoculum}$ ($15PSM_{SI-1}$). However, this increase was much more significant for SI 3 and 5 ($15PSM_{SI-3}$ and $15PSM_{SI-5}$).

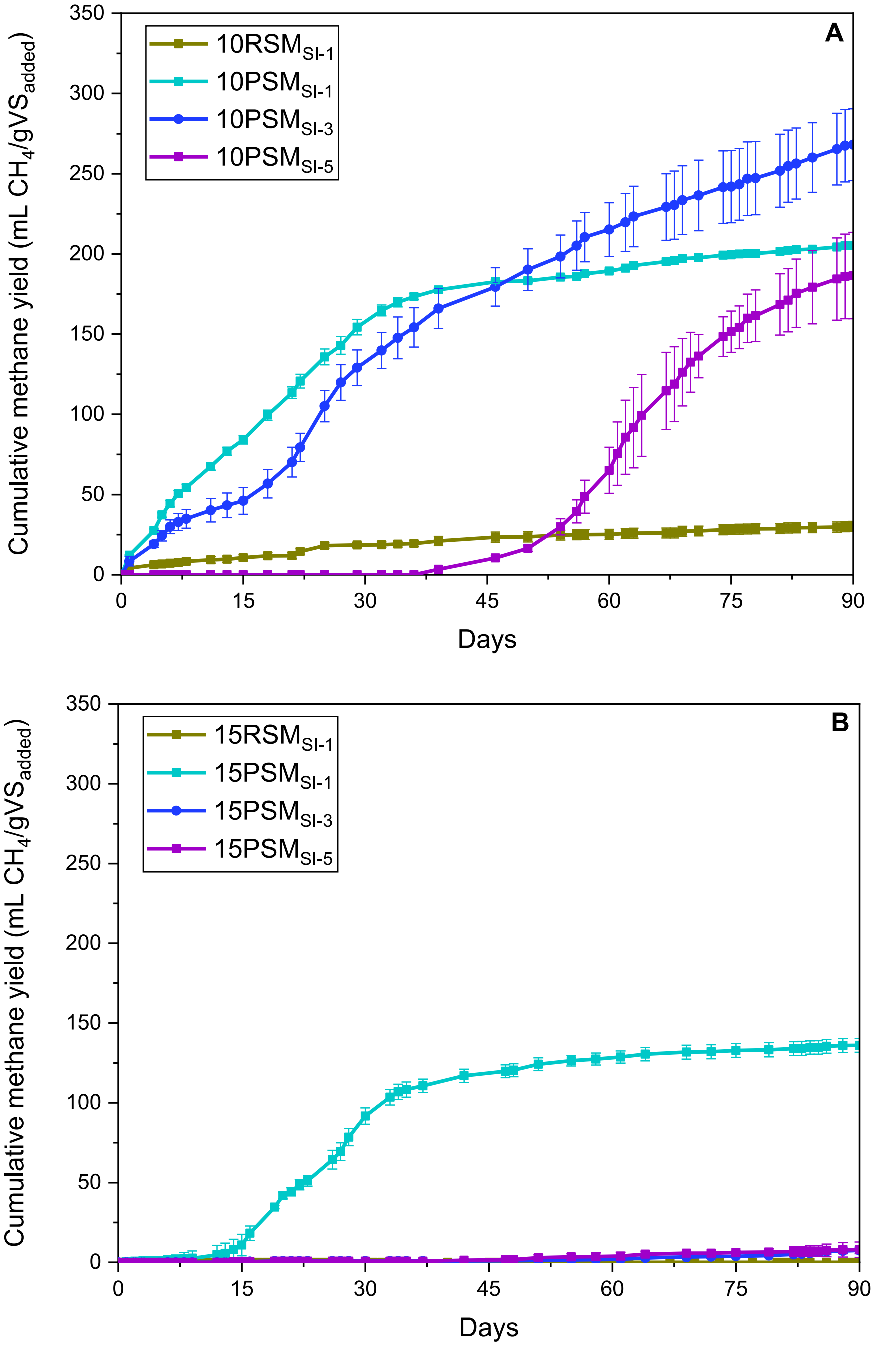
In general, more biogas was produced in the semi-dry AD than in the dry AD, in which the SI ratios 3 and 5 presented values similar to the control $CT_S$ (90 and 96 mL/gVS), which had no addition of substrate, besides to presenting just 1% of methane composition, producing only 7 and 11 mL $CH_4$/gVS, suggesting the presence of inhibitory compounds in swine manure and that these are more harmful to the process when the total solids content is higher (15%), due to greater difficulty in mass transfer (Xiao et al., 2019). So, increasing the SI ratio in dry AD is not feasible.

In contrast, in semi-dry AD, the increase SI to 3 increased methane production at the end of the process. In the reactors with pretreated substrate at 10% TS, methane composition was above 50% for all SI ratios tested. By increasing the SI ratio from 1 to 3, there was an increase of almost 31% in methane yield. However, increasing the SI to 5 showed no significant difference in methane yield.

In addition to methane production after 90 days of experiment, it is also important to discuss the cumulative and daily yield curve over time (Figures 3.2 and 3.3). In semi-dry AD, $10PSM_{SI-1}$ presented a greater slope in the curve at the beginning of the experiment and a higher cumulated methane yield until the 50th day of the experiment, when the $10PSM_{SI-3}$ condition started to show higher methane production. This occurs because the excess organic matter was initially harmful to the process, but over time, the microorganisms adapted, increasing the conversion to methane. At $10PSM_{SI-5}$, methane began to be produced only from the 39th day onwards, indicating possible microbial adaptation over time (Cao et al., 2020), yet representing the lowest yield among the reactors (187 mL $CH_4$/gVS).

It can then be seen that in semi-dry AD, the different SI ratios tested presented different profiles, in which the increase in the SI ratio was initially not favorable to methane production, probably because the greater amount of substrate can lead to an increase in methane inhibitory factors (André; Pauss; Ribeiro, 2018). However, after a period of microbial adaptation, increasing the SI ratio from 1 to 3 proved effective in promoting increased methane production.

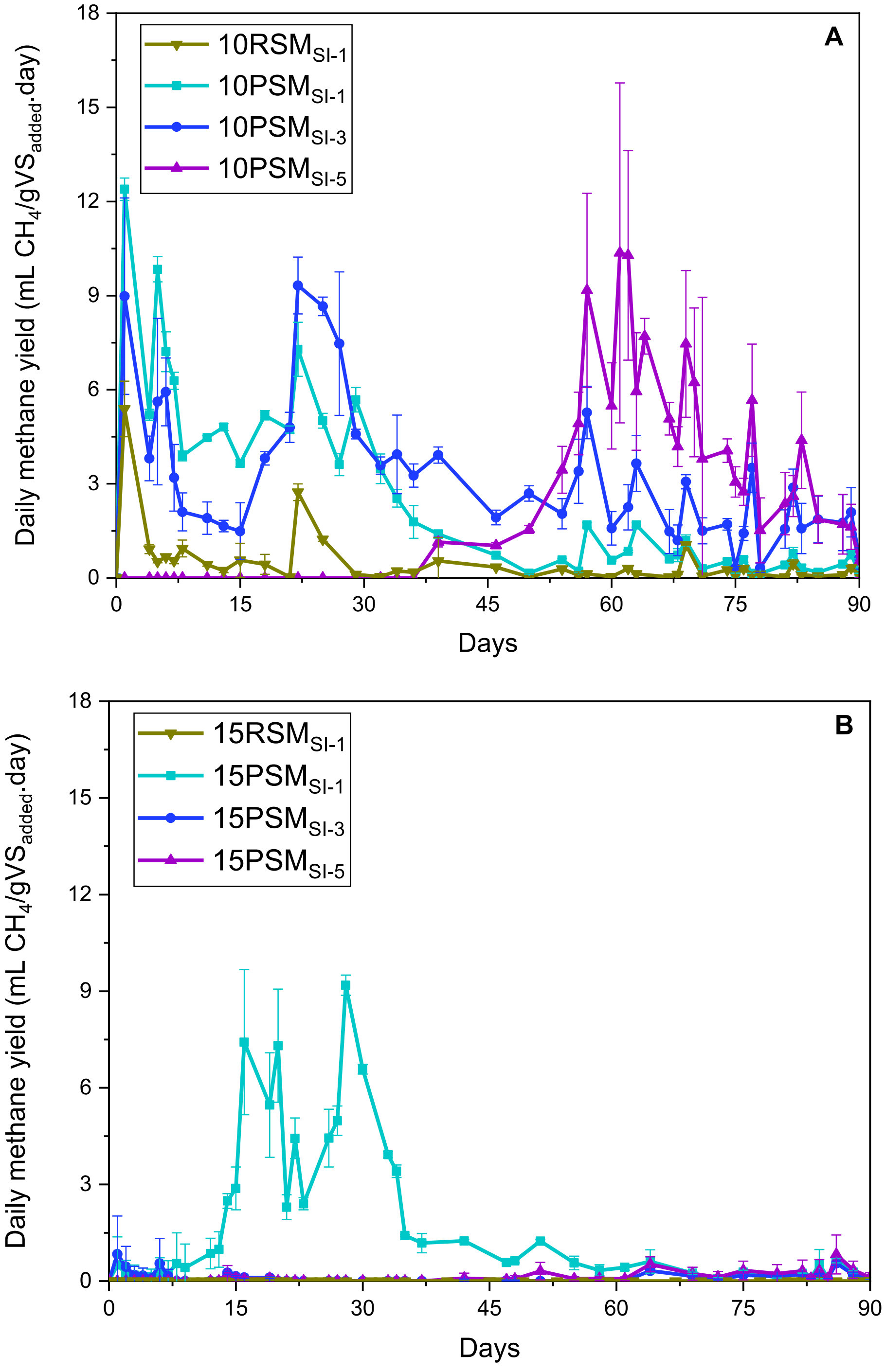
Figure 3.2 – Cumulative methane yield in semy-dry (A) and dry (B) anaerobic digestion.



Legend: 10RSM$_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 10% TS; 10PSM$_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 10% TS; 15RSM$_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 15PSM$_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 15% TS; 15PSM$_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 15% TS; 15PSM$_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 15% TS).

Source: Author (2023).

Figure 3.3 – Daily methane yield in semi-dry (A) and dry (B) anaerobic digestion.



Legend: $10RSM_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; $10PSM_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 10% TS; $10PSM_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 10% TS; $10PSM_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 10% TS; $15RSM_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; $15PSM_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 15% TS; $15PSM_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 15% TS; $15PSM_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 15% TS).

Source: Author (2023).

Furthermore, the major daily methane yield was achieved for 10PSM$_{SI-1}$ (12,4 mL $CH_4$/gVS.d) on the first day, then showed fluctuations until the 32nd day (3.5 mL $CH_4$/gVS.d), when it started to show a sharper drop in daily productivity with some fluctuations between days 55 and 72 when productivity was about 1.7 mL $CH_4$/gVS.d. This behavior is because swine manure, even pretreated, is still a complex substrate, initially with organic fractions of different levels of complexity, where simpler fractions are quickly converted into methane and the complex organic molecules take longer to be hydrolyzed and then converted into methane after several steps involving AD. On the other hand, 10RSM$_{SI-1}$ also showed its maximum daily methane yield on the first day – 5.4 mL $CH_4$/gVS.d, a value 56% lower than 10PSM$_{SI-1}$ value, but soon there was a drop in daily productivity, showing just one more peak (2.7 mL $CH_4$/gVS.d) around the 22nd day. By changing the SI ratio to 3 with pretreated swine manure (10PSM$_{SI-3}$), the highest daily methane productivity was 9.3 mL $CH_4$/gVS.day on the 22nd day, although it reached 9.0 mL $CH_4$/gVS.day on the first day, and for 10PSM$_{SI-5}$ – 10.4 mL $CH_4$/gVS.day on the 61st day.

On the other hand, in dry AD, there was only significant methane production in the SI ratio of 1 gVS$_{substrate}$ /gVS$_{inoculum}$. The cumulative methane yield grew slowly until the 15th day, where the curve presents an inflection point, and production becomes exponential, with a maximum productivity of 8.7, 8.3, and 9.2 mL $CH_4$/gVS.day on the 16th, 20th, and 28th days, respectively. Around the 33rd day, there is a new inflection point, where the curve begins to show a less accentuated growth trend, which is consistent with lower daily methane yield (3.4 mL $CH_4$/gVS.day) until it begins stabilizing around the 55th day.

These results agree with the literature, in which pretreatment of the substrate promotes improved production of methane-rich biogas. Khan & Ahring (2020) managed to increase methane production by 180% when using pretreated digested manure fibers at 100 ºC for 6h with NaOH in AD, while Hu et al. (2019) reported a 390% increase in methane production via AD when adopting thermal pretreatment of swine manure at 70 ºC for 3 days. González-Fernández et al. (2008) observed a 13% increase in biogas production using an alkaline treatment and a 35% increase when applying thermal treatment.

### *3.3.2 pH and soluble ammonia nitrogen*

As swine manure is a substrate rich in nitrogen, it is important to observe the ammonia nitrogen in the process to verify a possible methanogenesis inhibition due to excess ammonia.

The pH and soluble ammonia nitrogen (total - TAN$_S$ and free - FAN$_S$) values are shown in Table 3.6.

Table 3.6 – pH and total and free ammonia nitrogen.

| **Reactors** | **pH** | | **TAN$_S$ (mg/L)** | | **FAN$_S$ (mg/L)** | |
|---|---|---|---|---|---|---|
| | **Initial** | **Final** | **Initial** | **Final** | **Initial** | **Final** |
| **CT$_S$** | 7.38 | 8.76 | 1,332 | 2,583 | 39 | 1085 |
| **CT$_{RSM}$** | 7.01 | 7.13 | 326 | 1,997 | 4 | 34 |
| **CT$_{PSM}$** | 7.29 | 7.08 | 298 | 1,901 | 7 | 31 |
| **10RSM$_{SI-1}$** | 7.03 | 6.93 | 385 | 3,099 | 5 | 33 |
| **10PSM$_{SI-1}$** | 7.04 | 7.79 | 304 | 2,405 | 4 | 175 |
| **10PSM$_{SI-3}$** | 7.16 | 7.83 | 318 | 2,018 | 6 | 159 |
| **10PSM$_{SI-5}$** | 7.27 | 7.58 | 394 | 2,751 | 9 | 135 |
| **15RSM$_{SI-1}$** | 7.18 | 8.08 | 1,648 | 3,941 | 31 | 599 |
| **15PSM$_{SI-1}$** | 7.17 | 8.36 | 1,351 | 1,530 | 25 | 345 |
| **15PSM$_{SI-3}$** | 7.22 | 8.13 | 1,362 | 1,598 | 28 | 266 |
| **15PSM$_{SI-5}$** | 7.18 | 7.91 | 1,382 | 1,600 | 26 | 150 |

Legend: TAN$_S$ – soluble ammoniacal nitrogen; FAN$_S$ – soluble free ammoniacal nitrogen; CT$_S$ – sludge; CT$_{RSM}$ – raw swine manure; CT$_{PSM}$ – pretreated swine manure; 10RSM$_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 10% TS; 10PSM$_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 10% TS; 15RSM$_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 15PSM$_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 15% TS; 15PSM$_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 15% TS; 15PSM$_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 15% TS).

Source: Author (2023).

Regarding pH, in semi-dry AD, 10RSM$_{SI-1}$ kept its pH close to 7, while 10PSM$_{SI-1}$, 10PSM$_{SI-3}$, and 10PSM$_{SI-5}$ showed a final pH of 7.8, 7.8, and 7.6, respectively, which is still in the stability range for methanogenesis. However, in dry AD, the pH suffered greater variations (final pH: 15RSM$_{SI-1}$ – 8.08; 15PSM$_{SI-1}$ – 8.36; 15PSM$_{SI-3}$ – 8.13; 15PSM$_{SI-1}$ – 7.91), some being close to the inhibition range above 8.3. Such pH variations also influence the form of ammonia nitrogen (ionized – $NH_4^+$ or free – $NH_3$), as an increase in pH favors the formation of free ammonia, which is toxic to microorganisms.

Inhibition of anaerobic digestion by ammonia varies depending on the process's operational parameters. According to the literature, TAN inhibitory concentration is between 1,700 and 14,000 mg/L (Xiao et al., 2022b). In semi-dry AD, all reactors were within this inhibition range at the end of the process, although at the beginning of this range. The FAN inhibitory range is 150–1,200 mg/L (Meng et al., 2018). The semi-dry AD reactors with pretreated swine manure and SI 1 and 3 at the experiment completion were just a little over 150 mg/L ($10PSM_{SI-1}$: 175; $10PSM_{SI-3}$: 159 mg/L), and SI 5 was below this value ($10PSM_{SI-5}$: 135 mg/L), indicating that inhibition by ammonia was not the main reason for methanogenesis inhibition, that could be associated to heavy metals and antibiotics presents in the swine manure (Romero et al., 2020).

In dry AD with pretreated substrate, the TAN values at the end of the process were all below the inhibition range, although for FAN, the values remained within the range, even at the beginning. Once again, it is observed that the low methane production was not related to the presence of ammonia nitrogen, as the dry condition $15PSM_{SI-1}$, which exhibited a higher FAN value compared to the SI 3 and 5 ratios also with 15% TS, presented greater production of biogas rich in methane, possibly because in this condition not only methanogenesis was more effective, but also hydrolysis and, therefore, more nitrogenous compounds became soluble in the process.

### *3.3.3 Kinetic Study*

The kinetic parameters and error functions for the mathematical models used to describe methane production are found in Table 3.7. Conditions $15RSM_{SI-1}$, $15PSM_{SI-3,}$ and $15PSM_{SI-5}$ were not included in this analysis because their methane production was not significant. In general, the Modified Gompertz, a sigmoidal model, was the most promising for describing the kinetics of methane production in AD, with a high coefficient of determination ($R^2 > 0.9$) and lower error function values - AIC and RMSE. This model is the most used for estimating methane production from complex substrates. It allows for estimating the lag phase time ($\lambda$) and the maximum methane production rate ($\mu_m$), parameters that will be discussed below. The Gompertz Modified function has a fixed inflection point, where the curvature sign changes from concave to convex or vice versa, that is, maximum growth rate, being asymmetric concerning its inflection point. The curve of this function is modeled according to the location of the inflection point (Morais et al., 2020).

Table 3.7 – Kinetic parameters estimated by kinetic modeling of methane production by anaerobic digestion.

| **Kinetic model** | **Parameters** | **Reactors** | | | | |
|---|---|---|---|---|---|---|
| | | **10RSM$_{SI-1}$** | **10PSM$_{SI-1}$** | **10PSM$_{SI-3}$** | **10PSM$_{SI-5}$** | **15PSM$_{SI-1}$** |
| **First-order** | **k (1/d)** | 0.032$^{a}$ | 0.043$^{b}$ | 0.025$^{c}$ | 0.012$^{d}$ | 0.029$^{a}$ |
| | **$R^2$** | 0.980 | 0.992 | 0.958 | 0.567 | 0.881 |
| | **RMSE** | 1.233 | 5.793 | 18.337 | 47.382 | 19.095 |
| | **AIC** | 19.985 | 156.589 | 257.983 | 350.720 | 279.244 |
| **Second-order** | **k'' (1/d)** | 0.002$^{a}$ | 0.000$^{b}$ | 0.000$^{b}$ | 0.000$^{b}$ | 0.000$^{b}$ |
| | **$R^2$** | 0.901 | 0.890 | 0.842 | 0.486 | 0.758 |
| | **RMSE** | 2.763 | 20.886 | 35.636 | 51.897 | 27.160 |
| | **AIC** | 89.389 | 269.438 | 316.454 | 359.224 | 312.365 |
| **Monomolecular** | **k (1/d)** | 0.032$^{a}$ | 0.045$^{b}$ | 0.028$^{a}$ | 0.016$^{c}$ | 0.036$^{d}$ |
| | **$\lambda$ (d)** | 0.000$^{a}$ | 0.730$^{b}$ | 3.341$^{c}$ | 13.326$^{d}$ | 5.503$^{e}$ |
| | **$R^2$** | 0.980 | 0.993 | 0.969 | 0.713 | 0.927 |
| | **RMSE** | -4.211 | 5.409 | 15.675 | 38.571 | 14.976 |
| | **AIC** | 21.985 | 152.544 | 246.182 | 339.895 | 258.409 |
| **Logistics** | **$\mu_m$ (mL/gVS.d)** | 0.543$^{a}$ | 5.328$^{b,c}$ | 4.322$^{b}$ | 6.323$^{c}$ | 5.568$^{b,c}$ |
| | **$\lambda$ (d)** | 0.000$^{a}$ | 0.000$^{a}$ | 3.944$^{b}$ | 49.184$^{c}$ | 14.292$^{d}$ |
| | **$R^2$** | 0.960 | 0.984 | 0.986 | 0.892 | 0.992 |
| | **RMSE** | 1.753 | 7.976 | 10.517 | 15.486 | 5.042 |
| | **AIC** | 52.254 | 186.722 | 211.067 | 174.727 | 156.075 |
| **Modified Gompertz** | **$\mu_m$ (mL/gVS.d)** | 0.591$^{a}$ | 5.622$^{b,c}$ | 4.620$^{b}$ | 6.819$^{c}$ | 5.289$^{b,c}$ |
| | **$\lambda$ (d)** | 0.000$^{a}$ | 0.000$^{a}$ | 3.473$^{a}$ | 49.406$^{b}$ | 12.965$^{c}$ |
| | **$R^2$** | 0.967 | 0.992 | 0.994 | 0.901 | 0.997 |
| | **RMSE** | 1.600 | 5.673 | 6.756 | 15.857 | 3.236 |
| | **AIC** | 44.405 | 156.743 | 172.117 | 195.764 | 114.395 |
| **Transfer** | **$\mu_m$ (mL/gVS.d)** | 0.969$^{a}$ | 9.192$^{b}$ | 7.363$^{c}$ | 2.626$^{d}$ | 4.951$^{e}$ |
| | **$\lambda$ (d)** | 0.000$^{a}$ | 0.725$^{b}$ | 3.296$^{c}$ | 9.049$^{d}$ | 5.498$^{e}$ |
| | **$R^2$** | 0.981 | 0.993 | 0.970 | 0.609 | 0.925 |
| | **RMSE** | 1.206 | 5.379 | 15.230 | 43.472 | 14.949 |
| | **AIC** | 20.134 | 152.060 | 243.649 | 344.053 | 258.233 |

Legend: 10RSM$_{SI-1}$ – raw swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 10% TS; 10PSM$_{SI-3}$ – pretreated swine manure + sludge at SI ratio 3 and 10% TS; 10PSM$_{SI-5}$ – pretreated swine manure + sludge at SI ratio 5 and 10% TS; 15PSM$_{SI-1}$ – pretreated swine manure + sludge at SI ratio 1 and 15% TS; k: methane production rate first-order constant (1/d). k'': methane production rate second-order constant (1/d). $\lambda$: time of the lag phase (d). $\mu_m$: maximum rate of methane production (mL $CH_4$/gVS.d). $R^2$: coefficient of determination; RMSE: root-mean-squared error; AIC: Akaike Information Criterion.
Source: Author (2023).

The value of $\mu_m$ is influenced by the anaerobic substrate biodegradability (Morais et al., 2021). In semi-dry AD, $\mu_m$ in 10PSM$_{SI-1}$ was 870% greater than in 10RSM$_{SI-1}$, indicating that with the pretreated manure, the organic matter in the substrate became more accessible to microorganisms present in the sludge, increasing the rate of maximum methane production. However, all the other reactors presented similar values, indicating that this parameter was not impacted by an increase in the SI ratio at 10% TS nor by the increase in total solids content for 15% at an SI ratio of 1. However, it is also important to consider the lag phase ($\lambda$), which increased drastically with SI ratio increment in semi-dry AD, demonstrating that the sludge presents greater difficulty in adapting at the beginning of the process when there is more substrate because it consequently increases the amount of inhibitory compounds present in the reaction medium, even though it is a mature sludge it was adapted to the brewery's substrate and not to swine manure (Silva et al., 2021). Comparing the same SI ratio 1, in 15% TS, the lag phase was 13 days. In comparison, in 10% TS, there was no lag phase, also suggesting greater difficulty in adapting the sludge due to the higher total solids content, limitations for substrate diffusion in the system, and the negative effects of low water content on microbial metabolism (Momayez; Karimi; Taherzadeh, 2019).

### ***3.3.4 Analysis of microbial communities***

As described in Chapter 3.3.1, pretreatment of swine manure promoted increased methane production via AD, and increasing the substrate inoculum ratio from 1 to 3 can increase methane yield. However, the kinetics are somewhat impaired; increasing SI to 5 greatly overloads the system, further harming the methane production kinetics. Furthermore, increasing the total solids content also harms the methane production speed. In this way, microbial communities of sludge (CT$_{S}$), raw swine manure (CT$_{RSM}$), pretreated swine manure (CT$_{PSM}$), sludge + raw swine manure with SI 1 (10RSM$_{SI-1}$) and sludge + pretreated swine manure with SI 3 (10PSM$_{SI-3}$) -as it was the one with the highest volumetric production of methane, were analyzed all at 10% TS at the beginning of the experiment to compare with samples from the end of the experiment including the conditions sludge + pretreated swine manure with SI 1 and 5 (10PSM$_{SI-1}$ and 10PSM$_{SI-5}$) at 10% TS and sludge + raw swine manure with SI 1 and sludge + pretreated swine manure with SI 1 at 15% TS (15RSM$_{SI-1}$ and 15PSM$_{SI-1}$).

#### ***3.3.4.1 Bacterial community structure***

Taxonomic classification of bacteria at the phylum level is shown in Figure 3.4(A). At the beginning of the experiment, *Chloroflexi* and *Actinobacteriota* were the dominant phyla in inoculum source sample (CT$_{S}$), also presenting the groups *Acidobacteriota*, *Bacteroidota*, *Caldatribacteriota*, *Desulfobacterota*, *Firmicutes*, *Planctomycetota*, *Proteobacteria*, *Spirochaetota*, *Synergistota* and *Thermotogota*. *Chloroflexi* are hydrolytic-fermentative bacteria widely reported in AD systems that treat livestock wastewater and sludge, and their enrichment is considered beneficial for the degradation of macromolecular organic materials and the subsequent methanogenesis process (Zhuravleva et al., 2022). *Actinobacteriota* acts in the acidogenesis process and converts organic matter into acetate and hydrogen (Qin et al., 2021). *Firmicutes* and *Actinobacteriota* were the major bacteria in raw swine manure ($CT_{RSM}$), while in pretreated swine manure ($CT_{PSM}$), there is almost no *Actinobacteriota*, with an almost absolute predominance of Firmicutes. This possibly occurred because *Firmicutes* had greater tolerance to high temperatures (Hu et al., 2019b). *Firmicutes* can produce a variety of enzymes related to the degradation of complex organic products, generally appearing in the AD system with high ammonia content (Xiao et al., 2021).

Analyzing the samples at the end of the experiment, in $CT_{RSM}$, the abundance of *Firmicutes* increased, while in $CT_{PSM}$, it decreased, increasing *Actinobacteriota* and *Bacteroidota*. This last one was reported to exist widely in different AD reactors and had high tolerance to high ammonia content and high VFAs, being essential for protein degradation (Hu et al., 2019a). Thus, the high abundance of *Bacteroidota* indicated sufficient protein degradation. The abundance of *Firmicutes*, *Proteobacteria*, and *Synergistota* was increased at $10RSM_{SI-1}$ while decreasing *Actinobacteriota* and *Chloroflexi* presence. *Proteobacteria* are also hydrolytic-fermentative bacteria of AD (Zhuravleva et al., 2022). At $10PSM_{SI-3}$, the increase in *Thermotogota* and *Synergistota* stands out. Changes in substrate properties resulting from pretreatment can affect the cultivation of *Thermotogae*, as organic compounds are degraded, releasing more soluble substances and ions (Liu; Lee; Kim, 2023).

Comparing some samples all at the end of the experiment: $10RSM_{SI-1}$ presents a great predominance of *Firmicutes*, while $10PSM_{SI-1}$ shows a more diverse microbiota with a greater abundance of *Actinobacteriota* and *Chloroflexi* mainly, which contributed to the greater methane production in this condition. In semi-dry AD, comparing the different SI, only 1 and 3 have *Acidobacteriota* and *Thermotogota*, while in SI 5, the greatest abundance is of *Firmicutes*. Increasing the solids content to 15% with both raw and pretreated manure increased the abundance of *Firmicutes* and reduced *Chloroflexi*, *Proteobacteria*, and *Synergistota* for the raw substrate and *Actinobacteriota*, *Chloroflexi*, and *Thermotogota* for the pretreated one, although this increased in *Bacteroidota*. The predominance of *Firmicutes* with a lower abundance of other species is likely linked to lower methane production.

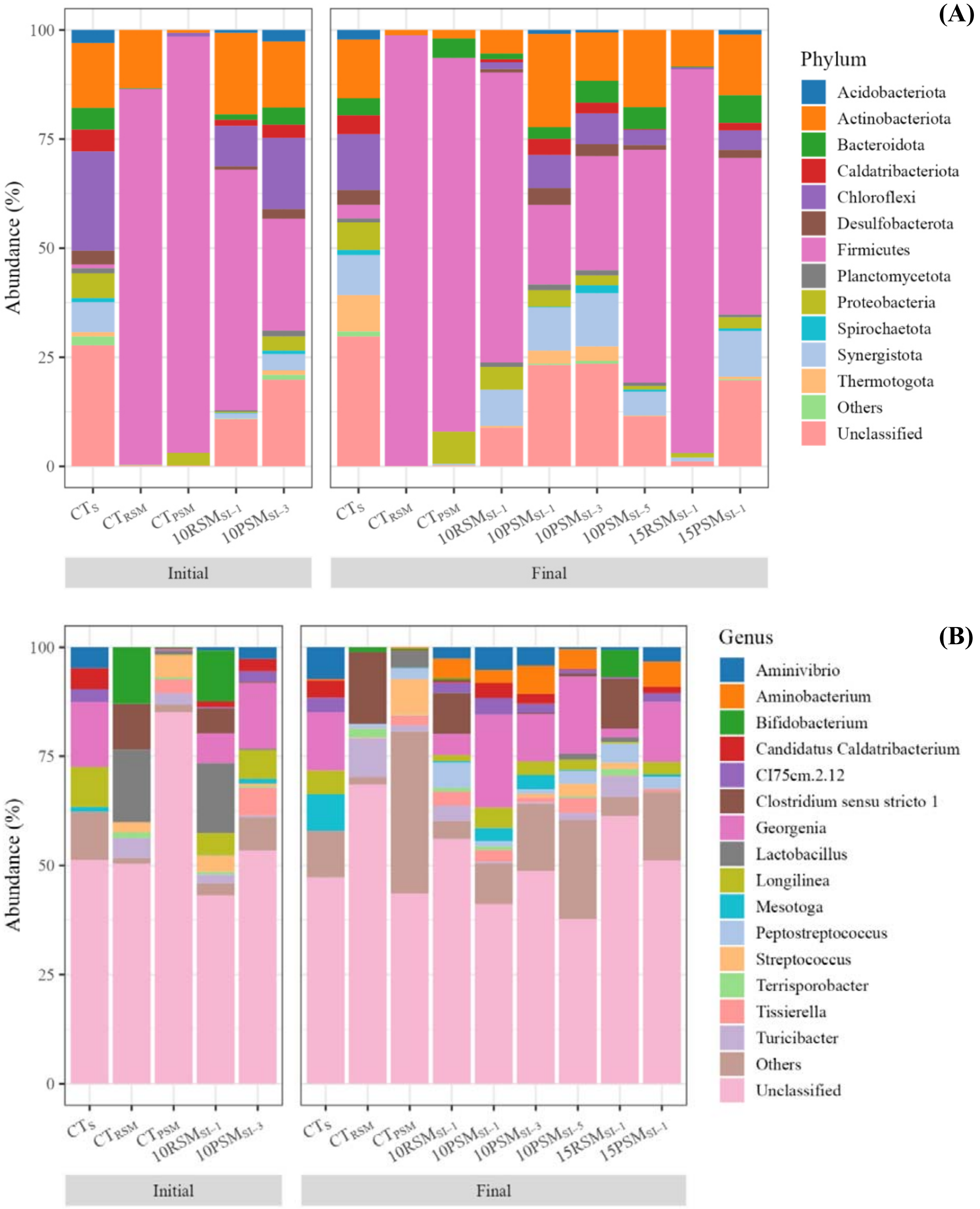
At genus level - Figure 3.4(B), at the beginning of the experiment, the inoculum showed greater abundances of *Georgenia* and *Longilinea*, raw manure - *Lactibacillus* and *Bifidobacterium*, pretreated manure had more unclassified bacteria, but the group in which it was possible to classify had more *Streptococcus* and *Tissierella*. Among the groups identified, the main changes throughout the experiment were: (1) $CT_{RSM}$ incremented the abundance of

*Clostridium sensu stricto* 1 and *Turicibacter* while decreasing that of *Bifidobacterium*, *Lactobacillus* and *Streptococus*; (2) $CT_{PSM}$ increased *Lactobacillus* and *Streptococus*; (3) $10RSM_{SI\text{-}1}$ incremented the abundance in *Aminivibrio*, *Aminobacterium*, CI75cm.2.12, *Clostridium sensu stricto 1*, *Peptostreptococus* and *Tissierella* while decreased in *Bifidobacterium*, *Georgenia*, *Lactobacillus*, *Longilinea* and *Streptococcus*; (4) $10PSM_{SI\text{-}3}$ increased *Aminivibrio*, *Aminobacterium*, *CI75cm.2.12*, *Georgenia* and *Mesotoga* while decreasing *Longilinea* and *Tissierella*. Members of the Clostridium sensu stricto 1 and *Turicibacter* groups are common acetogens under mesophilic conditions in AD systems with high ammonium concentrations using swine manure as substrate, which explains their increase throughout the experiment.

Furthermore, *Clostridium sensu stricto* 1 bacteria can also carry out extracellular electron transfer and reduce $Fe^{3+}$ (Zhuravleva et al., 2022). *Bifidobacterium* and *Streptococcus* are not often found in AD articles. The genus *Lactobacillus* is related to hydrolysis-acidogenesis (producing mainly lactic acid) and acetogenesis (Cuetero-Martínez et al., 2023). *Aminivibrio* can produce several VFAs and are also known to ferment a variety of amino acids, especially in co-culture with Methanobacterium as a hydrogen scavenger (Alalawy et al., 2021). *Aminobacterium* are capable of metabolizing amino acids into VFAs and ethanol (Wang; Zhao; Zhang, 2021). *Georgenia*, a genus of *Actinobacteriota*, is not commonly reported as a major organism in AD but has been implicated as important in protein degradation in an environment with an adequate SI ratio (Liang et al., 2023). *Longilinea* belongs to the *Chloroflexi* phylum and is known for its ability to hydrolyze various proteins and carbohydrates (Wang et al., 2022).

Comparing the samples at the end of the experiment, $10PSM_{SI\text{-}1}$ presents more abundance of *Aminivibrio*, *Candidatus Caldatribacterium*, *Georgenia*, *Longilinea*, and *Mesotoga* than $10RSM_{SI\text{-}1}$. *Candidatus Caldatribacterium* is a fermentative bacteria that produces acetate, and it is present in the sludge used as an inoculum source in this work (Zhuravleva et al., 2022). Increasing the SI ratio for manure pretreated to 10% TS also resulted in some variations in the microbiota, such as a reduction in *Aminivibrio* and *Candidatus Caldatribacterium* and an increase in *Streptococcus*. Increasing the solids content to 15% increased the abundance of *Bifidobacterium* and *Clostridium* sensu stricto 1 mainly and a reduction of *Aminivibrio*, *Aminobacterium*, and *Georgenia* for the raw substrate. In contrast, pretreated manure increased the abundance of *Aminobacterium* and decreased *Aminivibrio*, *Candidatus Caldatribacterium*, and *Georgenia*. It can then be seen that *Aminivibrio* and *Candidatus Caldatribacterium* are linked to greater methane production.

Figure 3.4 – Taxonomic classification of bacteria at phylum level (A) and genus level (B).



Source: Author (2023).

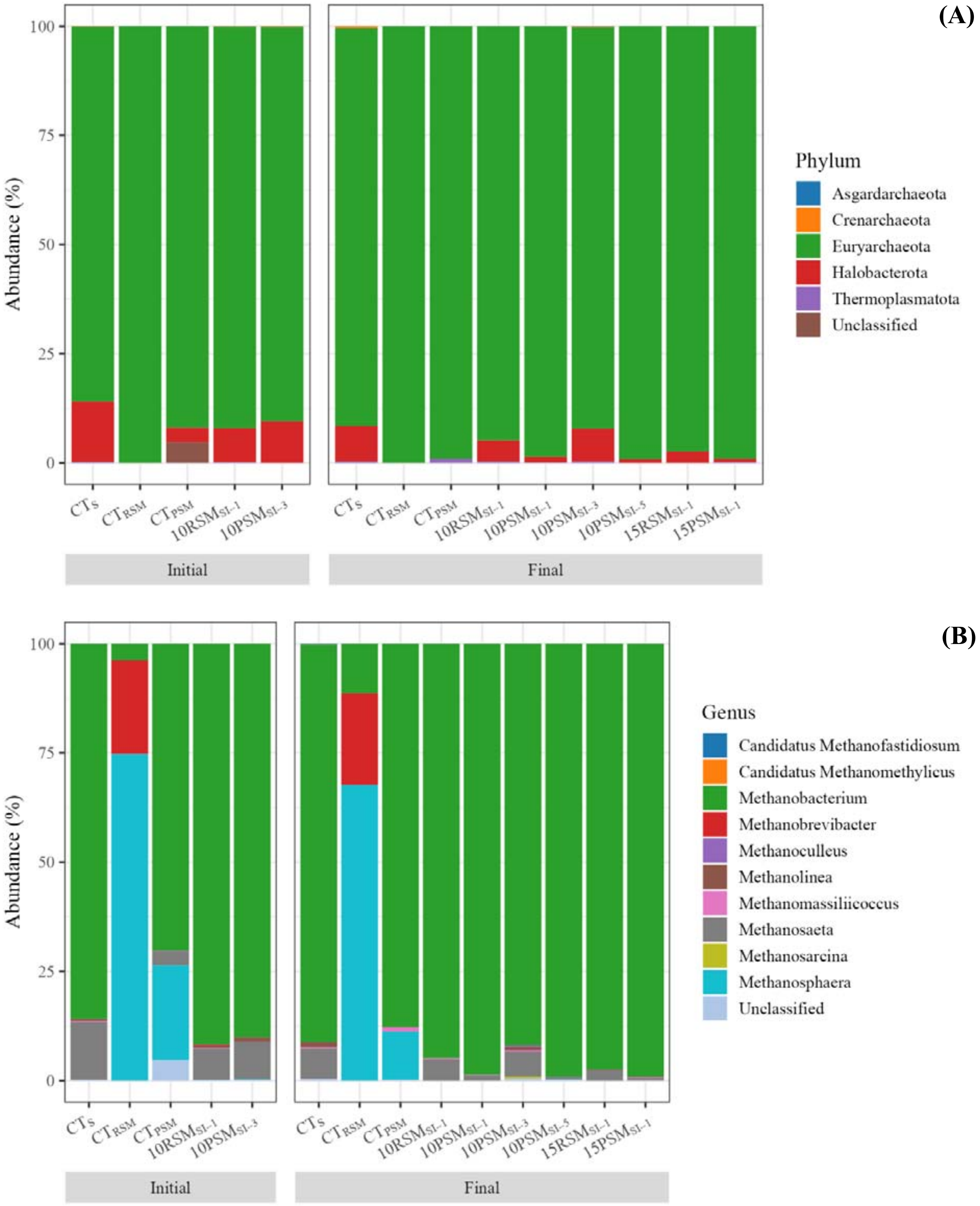
#### *3.3.4.2 Archaea community structure*

The taxonomic archaea classification at the phylum level is shown in Figure 3.5(A). *Euryarchaeota* is the most abundant phylum in all samples, followed by *Halobacterota*, which is more abundant in the sludge. It also appears in the pretreated manure at the beginning of the experiment but not in the raw manure. However, its abundance reduces over time. Both phyla are typical microbial components in anaerobic systems (Liang et al., 2023).

At the genus level - Figure 3.5(B), *Methanobacterium* is the most abundant in the sludge samples, followed by *Methanosaeta*. The raw swine manure has a greater abundance of *Methanosphaera* and *Methanobrevibacter* and a small quantity of *Methanobacterium*. In contrast, the pretreated one has more *Methanobacterium* and less *Methanosphaera*, in addition to presenting *Methanosaeta*. *Methanobacterium* and *Methanobrevibacter* are associated with hydrogenotrophic methanogenesis, which uses hydrogen and carbon dioxide to form methane, and have high tolerance to elevated VFAs and ammonia (Hu et al., 2019a). *Methanosaeta* is an acetoclastic methanogenic genus and is more sensitive to VFAs, ammonia, and other compounds than the hydrogenotrophic methanogens (Xiao et al., 2021). Due to this, its abundance decreased in the swine manure AD. *Methanosphaera* obtains energy for growth by using $H_2$ to reduce methanol to methane (Zhang et al., 2019b).

At the end of the process, except for $CT_{RSM}$, the dominant genus was *Methanobacterium*. Comparing the samples for the semi-dry condition and SI ratio 1, pretreated manure increased the abundance of *Methanobacterium*. However, it decreased *Methanosaeta* abundance, possibly due to the higher free ammonia concentration in $10PSM_{SI-1}$. By increasing the SI ratio from 1 to 3, the abundance of *Methanosaeta* increased from 1.3 to 5.8%, in addition to being the only condition that presented *Methanosarcina* and *Methanolinea*, although with low abundance (0.3% and 0.8% respectively). This greater presence of acetoclastic methanogens possibly caused greater methane production in this condition ($10PSM_{SI-3}$) at the experiment completion. When the total solids content was increased, there was a reduction in the abundance of *Methanosaeta* for both the raw and pretreated substrates.

Figure 3.5 – Taxonomic classification of archaea at phylum level (A) and genus level (B).



Source: Author (2023).

## 3.4 Conclusion

The biogas production from anaerobic digestion (AD) of thermo-alkaline pretreated swine manure is an alternative to dependence on oil platforms. Using thermal-alkaline pretreatment with a 3% NaOH solution in an autoclave at 121 °C resulted in an increase in the production of methane-rich biogas during the anaerobic digestion of swine manure in mesophilic conditions from 30 to 205 mL $CH_4$/gVS at 10% TS and from 0 to 136 mL $CH_4$/gVS at 15% TS. Studying the effect of SI ratio using pretreated substrate, it is highlighted that in semi-dry AD with SI ratio of 1 $gVSs_{ubstrate}/gVS_{inoculum}$ achieved a cumulative methane yield of 205 mL/gVS. Increasing the SI ratio to 3 and 5, this yield was 268 and 187 mL $CH_4$/gVS respectively. So, the SI ratio 3 showed higher yield. As for kinetics, the maximum methane production rate was not impaired with the increase in SI, but there was a large increase in the lag phase. Therefore, it would be important to carry out an economic study considering the methane yields and the kinetic parameters obtained to better direct which condition is most favorable for this AD process. Furthermore, in future experiments, the influence of this parameter can be analyzed using a sludge adapted to high loads of pig manure. About the dry condition, the production of biogas rich in methane was greatly harmed when the SI ratio increased, with the cumulative methane yield of SI ratios 1, 3, and 5, respectively, 136, 7, and 11 mL $CH_4$/gVS. In addition, in SI 1 with 15% TS compared to 10% TS condition, there was no significant difference in $\mu_m$, but the lag phase increased from 0 to 14 days. Regarding microbiological analysis, bacteria genus *Aminivibrio*, *Candidatus Caldatribacterium* are linked to a greater methane production. At the end of the process, the dominant genus was *Methanobacterium* in almost all conditions and by increasing the SI ratio from 1 to 3 at 10% TS, the abundance of *Methanosaeta* increased and this may also have favored greater methane production. In general, the Modified Gompertz kinetic model best fit the methane yield curve of reactors and the kinetic parameter obtained can be used to optimize and scale the anaerobic digestion processes of thermo-alkaline pretreated swine manure, that is an interesting alternative for treating swine manure, with potential added economic value.

# 4 INFLUENCE OF GRANULAR ACTIVATED CARBON ADDITION ON METHANE PRODUCTION IN DRY AND SEMI-DRY ANAEROBIC DIGESTION OF SWINE MANURE

## ABSTRACT

Pig farming is an important agricultural activity responsible for one of the world's largest supplies of animal protein. Managing swine waste is a big challenge, and anaerobic digestion (AD) is an important alternative to treat waste and recover resources. Due to the complexity of this waste, thermal-alkaline pretreatment is usually required to improve the substrate bioavailability. Metal and carbon-based conductive materials have shown promising results in AD by assisting in microorganisms' syntrophic activity, resulting in higher biogas yields. This work aimed to assess the effect of granular activated carbon (GAC) on biogas production and composition in semi-dry and dry AD of swine manure. Molecular biology tools were also applied for a better comprehension of the different microbial population involved and ecology dynamics changes. The experiment was conducted in batches, inoculated with raw or pretreated swine manure and anaerobic sludge at a substrate/inoculum ratio of 1 $g.VS_{substrate}/g.VS_{inoculum}$ with total solids content of 10 and 15%, at a mesophilic temperature of 37 °C under orbital agitation at 150 rpm for 90 days. Tests were carried out without additive, with non-conductive material (nylon) and with GAC (10, 20 and 30 g/L). The addition of 20 g GAC/L promoted an increase in methane yield from 3 to 154 and 155 mL $CH_4$/gVS with raw and pretreated manure, respectively, in semi-dry condition (10% TS). Therefore, there was no significant difference when carrying out pretreatment. Regarding the study of GAC dosage for pretreated manure, in semi-dry AD the addition of 10 g GAC/L yielded 190 mL $CH_4$/gVS, a value 21% higher than with 20 and 30 g GAC/L, although the kinetics were more favorable for the concentration of 20 g GAC/L, according to the Modified Gompertz model, which best described the process. In dry AD, the best condition was with 30 g GAC/L, producing 157 mL $CH_4$/gVS, followed by 20g GAC/L (127 mL $CH_4$/gVS), while for 10 g GAC/L the methane production was much lower (18 mL $CH_4$/gVS). Thus, it was concluded that the TS content influences the ideal GAC concentration in AD, and a higher GAC concentration makes the system more resilient to increasing total solids content.

**Keywords**: Anaerobic digestion; Swine manure; Conductive material; Granular activated carbon; Pretreatment; Biogas.

## 4.1 Introduction

According to the United States Department of Agriculture, pig farming is one of the world's largest agricultural activities, with global animal protein exports totaling 12.3 million tons in 2022 (USDA, 2023). The intensification of this activity brings environmental, social, and economic consequences, and an assessment of parameters related to sustainability is needed (De Camargo et al., 2018). The large amount of waste produced by the sector is an important source of pollution, as many pigs are kept in confined spaces in intensive farming systems, accumulating manure and urine. In addition, large amounts of water are used in pig farming, which is generally contaminated with manure and other pollutants, causing surface and groundwater pollution (Hu et al., 2019a).

It is estimated that around 300 million liters of liquid pig manure are generated daily, containing a variety of pollutants, such as organic matter, nitrogen, phosphorus, heavy metals, antimicrobials, and pathogens (Hu et al., 2019a; Zhang et al., 2022). Therefore, if not managed properly, such pollutants can contaminate the soil, groundwater, and surface water, besides contributing to air pollution by releasing ammonia and other gases (Jongbloed, 2008; Zhu et al., 2013). Then, correctly managing organic waste is a major challenge for the livestock sector.

Since swine manure is rich in proteins, lipids and carbohydrates, it is an excellent substrate for the production of biogas from AD, a biotechnological process capable of converting organic matter present in manure into biogas rich in methane, which can be converted into heat and electricity, reducing dependence on fossil fuels and emissions associated with its use (Chukwuma et al., 2021; Kougias; Angelidaki, 2018).

The AD has three classifications based on the total solids content of the system: wet ($TS < 10\%$), semi-dry ($10\% \leq TS < 15\%$) and dry ($TS \geq 15\%$), and this content influences the type of reactor to be used, energy consumption, digestate final quality, the different endogenous inhibitions during biodegradation and process profitability (Li et al., 2021b; Wang et al., 2023). Dry and semi-dry AD have advantages over wet AD, for example, the possibility of execution in smaller reactors, reduced water used, and energy savings for heating (Angelonidi; Smith, 2015; Chen et al., 2015; Hu et al., 2019a). On the other hand, it presents biological and technological disadvantages due to the excess of solids in the reactor, such as the difficulty of mixing and homogenizing the medium, consequently affecting methane production due to the deficiency in the diffusive transport of soluble and intermediate compounds (Rocamora et al., 2020).

It is considered that AD is divided into four stages: hydrolysis, acetogenesis, and methanogenesis, in which bacteria carry out the first three steps while the last one is by archaea (Adekunle; Okolie, 2015; Chernicharo, 2007). The syntrophy between the microorganisms involved in AD process must be the best possible. Fermentative bacteria and methanogenic archaea have complementary metabolisms, requiring syntrophy for electron exchange, which can be indirect or direct (Mostafa et al., 2020). The indirect pathway that transfers electrons through diffusive transport from chemical compounds (hydrogen or formate) is considered a bottleneck for producing $CH_4$ since it only occurs when there is low partial pressure of $H_2$, a phenomenon regulated by hydrogenotrophic archaea. It is a thermodynamically unfavorable pathway for methane generation, leading to volatile fatty acids (VFAs) accumulation and thus reducing AD efficiency (Feng et al., 2023b; Li et al., 2021a).

On the other hand, the direct pathway - Direct Interspecies Electron Transfer (DIET) is considerably faster and more energy efficient than indirect mechanisms, as it does not require complex enzymatic steps to produce, consume, and diffuse process redox mediators (Baek et al., 2018). This direct mechanism can occur through biotic (cytochromes and conductive pili) and abiotic (mediated by conductive materials - CMs) pathways (Feng et al., 2023b). Favoring DIET presents a short lag phase of acclimatization of microorganisms at the beginning of the process, increased methane production rate, improved methane yield, process stability through organic load variability, and resistance promotion to endogenous inhibitions (Park et al., 2018). The different electron transfer mechanisms involved in methane production via anaerobic digestion are shown in Figure 4.1.

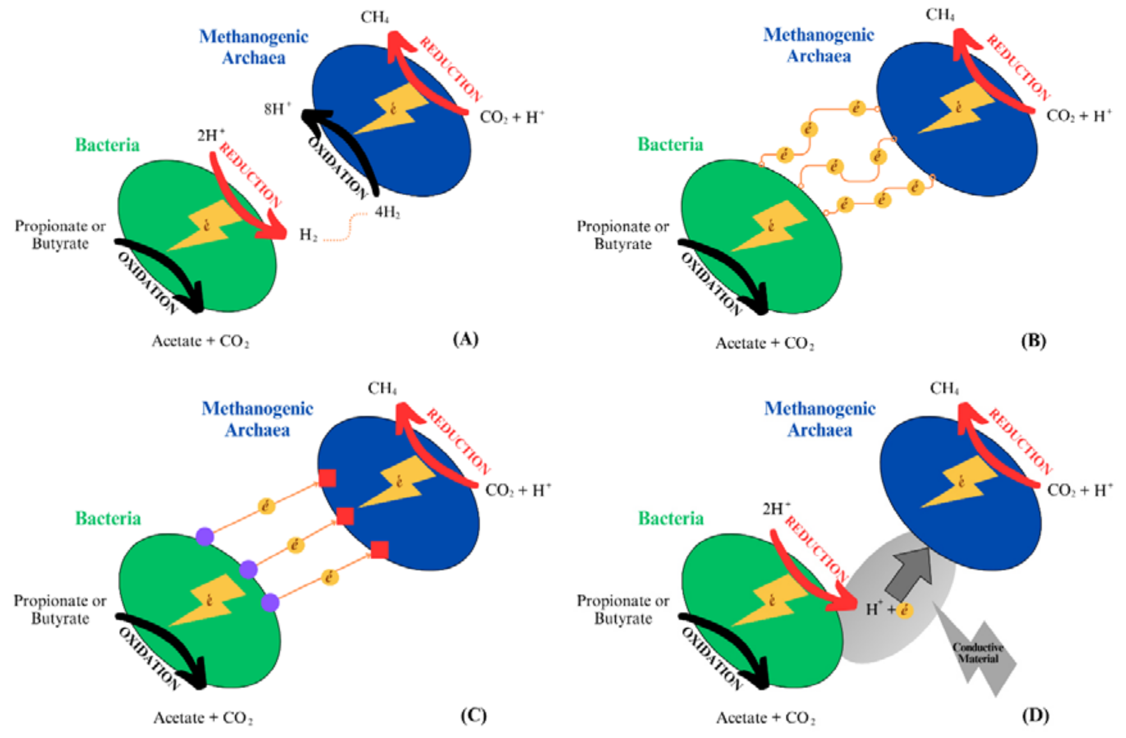
So, a promising technique to optimize anaerobic digestion is the addition of conductive materials that can be metal-based, such as iron oxide and hematite, or carbon-based, such as granular activated carbon (GAC) and biochar (Cavalcante; Gehring; Zaiat, 2021). The mechanisms of DIET via conductive materials can be classified into three main categories (Figure 4.2): (1) mechanism of attachment and growth of relevant microorganisms on the surface of relatively large CMs (e.g., GAC) and electron transfer; (2) electron transfer acceleration mechanism by replacing c-type cytochrome with nanometer-sized CMs (e.g., magnetite); and (3) electron transfer mechanism through an electron conduit of nanometer-sized CMs (Kang et al., 2021).

Therefore, CMs such as GAC and biochar, due to their larger size in relation to the microorganisms involved in the process, allow the connection of multiple microorganisms and enable interaction without physical contact between the bacteria that donate electrons and the archaea that receive them. Thus, the connection with the conductive material is sufficient to promote the DIET (Lovley, 2017). In addition, GAC is a low-cost, lightweight, chemically stable conductive material that has high biocompatibility, can act as an adsorbent for toxic compounds, allows the fixation of microorganisms without forming aggregates, and can replace conductive *pili* during DIET as it has excellent electrical conductivity, that is, it allows the transfer of electrons from cell to cell (Gahlot et al., 2020).

The literature already presents some work using conductive materials to optimize anaerobic digestion with various wastes, such as swine manure, which showed increases in methane production of 25% with the addition of biochar (Yang; Chen; Wen, 2021) and 42% with GAC (Romero et al., 2020). Cui et al. (2021) reported a 214% increase in methane yield from AD of food waste with biochar addition, and according to Sun et al. (2020), the use of GAC improves methane yield by 146%.

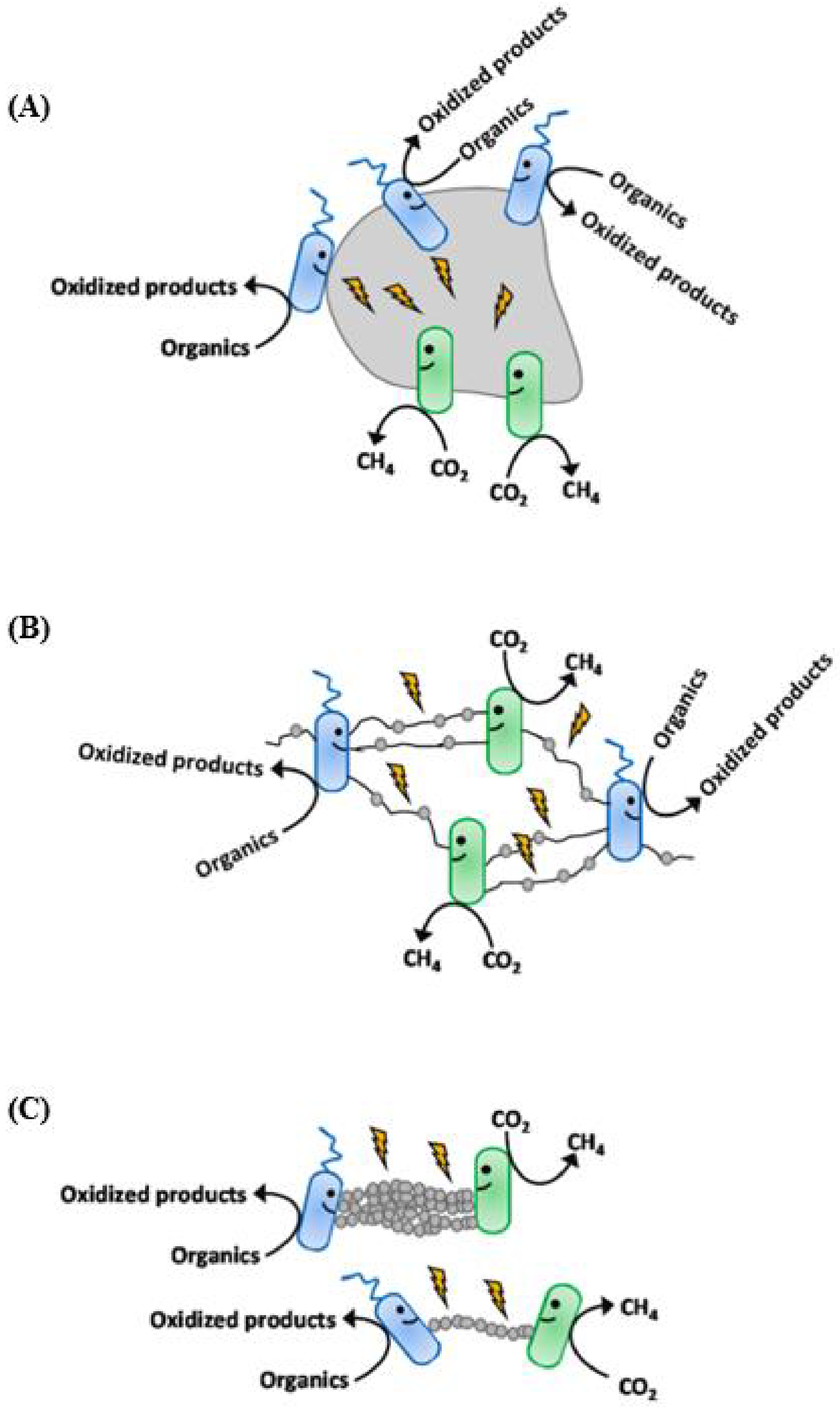
In addition, for a greater understanding of the steps of this entire biochemical process, kinetic analysis based on mathematical models is an important tool for predicting methane production, designing, and optimizing reactor performance (Hu et al., 2019c). The result of this analysis makes it possible to quantify the impact of variables and inhibitions that occurred during the bioprocess on the biogas production rate and yield (Bedoić et al., 2020).

Figure 4.1 – Schematic of the different electron transfer mechanisms between microorganisms via indirect transfer (A), conductive pili (B), transport proteins (C), and conductive material (D).



Source: Adapted from Zhao et al. (2020).

Figure 4.2 – DIET mechanisms via (A) large conductive materials, (B) nanometer-sized conductive materials, and (C) electron conduits of conductive materials.



Source: Kang et al. (2021).

Therefore, this work sought to evaluate strategies to enhance the anaerobic biometanization of swine manure through the addition of granular activated carbon, investigating the influence of thermo-alkaline pretreatment, total solids content, and GAC concentration addition, applying kinetic modeling analysis to understand the influence of the evaluated parameters better and generate important kinetic coefficients for process optimization and scale-up. Molecular biology tools were also applied for a better comprehension of the different microbial population involved and ecology dynamics changes.

## 4.2 Material and methods

### *4.2.1 Substrate and inoculum*

Swine manure (SW) was collected in a pig farm located at the Federal University of Ceará (Fortaleza - CE), in the Department of Animal Science, by scraping the residue in the pens. Soon after collection, this raw manure was subjected to physical-chemical characterization with series analyses of solids (total solids – TS and volatiles – VS), pH, chemical oxygen demand, and ammonia nitrogen of the soluble fraction ($COD_S$ and $TAN_S$) (Table 4.1) and refrigerated at 4ºC until use.

Table 4.1 – Physicochemical characterization of fresh and pretreated swine manure and inoculum.

| Parameter | Raw swine manure | Pretreated swine manure | Inoculum |
|---|---|---|---|
| **Total solids - TS (%)** | 23.5 | 14.0 | 12.4 |
| **Volatile solids - VS (%)** | 18.9 | 8.4 | 10.2 |
| **VS/TS (%)** | 80.5 | 59.9 | 82.5 |
| **pH** | 5.7 | 7.0 | 6.8 |
| **$COD_S^*$ (g/L)** | 65.2 | 39.3 | 3.2 |
| **$TAN_S^*$ (mg/L)** | 446.9 | 351.7 | 288.4 |

$^*$ $COD_S$ - soluble Chemical Oxygen Demand; $TAN_S$ - soluble ammoniacal nitrogen.
Source: Author (2023).

The thermal-alkaline pretreatment of swine manure was carried out with 3% w/v NaOH at a proportion of 60% mass/volume and incubation in an autoclave at 121 °C for 30 minutes.

The anaerobic inoculum was sludge collected from the wastewater treatment plant of a brewery located in Pacatuba, Ceará, Brazil. The physical-chemical characterization of the inoculum consisted of the same parameters submitted to swine manure. Before the solids analysis, the sludge was dewatered through sieves to obtain a higher total solids content (Table 4.1).

### *4.2.2 Conductive material*

Commercial granular activated carbon (12-20 *mesh*) (Sigma-Aldrich, Saint Louis, MO, USA) was used as conductive material. The particle size was 0.8-1.7 mm, bulk density was 1.8-2.4 g/cm$^{3,}$ and surface area was 650 m$^2$ /g. The GAC was washed three times with deionized water to remove impurities and dried in an oven at 105 ºC for 24 hours to remove moisture.

In order to use a material without electrical conductivity for comparison, granular nylon of size 1.1-2.4 mm and apparent density of 1.1 g/cm$^3$ was used.

### *4.2.3 Biochemical Methane Potential Tests*

The experiments were carried out in batch mode and triplicate in 300 mL borosilicate bottles with a useful volume of 70 mL and 230 mL of headspace. The concentrations of TS were 10 and 15%, adjusted with the addition of deionized water. All assays were prepared with a substrate/inoculum ratio of 1 $gVS_{substrate}/gVS_{inoculum}$, macro and micronutrient solution addition according to Angelidaki et al. (2009), pH adjustment to 7, and addition of sodium bicarbonate to buffer the system.

The control reactors were made up of sludge at 10% TS ($CT_S$); sludge and glucose (0,35 gCOD/gVS) at 10% TS ($CT_{SG}$); sludge, glucose (0,35 gCOD/gVS) and GAC – 20g/L – at 10% TS ($CT_{SG\text{-}GAC}$); sludge and raw swine manure at 10 and 15% TS (10RSM and 15RSM) and with GAC addition at a concentration of 20 g/L ($10RSM_{GAC20}$ and $15RSM_{GAC20}$); sludge and pretreated swine manure at 10 and 15% TS (10PSM and 15PSM); sludge, pretreated swine manure and nylon at 10 % TS ($10PSM_N$), 20g/L of the respective additive were added to their reactors. The other media contained sludge and pretreated manure at 10 and 15% TS with the addition of GAC at a concentration of 10 g/L ($10PSM_{GAC10}$ and $15PSM_{GAC10}$), 20 g/L ($10PSM_{GAC20}$ and $15PSM_{GAC20}$) and 30 g/L ($10PSM_{GAC30}$ and $15PSM_{GAC30}$). The bottles were sealed with butyl rubber stoppers and purged with $N_2$ for 1 minute to condition in an anaerobic environment. Then, they were placed in a shaker incubator (MA-420, Marconi LTDA, Brazil) and kept under orbital agitation to promote continuous mixing at 150 rpm at a mesophilic temperature of 37 °C for 90 days until methane production stabilizes.

### *4.2.4 Analytical Methods*

At the beginning and end of the experiment, the pH and solids series of the raw samples were analyzed. Samples were centrifuged at 13,000 rpm for ten minutes (Eppendorf AG, Germany) followed by filtration through a glass fiber membrane with a pore size of 0.45 µm (EMD Millipore, USA) to proceed with the analyses of CODs, TANs, Total Organic Carbon – COT (TOC-L CSN, Shimadzu Corporation, Japan).

Biogas quantification was carried out by measuring gauge pressure in each reactor and biogas composition was analyzed on a gas chromatograph with discharge detection by dielectric barrier ionization (GC-BID, *gas chromatography-barrier ionization discharge*) (GC BID-2010 Plus, Shimadzu Corporation, Japan), equipped with a Select Biodiesel GC Column, (15 mx 0.32 mm) (Agilent Technologies Inc., USA). The oven, injector, and detector temperatures were 250, 50, and 100 ºC, respectively. The carrier gas used was helium gas (White Martins LTDA, Brazil) at a flow of 2 mL /min, and the method run time was nine minutes.

### *4.2.5 Kinetic models*

The kinetic models' equations used to describe the anaerobic digestion bioprocess of swine manure are shown in Table 4.2.

Table 4.2 – Kinetic models selected to describe methane production.

| **Kinetic model** | **Kinetic model equation** |
|---|---|
| **First-order** | $P_t = P[1 - exp(-kt)]$ |
| **Second-order** | $P_t = \frac{k''(P)^2 t}{1 + k''(P)t}$ |
| **Monomolecular** | $P_t = P\left[1 - \exp\left(-k(t - \lambda)\right)\right]$ |
| **Modified Gompertz** | $P_t = Pexp\left\{-exp\left[\frac{\mu_m e}{P}(\lambda - t) + 1\right]\right\}$ |
| **Logistic** | $P_t = \frac{P}{1 + exp\left[\frac{4\,\mu_m(\lambda - t)}{P} + 2\right]}$ |
| **Transfer** | $P_t = P\left\{1 - exp\left[-\frac{\mu_m(t - \lambda)}{P}\right]\right\}$ |

Legend: $P_t$: methane accumulated during the incubation period (mL/gVS). P: volume of methane generated during the experiment (mL/gVS). k: methane production rate first-order constant (1/d). K": methane production rate second-order constant (1/d) t: digestion time (d). e: Euler number (dimensionless). λ: time of the lag phase (d). $\mu_m$: maximum rate of methane production (mL/gVS.d).
Source: Author (2023).

### *4.2.6 Statistical analyzes*

In order to estimate the parameters of the chosen kinetic models, a non-linear least squares analysis was carried out with the Solver tool in Microsoft Excel 2021®. The values predicted by this model were correlated with the experimental ones by calculating the coefficient of determination ($R^2$), according to Eq. (1):

$$R^2 = 1 - \frac{\Sigma_i (Y_{i,exp} - Y_{i,est})^2}{\Sigma_i \left(Y_{i,exp} - \bar{Y}\right)^2} \qquad (1)$$

On what:

$Y_{i,exp}$ is the experimental data value; $Y_{i,est}$ is the value estimated by the model; $\bar{Y}$ is the mean of the experimental data.

To select the model that best describes the bioprocess, the following error functions were performed: root mean square error (RMSE) and Akaike Information Criterion (AIC) (Akaike, 1998; Jahedsaravani; Marhaban; Massinaei, 2014; Lima; Mamede; Lima Neto, 2018). The greater the adequacy of the data estimated by the kinetic model to the experimental data, the smaller the error value. The error functions were calculated according to Eq. (2) and (3):

$$RMSE = \sqrt{\frac{\Sigma_i (Y_{i,exp} - Y_{i,est})^2}{n}} \qquad (2)$$

On what:

$n$ is the number of experimental data points (observations).

$$AIC = n \cdot ln(\frac{SS}{n}) + 2np \qquad (3)$$

On what:

AIC is the Akaike Information Criterion (dimensionless); SS is the squared sum of the residuals; $n$ is the number of experimental data observations; $np$ is the number of model parameters.

All work results were analyzed statistically using the Microcal software Origin 8.1 (Microcal Software Inc., Northampton, MA, USA) using analysis of variance (ANOVA) with a confidence level of 95% and a probability of 5% ($p < 0.05$). Tukey tests compared the different dosages (GAC concentrations of 3, 6, and 9 g/L). Thus, data was presented using the average value followed by the letter of the statistical treatment, in which equal letters mean no significant difference for $p < 0.05$.

### *4.2.7 DNA extraction, 16S rRNA gene sequencing, and data processing*

Genetic sequencing analyses and data processing were carried out in the Microbial and Molecular Ecology Laboratory of the UFC Biology Department. DNA was extracted from samples collected from the reactors using PowerSoil® (MoBio Laboratories Inc., USA) according to the manufacturer's instructions. The amplicon library for the V4 region of the 16S rRNA gene was prepared as described by Illumina (2013) using region-specific primers (515F/806R). After indexing, PCR products were cleaned with Agencourt AMPure XP - PCR purification beads (Beckman Coulter, Brea, CA, USA) based on the manufacturer's instructions and quantified with the dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Libraries were sequenced using the MiSeq 300-Cycle Reagent Kit v2 (Illumina, 2013) with a MiSeq Desktop Sequencer (ILLUMINA).

The data obtained by sequencing were analyzed with bioinformatics tools as follows. Sequencing reads were trimmed, assembled, and denoised using DADA2 v1.28.0 (Callahan et al., 2016). The resulting amplicon sequencing variants (ASVs) were taxonomically classified using the IDTAXA classifier (Murali; Bhargava; Wright, 2018) from DECIPHER package v2.28.0 (Wright, 2016) based on release 138 of SILVA rRNA database (Quast et al., 2013).

## 4.3 Results and Discussion

### *4.3.1 Degradation of organic matter and methane-rich biogas yield*

The total solids, volatile solids, and total organic carbon contents of the soluble fraction measured at the beginning and end of the experiment are shown in Table 4.3. At the same time, the yield of biogas and methane and biogas composition are shown in Table 4.4, where the difference in letters at columns for groups separated by line gives the statistical difference.

According to the data obtained, there was a greater reduction in total and volatile solids in reactors with the addition of granular activated carbon (GAC) in both semi-dry and dry AD. However, this reduction was greater in the semi-dry condition, indicating greater organic matter degradation.

Concerning the total organic carbon of the soluble fraction (TOCs), there was an increase in the control reactor $CT_S$, which characterizes the hydrolysis of the organic matter remaining in the sludge and possible cellular hydrolysis due to substrate scarcity. In reactors with glucose addition, the final concentration of TOCs decreased compared to the initial concentration, which can be explained by the consumption of this soluble substrate throughout the bioprocess. However, there was no difference when adding GAC, which corroborates the similar amount of methane produced in $CT_{SG}$ and $CT_{SG\text{-}GAC}$ (Table 4.4.).

So, the sludge used had satisfactory methanogenic activity as the control of sludge and glucose ($CT_S$) produced 18 mL $CH_4$/gVS more than the control of sludge ($CT_S$), and the maximum theoretical production estimated for the addition of glucose would be around 21 mL $CH_4$/gVS. The conductive material did not cause a significant difference in the final yield of biogas and methane of the positive control of sludge with glucose (Table 4.4).

Table 4.3 – Analysis of solid content and total organic carbon (TOC).

| **Reactors** | **TS (%)** | | **VS (%)** | | **TOCs (mg/L)** | |
|---|---|---|---|---|---|---|
| | **Initial** | **Final** | **Initial** | **Final** | **Initial** | **Final** |
| $CT_S$ | 12.4 | 11.2 | 9.4 | 8.4 | 771 | 1,195 |
| $CT_{SG}$ | 11.8 | 9.6 | 9.3 | 6.7 | 6,516 | 932 |
| $CT_{SG\text{-}GAC}$ | 13.4 | 11.6 | 9.6 | 8.8 | 5,638 | 1,066 |
| 10RSM | 11.1 | 9.5 | 6.6 | 6.8 | 5,328 | 11,687 |
| $10RSM_{GAC20}$ | 12.2 | 9.0 | 8.7 | 6.2 | 4,684 | 1,664 |
| 10PSM | 10.9 | 9.7 | 6.5 | 6.5 | 7,356 | 12,743 |
| $10PSM_N$ | 11.3 | 10.3 | 6.9 | 7.2 | 6,410 | 12,612 |
| $10PSM_{GAC10}$ | 11.5 | 8.4 | 7.1 | 5.2 | 7,460 | 4,405 |
| $10PSM_{GAC20}$ | 12.5 | 8.7 | 8.6 | 5.4 | 6,744 | 2,695 |
| $10PSM_{GAC30}$ | 13.4 | 9.9 | 8.8 | 7.0 | 6,388 | 2,168 |
| 15RSM | 15.2 | 14.1 | 11.3 | 10.7 | 5,990 | 15,284 |
| $15RSM_{GAC20}$ | 17.3 | 15.2 | 13.1 | 11.5 | 5,813 | 4,047 |
| 15PSM | 14.5 | 13.2 | 8.4 | 9.5 | 9,195 | 15,327 |
| $15PSM_{GAC10}$ | 15.3 | 13.4 | 11.1 | 9.8 | 6,994 | 15,493 |
| $15PSM_{GAC20}$ | 16.1 | 12.7 | 11.5 | 8.3 | 6,323 | 6,393 |
| $15PSM_{GAC30}$ | 17.1 | 12.7 | 11.1 | 9.0 | 5,989 | 4,182 |

Legend: $TOC_S$ - total organic carbon of soluble fraction; $CT_S$ – sludge; $CT_{SG}$ – sludge + glucose; $CT_{SG\text{-}GAC}$ – sludge + glucose + 20 g GAC/L; 10RSM – raw swine manure + sludge (10% TS); $10RSM_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (10% TS); 10PSM – pretreated swine manure + sludge (10% TS); $10PSM_N$ – pretreated swine manure + sludge + 20 g/L nylon (10% TS); $10PSM_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (10% TS); $10PSM_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (10% TS); $10PSM_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (10% TS); 15RSM – raw swine manure + sludge (15% TS); $15RSM_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (15% TS); 15PSM – pretreated swine manure + sludge (15% TS); $15PSM_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (15% TS); $15PSM_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (15% TS); $15PSM_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (15% TS).
Source: Author (2023).

Table 4.4 – Biogas and methane yields and biogas composition.

| Reactors | Biogas Yield (mL/gVS) | Methane Yield (mL/gVS) | Methane composition (%) |
|---|---|---|---|
| CT$_{\text{S}}$ | 111$^{a}$ | 69$^{a}$ | 62$^{a}$ |
| CT$_{\text{SG}}$ | 189$^{b}$ | 96$^{b}$ | 51$^{b}$ |
| CT$_{\text{SG-GAC}}$ | 185$^{b}$ | 95$^{b}$ | 51$^{b}$ |
| 10RSM | 69$^{a}$ | 3$^{a}$ | 5$^{a}$ |
| 10RSM$_{\text{GAC20}}$ | 276$^{b}$ | 154$^{b}$ | 56$^{b}$ |
| 10PSM | 73$^{a}$ | 3$^{a}$ | 4$^{a}$ |
| 10PSM$_{\text{N}}$ | 76$^{a}$ | 3$^{a}$ | 4$^{a}$ |
| 10PSM$_{\text{GAC10}}$ | 361$^{c}$ | 190$^{c}$ | 53$^{b}$ |
| 10PSM$_{\text{GAC20}}$ | 276$^{b}$ | 155$^{b}$ | 56$^{b}$ |
| 10PSM$_{\text{GAC30}}$ | 276$^{b}$ | 158$^{b}$ | 57$^{b}$ |
| 15RSM | 56$^{a}$ | 2$^{a}$ | 4$^{a}$ |
| 15RSM$_{\text{GAC20}}$ | 201$^{d}$ | 111$^{d}$ | 55$^{b}$ |
| 15PSM | 97$^{a}$ | 8$^{a}$ | 8$^{a}$ |
| 15PSM$_{\text{GAC10}}$ | 72$^{a}$ | 18$^{a}$ | 25$^{c}$ |
| 15PSM$_{\text{GAC20}}$ | 251$^{b,d}$ | 127$^{d}$ | 51$^{b}$ |
| 15PSM$_{\text{GAC30}}$ | 290$^{b}$ | 157$^{b}$ | 54$^{b}$ |

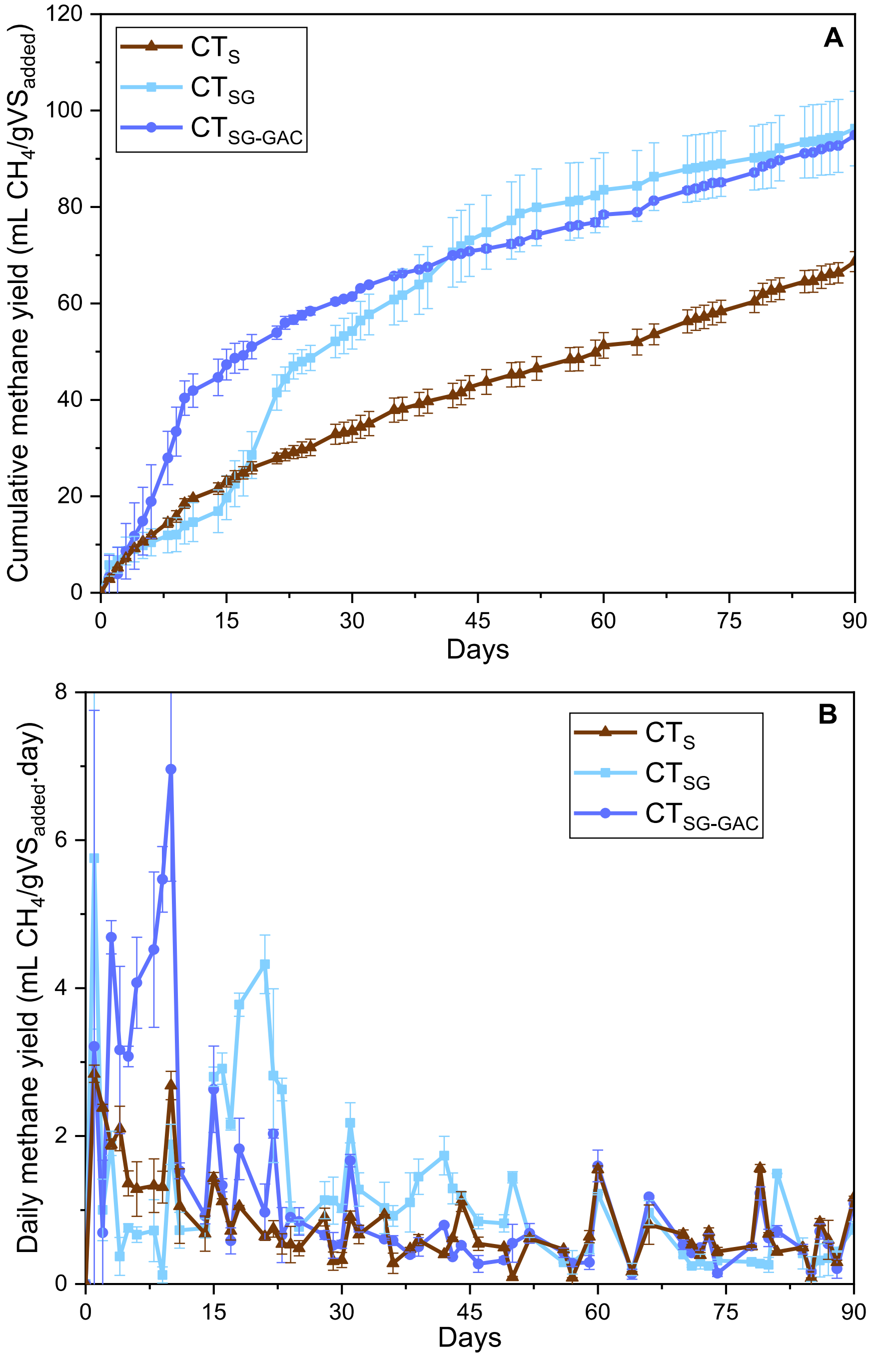
Legend: CT$_{\text{S}}$ – sludge; CT$_{\text{SG}}$ – sludge + glucose; CT$_{\text{SG-GAC}}$ – sludge + glucose + 20 g GAC/L; 10RSM – raw swine manure + sludge (10% TS); 10RSM$_{\text{GAC20}}$ – raw swine manure + sludge + 20 g GAC/L (10% TS); 10PSM – pretreated swine manure + sludge (10% TS); 10PSM$_{\text{N}}$ – pretreated swine manure + sludge + 20 g/L nylon (10% TS); 10PSM$_{\text{GAC10}}$ – pretreated swine manure + sludge + 10 g GAC/L (10% TS); 10PSM$_{\text{GAC20}}$ – pretreated swine manure + sludge + 20 g GAC/L (10% TS); 10PSM$_{\text{GAC30}}$ – pretreated swine manure + sludge + 30 g GAC/L (10% TS); 15RSM – raw swine manure + sludge (15% TS); 15RSM$_{\text{GAC20}}$ – raw swine manure + sludge + 20 g GAC/L (15% TS); 15PSM – pretreated swine manure + sludge (15% TS); 15PSM$_{\text{GAC10}}$ – pretreated swine manure + sludge + 10 g GAC/L (15% TS); 15PSM$_{\text{GAC20}}$ – pretreated swine manure + sludge + 20 g GAC/L (15% TS); 15PSM$_{\text{GAC30}}$ – pretreated swine manure + sludge + 30 g GAC/L (15% TS). Equal letters mean no significant difference ($p <$ 0.05) within the group separated by line.
Source: Author (2023).

However, from Figure 4.3A, it is possible to observe that the presence of granular activated carbon promoted a difference in the methane production curve, making it more pronounced in the first days of the experiment and providing a shorter lag phase.

For both conditions, with and without GAC (CT$_{\text{SG}}$ and CT$_{\text{SG-GAC}}$), a short lag phase was observed, characterized by the slope of the straight line in the first days of the bioprocess, due to the presence of glucose, an easily bioavailable substrate for microorganisms. Organic residues containing complex macromolecules must first be converted into glucose or other oligomers and then transformed into volatile fatty acids, which increases the lag phase (LIU et al., 2016). Even so, the presence of the conductive material enabled faster methane production compared to the reactor that did not have GAC.

Although the maximum daily methane yield was reached by CT$_{\text{SG}}$ on the first day (5.8 mL $CH_4$/gVS.d), this yield soon showed a sharp drop, remaining below 2 mL $CH_4$/gVS.d until the 13th day and reached a new peak again on the 21st day (4.3 mL $CH_4$/gVS.d) – Figure 4.3B. CT$_{\text{SG-GAC}}$ showed an increasing trend in daily methane yield until the 10th day, when it reached its maximum value (7.0 mL $CH_4$/gVS.d). So, the condition with GAC showed higher daily methane production until the 12th day and had a methane production curve with a greater slope of the straight line. This favoring of methanogenesis in the presence of GAC is linked to the promotion of direct interspecies electron transfer (DIET).

Figure 4.3 – Cumulative (A) and daily (B) methane yield of reactors of sludge and sludge and glucose with and without the addition of conductive material – granular activated carbon (GAC).



Legend: $CT_S$ – sludge; $CT_{SG}$ – sludge + glucose; $CT_{SG\text{-}GAC}$ – sludge + glucose + 20 g GAC/L.
Source: Author (2023).

In semi-dry AD, the reactors with pretreated manure without additives (10PSM) and with the addition of nylon ($10PSM_N$), there was an increase of 67-93% in TOCs, while for the reactors with GAC ($10PSM_{GAC10}$, $10PSM_{GAC20}$ and $10PSM_{GAC30}$) there was a reduction 42-66% of TOCs. The TOCs increase phenomenon is explained by the initial organic matter hydrolysis, which happened in all reactors. However, its reduction occurs with the conversion of organic matter into methane, which only occurs effectively in reactors with the addition of GAC.

Therefore, when using pretreated pig manure instead of glucose, the effect of the additive was even more pronounced. At 10% TS, when comparing reactors without GAC (10PSM – 73 and 3 mL /VS of biogas and methane) and 20g GAC/L ($10PSM_{GAC20}$ – 276 and 155 mL/VS of biogas and methane), a 2.8-fold increase in the accumulated biogas yield was observed with GAC addition and significant methane production. At the same time, almost no methane was generated in the absence of the conductive material.

This occurs because when a conductive material is present, the system is not dependent of indirect electron transport, that depends on the concentration of metabolites ($H_2$ and formate), which must be kept low enough to provide favorable thermodynamic conditions for the reaction, i.e., metabolites diffusion is a limiting factor (Su et al., 2023; Wu et al., 2020). This explains the low production in reactors without GAC, in which there is initial hydrolysis of the substrate by fermentative bacteria that releases electrons, then the protons generated in the hydrolysis are reduced to $H_2$ in the presence of hydrogenases, subsequently used by hydrogenotrophic methanogens to generate methane (Su et al., 2023).

Another feature of GAC that improves the biomethanization process is the property of being a porous material, which facilitates the formation of microbial biofilms on its surface, providing more places for the microbial community to attach, reducing diffusion restrictions and assisting in the exchange of electrons between species, i.e., facilitating syntrophic interactions between microbial communities and the proliferation of those that facilitate organic matter conversion (Dang et al., 2017; Florentino et al., 2019; Kalantzis et al., 2023).

In this study, a control group was also used with the addition of nylon, a non-conductive material, to verify whether the effect of adding GAC was, in fact, due to its capacity as a conductive material or just because it is a support medium that allows biofilm formation. The biogas and methane production yield of the nylon condition ($10PSM_N$) was similar to that of the condition without additive (10PSM). Compared to the reactor with GAC ($10PSM_{GAC20}$), it produced 72% less biogas and almost no methane – 3 mL $CH_4$/gVS. This result demonstrates that, even when added at the same concentration, the conductive characteristics of GAC prevailed over the physical support material property also present in nylon; that is, the high biogas production and its methane content from the $10PSM_{GAC20}$ reactor is due to the promotion of direct interspecies electron transfer (DIET) and previously described GAC properties.

A similar study was carried out elsewhere (Altamirano-Corona; Anaya-Reza; Durán-Moreno, 2021), where the conductive effect of GAC was compared to the addition of non-conductive materials, such as granular silica gel and glass beads. This is extremely important for better comparative effects, as the non-conductive material can also function as a support material for microorganisms, which use the additive as physical support for biofilm growth and facilitate syntrophic relationships (Wu et al., 2020).

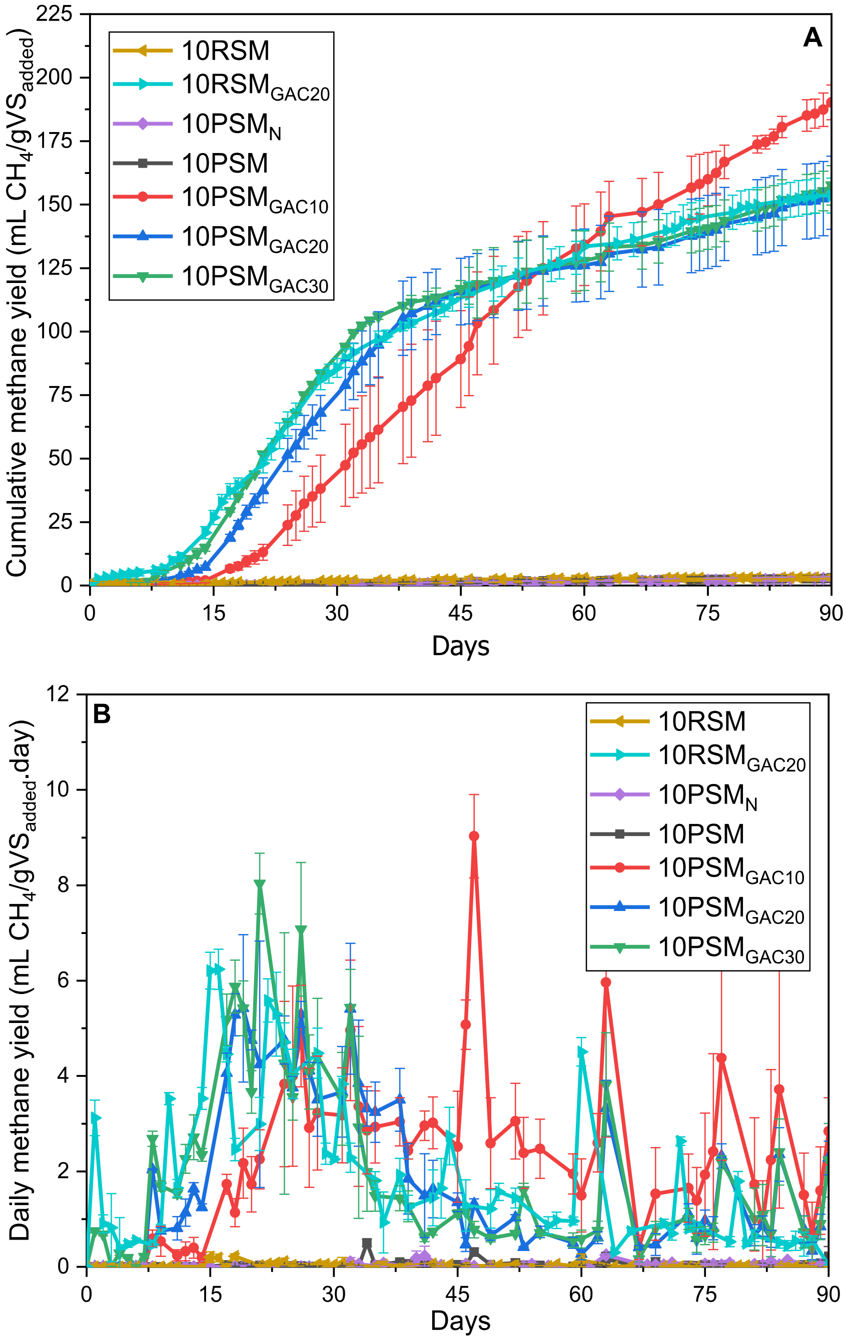
Regarding the GAC addition dosage study, the reactors with different dosages of granular activated carbon added (10, 20, and 30 g/L) resulted in significant differences in the volumetric production of biogas and methane (Table 4.4 and Figure 4.4). However, the biogas composition was similar (53-57 $\%CH_4$).

In the present study, at the end of the semi-dry anaerobic digestion process of pretreated swine manure, the dosage of 10 g GAC/L ($10PSM_{GAC10}$) resulted in a higher biogas production compared to dosages of 20 and 30 g GAC/L ($10PSM_{GAC20}$ and $10PSM_{GAC30}$), producing on average 30% more biogas and 23% more methane. However, it is important to note in Figure 4.4A that at the beginning of the experiment, $10PSM_{GAC30}$ has a cumulative methane yield slightly higher than $10PSM_{GAC20}$ and much higher when compared to $10PSM_{GAC10}$. Therefore, a higher concentration of GAC promoted faster methane production. However, around day 55, methane production in $10PSM_{GAC10}$ surpassed the others, showing that over time, in which there is a greater adaptation of microorganisms, then a lower concentration of GAC (10 g/L) was more satisfactory considering 90 days of the experiment.

When analyzing the daily methane yield at 10% TS (Figure 4.4B), it is precisely observed that $10PSM_{GAC30}$ achieved better results up to the 32nd day, with a maximum value of 8.0 mL $CH_4$ gVS on the 21st day, followed by $10PSM_{GAC20}$ with a maximum productivity of 5.4 mL $CH_4$/gVS on the 19th day. However, from the 39th day onwards, $10PSM_{GAC10}$ starts to present the highest daily methane yield, reaching a maximum value of 9.0 mL $CH_4$/gVS on the 47th day, and continues with the best results until the end of the experiment.

Other studies that compared different dosages of GAC showed an optimal concentration of 20 g GAC/L during ethanol digestion (Lin et al., 2017), 15 g GAC/L in sewage sludge digestion (Azizi et al., 2023), 12 g GAC/L in the digestion of food waste (Zhang; Zhang; Loh, 2018), among others. Therefore, different substrates and operating conditions require specific GAC concentrations.

Figure 4.4 – Cumulative (A) and daily (B) methane yield of the reactors with the addition of different dosages of granular activated carbon (GAC) under semi-dry AD conditions.



Legend: 10RSM – raw swine manure + sludge (10% TS); 10RSM$_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (10% TS); 10PSM – pretreated swine manure + sludge (10% TS); 10PSM$_N$ – pretreated swine manure + sludge + 20 g/L nylon (10% TS); 10PSM$_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (10% TS); 10PSM$_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (10% TS); 10PSM$_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (10% TS).
Source: Author (2023).

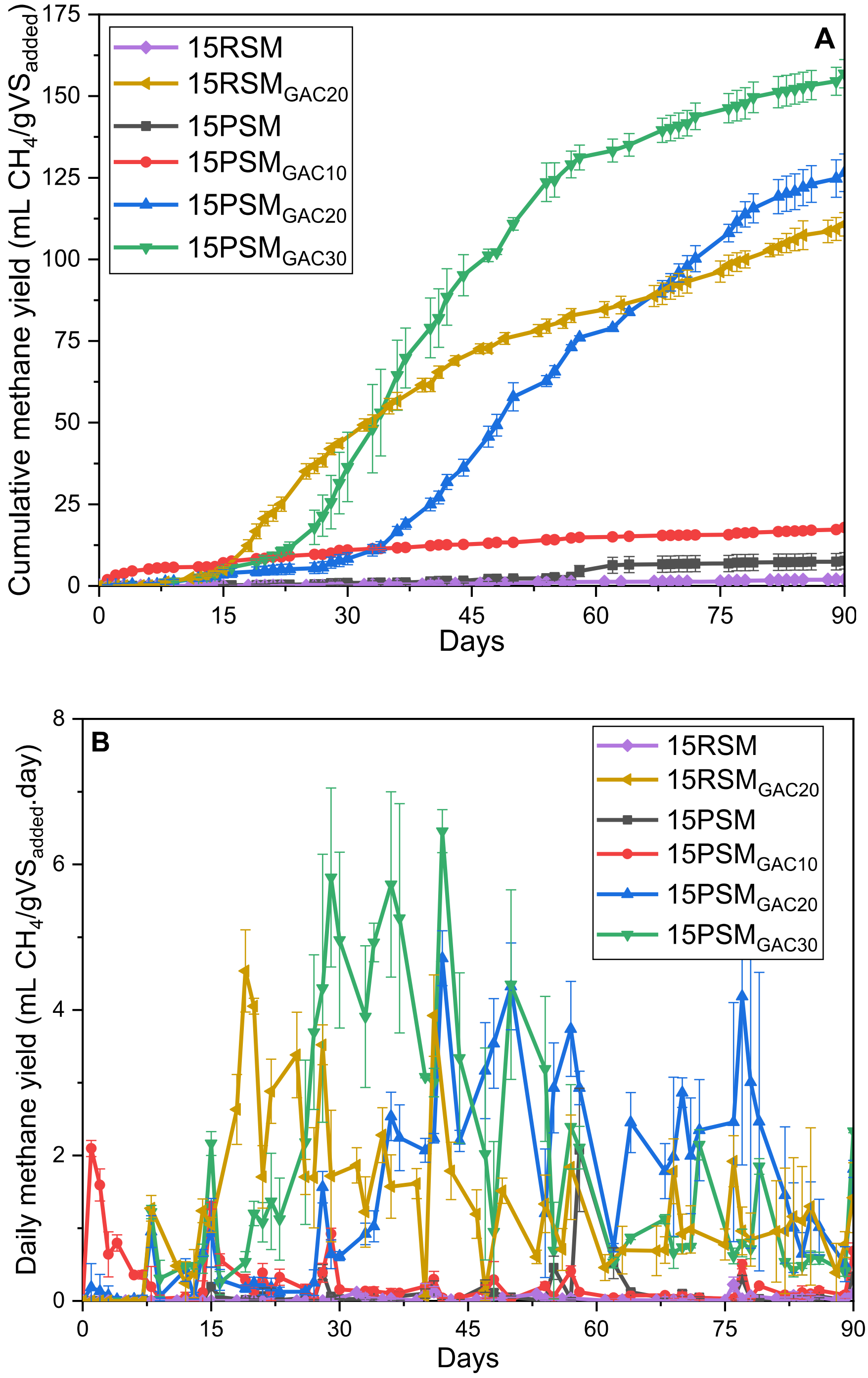
On the other hand, in dry AD, the effect of GAC dosage was different. By increasing the TS content from 10 to 15%, using a concentration of 10 g GAC/L, there was a reduction of 80 and 90% in cumulative biogas and methane yield, respectively. Biogas composition was only 34%, a value below that expected for anaerobic digestion, usually 50 to 70% (Unpaprom et al., 2021). However, the methane composition was satisfactory for concentrations of 20 and 30 g GAC/L (56-63% $CH_4$). Compared with semi-dry AD, reactors with 20 g GAC/L showed a 20% reduction in the final methane yield, while with 30 g GAC/L, there was no significant difference (Table 4.4). This corroborates with TOC results, in which there was only a reduction in TOC (30%) for the concentration of 30 g GAC/L ($15PSM_{GAC30}$). In comparison, $15PSM_{GAC20}$ presented very similar initial and final TOC values, and $10PSM_{GAC10}$ showed a 120% increase in TOC at the end of the process, indicating that the methanogenic step was not effective.

This occurs because increasing the total solids content is a challenge for AD, and low concentrations of GAC could not offer greater resilience to the system. Dry AD presents limitations for substrate diffusion and negative effects on microbial metabolism due to the lower water content. Thus, the mass transfer becomes more difficult, impacting intermediate product assimilation and reducing methane production efficiency (Momayez; Karimi; Taherzadeh, 2019; Rocamora et al., 2020).

Regarding the daily methane yield in dry AD (Figure 4.5B), $15PSM_{GAC10}$ reached maximum productivity on the first day. However, it was only 2.1 mL $CH_4$/gVS and presented low values until the end of the experiment. $15PSM_{GAC20}$ reached its maximum yield on the 42nd day (4.7 mL $CH_4$/gVS), and from the 55th day, it remained higher than $15PSM_{GAC30}$, which was the condition that presented the highest methane yields with a peak of 6.4 mL $CH_4$/gVS on the 42nd day. Analyzing the cumulative curve methane yield in dry AD (Figure 4.5A), it is observed that the concentration of 30 g GAC/L presented better performance, justified by the higher daily productivity between days 34 and 42.

Just as biogas production depends on the amount of organic matter present, the concentration of GAC is also directly related to the organic load content present. Previous studies have reported that increasing the concentration of activated carbon tends to result in a greater volume of biogas as the load of organic matter increases (Zhang; Zhang; Loh, 2018).

Figure 4.5 – Cumulative (A) and daily (B) methane yield of the reactors with the addition of different dosages of granular activated carbon (GAC) under dry AD conditions.



Legend: 15RSM – raw swine manure + sludge (15% TS); $\text{15RSM}_{\text{GAC20}}$ – raw swine manure + sludge + 20 g GAC/L (15% TS); 15PSM – pretreated swine manure + sludge (15% TS); $\text{15PSM}_{\text{GAC10}}$ – pretreated swine manure + sludge + 10 g GAC/L (15% TS); $\text{15PSM}_{\text{GAC20}}$ – pretreated swine manure + sludge + 20 g GAC/L (15% TS); $\text{15PSM}_{\text{GAC30}}$ – pretreated swine manure + sludge + 30 g GAC/L (15% TS).
Source: Author (2023).

When using raw swine manure, it is possible to see in the variation in solids contents that the reactor with GAC and 10% total solids achieved a more significant reduction in both TS and VS, 26 and 29%, respectively. This result indicates how GAC strengthened the efficiency of methanogenesis since VS is the leading organic fraction for methane production (Johnravindar et al., 2020). In the reactors without adding GAC, the TOCs increased by 120% (10RSM) and 155% (15RSM). On the other hand, the reactors with the addition of GAC showed a reduction in the concentration of soluble organic matter (65% – $10RSM_{GAC20}$ and 30% – $15RSM_{GAC20}$). These results are in line with methane production (Figures 4.4A and 4.5A).

The reactors without conductive material presented 56.4-68.8 mL/gVS of biogas but did not show significant methane production in the semi-dry or dry AD condition. The presence of GAC provided volumetric biogas yield of 275.6 and 201.1 mL/gVS for 10 and 15% of total solids, respectively, and volumetric methane yield of 153.6 and 110.1 mL $CH_4$/gVS for 10 and 15% of total solids, respectively. So, by increasing the TS content, methane production fell by about 35%.

Regarding daily methane production (Figures 4.4B and 4.5B), the best result was achieved by the $10RSM_{GAC20}$ condition, which reached a maximum value of 6.2 mL/gVS on the 15th day, maintaining significant values until the 30th day, when it starts to show a tendency to drop and stabilization of methane production. However, on the 60th day, there is a more pronounced peak (4.5 mL/gVS), after which the trend of production stabilization returns. After so many days of batch operation, this peak suggests a new hydrolysis phase of more complex material that was later hydrolyzed and only then converted into methane.

However, $15RSM_{GAC20}$ reached its maximum daily methane yield of 4.5 mL/gVS on the 19th day, showing a decrease and fluctuations in its production volume until the 41st when it reached a new peak (3.9 mL/gVS). Then, the reactor returned to display a stability trend. In general, the daily productivity of $15RSM_{GAC20}$ was lower than $10RSM_{GAC20}$. This is due to the characteristics of swine manure combined with a high solids content (15%), meaning that the substrate is not easily digested, taking longer to be hydrolyzed and converted into methane during AD. As mentioned, the reactors only obtained significant methane production by adding GAC.

Finally, it is also noted that, in the present study, the pretreatment did not promote a significant difference in biogas yield, methane yield, and methane composition between reactors without GAC addition with raw and pretreated manure nor between reactors with 20g GAC/L addition with raw and pretreated manure under two conditions evaluated (10 and 15%

TS). So, to optimize the production of methane-rich biogas, only the addition of the conductive material was necessary.

### *4.3.2 Ammoniacal nitrogen concentration and pH*

The ammonia nitrogen and pH values at the beginning and end of the bioprocess are shown in Table 4.5. In general, throughout the experiment there was an increase in the concentration of ammonia and consequently there was an increase in pH as well. The ammonia concentration inhibiting the anaerobic digestion is between 1,500 and 7,000 mg/L in varied substrates (Chowdhury et al., 2019). Even the control reactors $CT_S$ and $CT_{SG\text{-}GAC}$ were within this inhibition range, although at the beginning of it. This demonstrates that the sludge used is rich in nitrogenous compounds.

In dry AD, the ammonia nitrogen values were higher than in semi-dry AD, contributing to the lower methane production yields in reactors with 15% ST. Furthermore, reactors with activated carbon showed lower ammonia concentration at the end of the process, probably due to the adsorption capacity on the GAC surface (Dang et al., 2017; Florentino et al., 2019; Xu et al., 2022).

Certainly, ammonia is not the only inhibitor of the bioprocess, and GAC helped in the adsorption of other inhibitors throughout AD. In previous studies, granular activated carbon in the anaerobic digestion of swine manure also promoted the absorption of organic solvents and heavy metals through its porous and rough surface (Xiao et al., 2019).

Table 4.5 – Results of soluble ammonia nitrogen and pH of the reactors.

| Reactors | Soluble Ammonia Nitrogen (mg/L) | | pH | |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| $CT_S$ | 288 | 1,820 | 7.8 | 9.0 |
| $CT_{SG}$ | 291 | 714 | 7.9 | 8.9 |
| $CT_{SG\text{-}GAC}$ | 286 | 1,652 | 7.9 | 8.9 |
| 10RSM | 633 | 3,085 | 7.7 | 7.9 |
| $10RSM_{GAC20}$ | 535 | 2,114 | 7.8 | 8.6 |
| $10PSM_N$ | 392 | 2,235 | 7.6 | 8.0 |
| 10PSM | 501 | 2,352 | 7.7 | 8.2 |
| $10PSM_{GAC10}$ | 462 | 1,997 | 7.5 | 8.5 |
| $10PSM_{GAC20}$ | 476 | 2,091 | 7.7 | 8.6 |
| $10PSM_{GAC30}$ | 546 | 1,526 | 7.5 | 8.3 |
| 15RSM | 672 | 3,304 | 7.7 | 8.2 |
| $15RSM_{GAC20}$ | 679 | 3,033 | 8.0 | 9.0 |
| 15PSM | 553 | 2,968 | 7.7 | 8.6 |
| $15PSM_{GAC10}$ | 567 | 3,211 | 7.9 | 8.5 |
| $15PSM_{GAC20}$ | 465 | 2,417 | 8.0 | 9.0 |
| $15PSM_{GAC30}$ | 381 | 2,641 | 7.8 | 9.0 |

Legend: $CT_S$ – sludge; $CT_{SG}$ – sludge + glucose; $CT_{SG\text{-}GAC}$ – sludge + glucose + 20 g GAC/L; 10RSM – raw swine manure + sludge (10% TS); $10RSM_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (10% TS); 10PSM – pretreated swine manure + sludge (10% TS); $10PSM_N$ – pretreated swine manure + sludge + 20 g/L nylon (10% TS); $10PSM_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (10% TS); $10PSM_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (10% TS); $10PSM_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (10% TS); 15RSM – raw swine manure + sludge (15% TS); $15RSM_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (15% TS); 15PSM – pretreated swine manure + sludge (15% TS); $15PSM_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (15% TS); $15PSM_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (15% TS); $15PSM_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (15% TS).
Source: Author (2023).

### 4.3.3 Kinetic modeling of methane production

Table 4.6 presents the values of the reactor's kinetic parameters and the error functions. Analyzing the $CT_{SG}$ and $CT_{SG\text{-}GAC}$ control groups, it was observed that the selected models presented a good fit to the experimental data, especially Transfer. The addition of GAC in the glucose control promoted a 13% increase in methane first-order production rate, although there was no significant difference in the maximum rate of methane production ($\mu_m$), and reduction of the lag phase ($\lambda$) from 2.6 to zero days, then the presence of conductive material favored the kinetics of methane production.

The model that best described methane production from semi-dry and dry AD from swine manure was the Modified Gompertz, a sigmoidal model very used for describing AD of complex substrates, showing the best fit to experimental data, which had high values of $R^2$ and lower values of RMSE and AIC (Table 4.6).

Among the kinetic coefficients estimated by this model, when using raw swine manure with a GAC addition of 20g/L, the maximum methane production rate ($\mu_m$) was approximately 38% lower in dry conditions than in semi-dry conditions, and the lag phase increased from 6.5 to 10.6 days. For pretreated swine manure with the same GAC addition, there was no significant difference in $\mu_m$ when increasing the total solids content, but the lag phase increased from 10.4 to 32.0 days. Therefore, increasing the total solids content caused a reduction in the maximum methane production rate for raw manure and an increase in the lag phase for pretreated manure. As previously discussed, this occurs because increasing the total solids content is a challenge for AD, even with the addition of conductive material that promotes DIET and makes the methane production process faster and more efficient. In conditions of higher TS content, there is a greater limitation for metabolite diffusion in the system and negative effects on microbial metabolism due to the lower water content. Thus, the mass transfer becomes more difficult, impacting intermediate product assimilation and reducing methane production efficiency and velocity (Momayez; Karimi; Taherzadeh, 2019; Rocamora et al., 2020).

However, comparing the difference between raw and pretreated substrate both with 20g GAC/L, in semi-dry AD, there was no significant difference in parameters $\mu_m$ but the $\lambda$ increased from 6.5 to 10.4 days when pretreatment was applied. While in the dry condition, using pretreatment caused an increase of 44% in $\mu_m$, although it increased $\lambda$ from 10.6 to 32.0 days. Pretreatment can increase the rate of methane production because, in the pretreated substrate, the organic matter is in a simpler form and becomes more bioavailable to microorganisms. However, the pretreatment process can release more inhibitory compounds into the medium, such as hydroxymethylfurfural, phenol, trace elements, sulfide, humic acid, and heavy metals, and this makes it difficult for microorganisms to adapt to the environment, leading to a longer lag phase (Awogbemi; Vandi; Kallon, 2022; Kumar et al., 2022; Preethi et al., 2022).

Concerning the GAC dosage study with pretreated swine manure, there was no significant difference in $\mu_m$ in semi-dry AD with the increase in GAC concentration. At the same time, $\lambda$ reduced from 19.0 to 10.4 and 7.2 when the GAC concentration increased from 10 to 20 and 30 g/L, respectively. For dry conditions, the reaction with 10 g GAC/L had lower $\mu_m$ and $\lambda = 0$ but presented methane production only in the first few days, possibly because its GAC active sites were saturated and thus unable to promote the DIET throughout the AD process period. When increasing from 20 to 30 g GAC/L, there was an increase of 38% in $\mu_m$ and a reduction in $\lambda$ from 32.0 to 21.3 days. Therefore, for a higher TS content in the medium, the kinetics are more favored with a higher concentration of GAC.

Table 4.6 – Parameters estimated by kinetic modeling of methane production by anaerobic digestion.

| Model | Parameters | Reactors | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CT$_{SG}$ | CT$_{SG\text{-}GAC}$ | 10RSM$_{GAC20}$ | 10PSM$_{GAC10}$ | 10PSM$_{GAC20}$ | 10PSM$_{GAC30}$ | 15RSM$_{GAC20}$ | 15PSM$_{GAC10}$ | 15PSM$_{GAC20}$ | 15PSM$_{GAC30}$ |
| **First Order** | **k(1/d)** | $0.029^{a}$ | $0.036^{b}$ | $0,027^{a}$ | $0.017^{c}$ | $0.024^{a}$ | $0.026^{a}$ | $0,021^{c}$ | $0.031^{a}$ | $0.013^{c}$ | $0.019^{c}$ |
| | **R$^2$** | 0.966 | 0.969 | 0,940 | 0.841 | 0.908 | 0.930 | 0,905 | 0.968 | 0.733 | 0.815 |
| | **AIC** | 205,044 | 176,200 | 300,934 | 391,536 | 336,795 | 318,301 | 285,149 | -21.530 | 368,331 | 375,845 |
| **Second Order** | **k'' (L/g.d)** | $5.3\cdot10^{-4a}$ | $7.3\cdot10^{-4a}$ | $2.9\cdot10^{-4c}$ | $1.2\cdot10^{-4c}$ | $2.5\cdot10^{-4c}$ | $2.7\cdot10^{-4c}$ | $2.9\cdot10^{-4c}$ | $3.3\cdot10^{-3d}$ | $1.3\cdot10^{-4c}$ | $1.8\cdot10^{-4c}$ |
| | **R$^2$** | 0.854 | 0.955 | 0,820 | 0.730 | 0.793 | 0.822 | 0,788 | 0.929 | 0.630 | 0.692 |
| | **AIC** | 289,399 | 196,713 | 365,044 | 422,937 | 384,699 | 373,684 | 331,077 | 23,606 | 386,847 | 404,998 |
| **Monomolecular** | **k(1/d)** | $0.032^{a}$ | $0.036^{a}$ | $0,032^{a}$ | $0.021^{b}$ | $0.030^{a}$ | $0.031^{a}$ | $0,026^{a}$ | $0.031^{a}$ | $0.018^{b}$ | $0.025^{a,b}$ |
| | **λ (d)** | $2.617^{a}$ | $0.000^{b}$ | $4,698^{c}$ | $9.181^{d}$ | $6.243^{c}$ | $5.217^{c}$ | $7,067^{c}$ | $0.000^{b}$ | $11.906^{e}$ | $9.091^{d}$ |
| | **R$^2$** | 0.975 | 0.969 | 0,968 | 0.906 | 0.953 | 0.965 | 0,952 | 0.968 | 0.812 | 0.882 |
| | **AIC** | 188,924 | 178,200 | 266,614 | 362,751 | 299,130 | 280,177 | 248,228 | -19,530 | 350,277 | 352,172 |
| **Logistics** | **μ$_m$(mL/gVS.d)** | $1.846^{a}$ | $1,984^{a}$ | $3,253^{b}$ | $3.664^{b}$ | $3.616^{b}$ | $3.458^{b}$ | $2,007^{a}$ | $0.322^{c}$ | $2,940^{b}$ | $4,140^{b}$ |
| | **λ (d)** | $2.621^{a}$ | $0.000^{a}$ | $7,254^{b}$ | $21.154^{c}$ | $11.791^{b}$ | $8.131^{b}$ | $11,676^{b}$ | $0.000^{a}$ | $33.519^{d}$ | $22.606^{c}$ |
| | **R$^2$** | 0.980 | 0.854 | 0,978 | 0.989 | 0.968 | 0.954 | 0,971 | 0.908 | 0.994 | 0.993 |
| | **AIC** | 175,922 | 267,567 | 246,207 | 233,671 | 277,211 | 296,385 | 219,679 | 40,820 | 150,862 | 192,893 |
| **Modified Gompertz** | **μ$_m$(mL/gVS.d)** | $1.929^{a}$ | $2.142^{a}$ | $3.362^{b,d}$ | $3.655^{b,d}$ | $3.591^{b,d}$ | $3.506^{b,d}$ | $2.084^{a}$ | $0.347^{c}$ | $2.998^{d}$ | $4.145^{b}$ |
| | **λ (d)** | $2.211^{a}$ | $0.000^{b}$ | $6.503^{c}$ | $19.007^{d}$ | $10.425^{e}$ | $7.193^{c}$ | $10.617^{e}$ | $0.000^{b}$ | $31.983^{f}$ | $21.301^{d}$ |
| | **R$^2$** | 0.993 | 0.889 | 0,993 | 0.997 | 0.988 | 0.979 | 0,990 | 0.920 | 0.991 | 0.999 |
| | **AIC** | 119,131 | 251,324 | 178,661 | 165,941 | 220,946 | 250,618 | 161,628 | 32,791 | 169,704 | 93,628 |
| **Transfer** | **μ$_m$(mL/gVS.d)** | $3.042^{a}$ | $3.368^{a}$ | $4,846^{b}$ | $3.941^{a,b}$ | $4.566^{b}$ | $4,890^{b}$ | $2,809^{a}$ | $0.551^{c}$ | $2,260^{a}$ | $3,860^{a,b}$ |
| | **λ (d)** | $2.594^{a}$ | $0.000^{b}$ | $4,682^{c}$ | $9.145^{d}$ | $6.227^{c}$ | $5,200^{c}$ | $7,036^{c}$ | $0.000^{b}$ | $11.878^{e}$ | $9.076^{d}$ |
| | **R$^2$** | 0.975 | 0.968 | 0,968 | 0.906 | 0.953 | 0.964 | 0,953 | 0.968 | 0.810 | 0.881 |
| | **AIC** | 187,393 | 177,446 | 265,443 | 359,827 | 297,986 | 278,981 | 245,695 | -21.423 | 347,595 | 351,157 |

Legend: CT$_S$ – sludge; CT$_{SG}$ – sludge + glucose; CT$_{SG\text{-}GAC}$ – sludge + glucose + 20 g GAC/L; 10RSM – raw swine manure + sludge (10% TS); 10RSM$_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (10% TS); 10PSM – pretreated swine manure + sludge (10% TS); 10PSM$_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (10% TS); 10PSM$_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (10% TS); 10PSM$_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (10% TS); 15RSM – raw swine manure + sludge (15% TS); 15RSM$_{GAC20}$ – raw swine manure + sludge + 20 g GAC/L (15% TS); 15PSM – pretreated swine manure + sludge (15% TS); 15PSM$_{GAC10}$ – pretreated swine manure + sludge + 10 g GAC/L (15% TS); 15PSM$_{GAC20}$ – pretreated swine manure + sludge + 20 g GAC/L (15% TS); 15PSM$_{GAC30}$ – pretreated swine manure + sludge + 30 g GAC/L (15% TS); k: methane production rate first-order constant (1/d). K'': methane production rate second-order constant (1/d). λ: time of the lag phase (d). μ$_m$: maximum rate of methane production (mL CH $_4$/gVS.d). R$^2$: coefficient of determination; AIC: Akaike Information Criterion. Equal letters mean no significant difference ($p < 0.05$).

Source: Author (2023).

In the literature, it has been reported that some concentrations of GAC affect the *lag phase* or methane production rate, and other concentrations impact the final methane volume, indicating that the effects of material dosages are not proportional to methane production (Orrantia et al., 2023). According to findings by Romero et al. (2020), using granular activated carbon obtained a better lag phase performance for liquid swine effluents with a lower total solids content (on average 10%), using a concentration of 15 g GAC/L. In their experiments, the lag phase decreased by up to 66.6%, while the decrease was 18.7% for raw effluent. However, the final methane was better for the raw effluent, with an increase of 42.1%

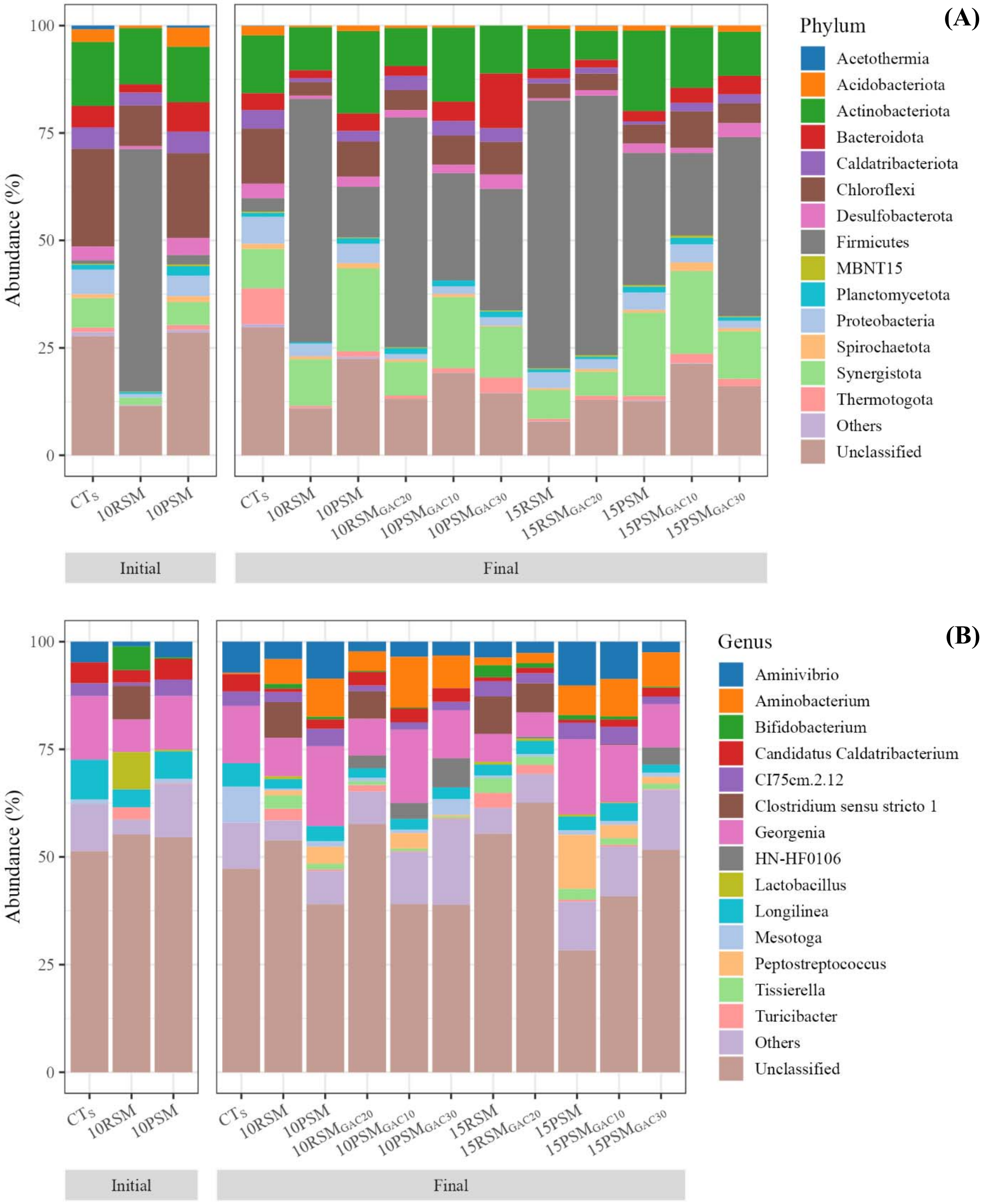
### *4.3.4 Microbial community analysis*

Analysis of the microbial community was carried out at the end of the experiments for samples of reactors CT$_S$, 10RSM, 10RSM$_{GAC20}$, 10PSM, 10PSM$_{GAC10}$, 10PSM$_{GAC30}$, 15RSM, 15RSM$_{GAC20}$, 15PSM, 15PSM$_{GAC10}$, and 15PSM$_{GAC30}$. The groups CT$_S$, 10RSM, and 10PSM were also analyzed before the experiments started.

#### *4.3.4.1 Structure of the bacterial communities*

As shown in Figure 4.6(A), at the bacterial phylum level, the compositions of CT$_S$ and 10PSM were approximately the same, including *Acidobacteriota*, *Actinobacteriota*, *Caldatribacteriota*, *Chloroflexi*, *Proteobacteria*, and *Synergistota*. For 10RSM, there was a major difference in the bacterial community composition, mainly due to *Firmicutes*, the most abundant phylum in this condition with raw swine manure. These bacteria are commonly found in animal manure (Subirats; Sharpe; Topp, 2022) and in anaerobic digestion, being associated with the stages of hydrolysis and acidogenesis (Cuetero-Martínez et al., 2023). At the end of the AD process, the abundance of *Firmicutes* was practically the same at 10RSM, but there was a reduction in *Actinobacteriota* and *Chloroflexi* and an increase in *Synergistota*; already at 10PSM, *Actinobacteriota*, *Firmicutes*, and *Synergistota* increased, and *Acidobacteriota*, *Bacteroidota*, and *Chloroflexi* decreased.

Figure 4.6 – Taxonomic classification of bacteria at phylum level (A) and genus level (B).



Source: Author (2023).

Analyzing the effect of adding GAC in the condition with raw manure, only small variations in the microbiota were observed at 10 and 15% TS, highlighting the reduction in the abundance of *Actinobacteriota* and the increase in *Caldatribacteriota*, *Chloroflexi* and

Desulfobacteriota, with this increase even more discreet in the dry condition, where *Acidobacteriota* also increased slightly with 20 g GAC/L. For the pretreated manure, in the semi-dry condition, when adding GAC 10 g GAC/L, there was an increase in the abundance of *Firmicutes*, and at 30 g GAC/L, it also increased *Bacteroidota* and *Thermotogota* but reduced *Actinobacteriota*. The dry condition showed different behavior concerning the abundance of Firmicutes. When adding 10 g GAC/L, it decreased, but with 30 g GAC/L, it increased. The same behavior occurred for *Thermotogota*. *Actinobacteriota* decreased with increasing GAC concentration as in semi-dry conditions. Overall, a greater abundance of *Firmicutes* may have contributed to greater production of methane-rich biogas from swine manure AD.

At the genus level, in almost all samples, more than 50% of the abundance was unclassified or others, and the dominant bacteria was *Georgenia*. At the beginning of the experiment, 10RSM is differentiated by the presence of *Bifidobacterium*, *Clostridium sensu stricto 1*, *Lactobacillus* and *Turicibacter*. At the end of AD, the microbiota changed, greatly reducing the abundance of *Bifidobacterium* and *Lactobacillus* and increasing *Aminivibrio*, *Aminobacterium*, *Preptostreptococcus*, and *Tissierella*, even so there was no significant production of methane in this condition. In the semi-dry conditions, when adding GAC, which promoted the effective production of methane, a greater abundance of *Candidatus Caldatribacterium* and *HN-HF0106*, fermentative bacteria that produce acetate, was observed. *HN-HF0106* are cellulolytic bacteria that can use cellulose as the only carbon and energy source while producing $H_2$ and acetate (Zhuravleva et al., 2022). Compared with the dry condition, although the microbiota was quite similar, the abundances of *Aminivibrio*, *Aminobacterium*, *Georgenia*, and mainly *HN-HF0106* were lower.

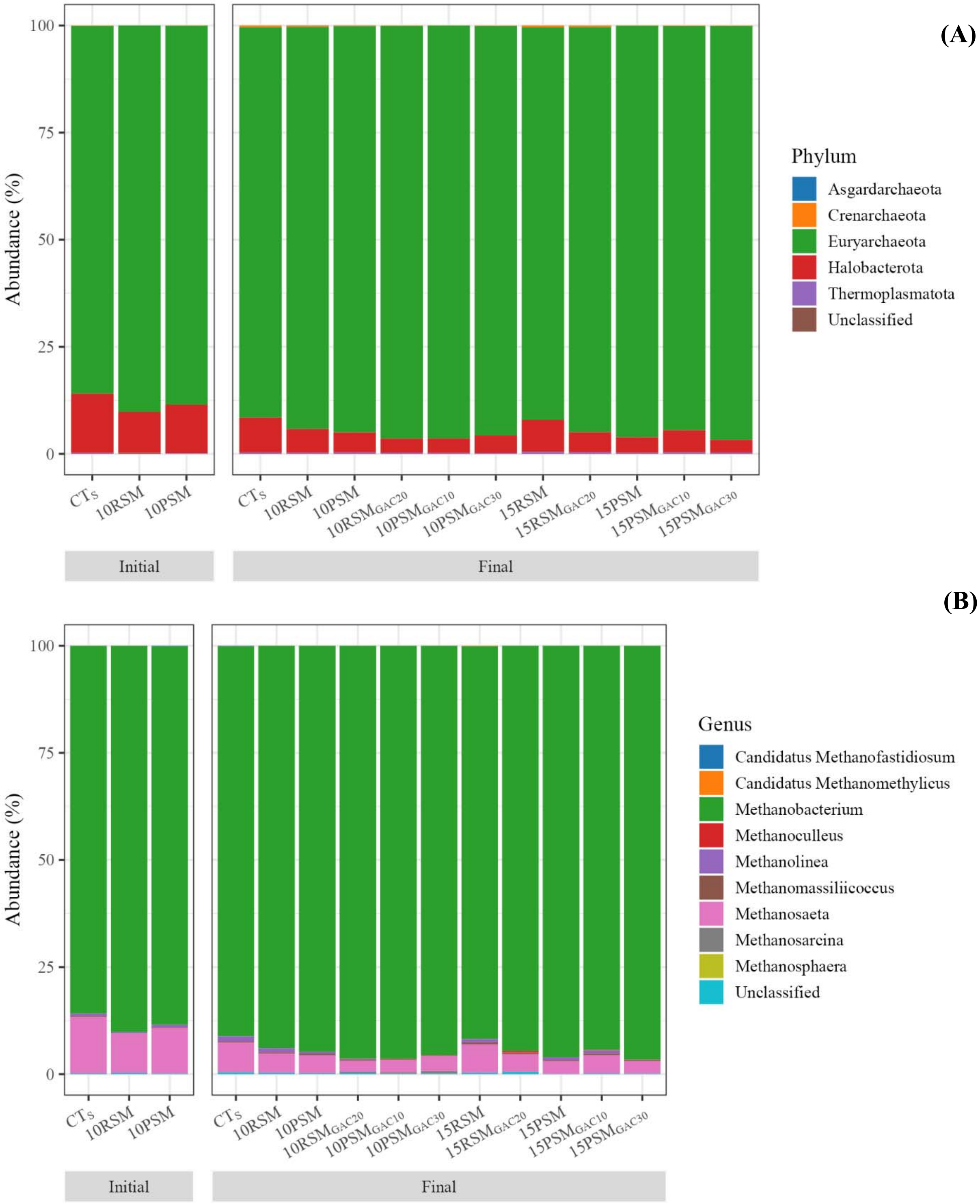
About reactors with pretreated manure, after 90 days of AD, 10PSM showed a greater diversity of abundances, such as *Bifidobacterium*, *Candidatus Caldatribacterium*, *and CI75cm.2.12*, *Preptostreptococcus* and *Tissierella*, even so, there was no significant methane production in this condition either. However, when adding GAC, which increased the methane yield, in the condition with 10% TS, an increase in *Aminobacterium* and *HN-HF0106* was observed mainly. For 15% TS, only *HN-HF0106* was identified during the batch assays with 30 g GAC/L.

### *4.3.4.2 Structure of the archaea communities*

As shown in Figure 4.7(A), *Euryarchaeota* was the most abundant archaea at the phylum level, and *Halobacterota*. Both phyla are typical microbial components in anaerobic systems (Liang et al., 2023). In general, at the end of the process, there was an increase in the abundance of *Euryarchaeota*. The addition of GAC also favored the greater predominance of this phylum. The phyla *Crenarchaeota* and *Thermoplasmatota* practically did not appear in the samples, with abundances of 0.1-0.5%.

At the genus level, *Methanobacterium* was the most abundant archaea for all samples studied, known as hydrogenotrophic methanogens that use hydrogen and carbon dioxide to form methane (Hu et al., 2019a). The second most abundant group was *Methanosaeta*, a typical acetoclastic methanogen, more sensitive to VFA, ammonia, and other compounds than hydrogenotrophic methanogens (Xiao et al., 2021). In general, with the addition of the conductive material, the *Methanobacterium* genus was slightly more favored.

Figure 4.7 – Taxonomic classification of archaea at phylum level (A) and genus level (B).



Source: Author (2023).

## 4.4 Conclusion

In this study, the effects of GAC on semi-dry and dry AD of swine manure were examined for raw and pretreated substrate. In all conditions without GAC addition, there was no significant methane production. With raw manure, when increasing the TS content from 10 to 15%, even in the presence of GAC, the methane yield was reduced by 35%, and the kinetics were unfavorable with a reduction in K and $\mu_m$ and an increase in the lag phase. For the manure pretreated with different dosages of GAC, in the semi-dry condition, the kinetics were more favored at the dosage of 20 g GAC/L, but, at experiment completion, the final methane production was higher at 10 g GAC/L. In dry AD, there was greater production of $CH_4$ and favored kinetics with the dosage of 30 g GAC/L. Therefore, the TS content influences the ideal GAC concentration in AD, and a higher GAC concentration makes the system more resilient to increasing total solids content. The dominant bacteria in most of the reactors was Georgenia. In the semi-dry condition for raw manure, when adding GAC, a greater abundance of *Candidatus Caldatribacterium* and *HN-HF0106* was observed; for pretreated substrate was *Aminobacterium* and *HN-HF0106*. The major archaea abundances for all samples studied were *Methanobacterium* and *Methanosaeta*. In general, with the addition of the conductive material, the *Methanobacterium* genus was slightly more favored. In addition, the Modified Gompertz kinetic model was the best fit for the methane yield curve of reactors, and the kinetic parameter obtained can be used to optimize and scale the anaerobic digestion processes of swine manure with GAC addition. Therefore, the investigation addressed here deepened the understanding of GAC effect on the anaerobic digestion of swine manure. However, further technical, economic, and environmental investigations are still needed regarding its future full-scale use.

# 5 FINAL CONSIDERATIONS

Anaerobic digestion (AD) of swine manure is an attractive alternative to recover resources, such as methane-rich biogas, in waste treatment. This work this work sought to evaluate strategies to enhance the anaerobic biometanization of swine manure, such as thermos-alkaline pretreatment (3% NaOH at 121 ºC for 30 min), total solids content (10 and 15% TS), SI ratio (1, 3 and 5 $g.VS_{substrate}$ / $g.VS_{inoculum}$) and addition of granular activated carbon - GAC (10, 20 and 30 g/L) as conductive material, applying kinetic modeling analysis to understand the influence of the evaluated parameters better and generate important kinetic coefficients for optimization and process scale-up. Molecular biology tools were also applied for a better comprehension of the different microbial population involved and ecology dynamics changes.

The first strategy evaluated was thermo-alkaline pretreatment; however, the results obtained in the two experimental phases were different. In the second phase, the pig manure used probably had more protein and less lignocellulose in its composition, and therefore, the pretreatment was not so evident in increasing the methane production, completely different from what occurred in the first phase. This highlights that the matrix used greatly influences the pretreatment to be adopted. Therefore, it is important also to investigate other pretreatment methods, such as steam explosion.

Regarding the study of the inoculum/substrate ratio (SI), values from 0.25 to 9.3 based on total solids were found in the literature, and based on this, values 1, 3, and 5 were chosen in this work. The increase in the SI ratio for the pretreated substrate proved to be viable only in the semi-dry condition (10% TS), as in the dry condition (15% TS), there was only significant methane production in SI 1 and even, so it was lower than in the semi-dry condition. The SI ratio 3 showed a higher yield at 10% TS. As for kinetics, the maximum methane production rate was not impaired with the increase in SI, but there was a large increase in the lag phase. Therefore, conducting an economic study considering the methane yields and the kinetic parameters obtained would be important to determine better which condition is most favorable for this AD process. Furthermore, in future experiments, the influence of this parameter can be analyzed using a sludge adapted to high loads of pig manure.

In the two phases of the research, the investigated parameters were evaluated in two conditions: semi-dry and dry, and the increase in total solids content made methane production difficult in all situations. However, this issue has been mitigated with higher concentrations of granular activated carbon (GAC). It was then concluded that the TS content influences the ideal concentration of GAC in AD, and a higher concentration of GAC makes the system more resilient to increasing total solids content. Furthermore, it was observed that GAC promoted the improvement of anaerobic digestion for raw swine manure, making it unnecessary to adopt pretreatment techniques that not only increase the cost of the process but can also release inhibitory compounds to the microorganisms involved in anaerobic digestion.

However, using GAC also generates an extra cost in the process, and therefore, the suggestion is that in future work, the possibility of replacing GAC for biochar can be investigated, which can be produced from the pyrolysis of the digestate remaining at the end of AD. This residue needs to receive adequate treatment and, with this application, could add even more value to the treatment of swine manure.

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