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SHIFTS ON BACTERIAL POPULATIONS AND BIOGAS PRODUCTION BY ADDING TWO INDUSTRIAL RESIDUES IN CO-DIGESTION WITH CATTLE MANURE

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Dissertação apresentada ao Programa de Pós-graduação em Ecologia Aplicada ao Manejo e Conservação dos Recursos Naturais, da Universidade Federal de Juiz de Fora como parte dos requisitos necessários à obtenção do título de Mestre em Ecologia aplicada ao Manejo e Conservação dos Recursos Naturais.

Orientador: Dr. Nathan Oliveira Barros

Coorientador: Dr. Marcelo Henrique Otenio

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RESUMO

A biodigestão anaeróbia (BA) figura como alternativa sustentável para evitar o lançamento de dejeto bovino (DB) no meio ambiente, resultando em biogás e biofertilizante. A bioconversão anaeróbia de biomassa em metano (CH_4) via BA requer um processo biológico de múltiplas etapas, incluindo microrganismos com funções distintas. Estudou-se a dinâmica de populações bacterianas acidogênicas por microbiologia clássica, bem como a produtividade de biogás por gasômetro e cromatografia, no processo de co-digestão anaeróbia (co-AD). Este trabalho apresenta uma avaliação de desempenho de sistemas co-AD para a produção de biogás utilizando DB em conjunto com resíduos da Estação de Tratamento de Esgoto (ETE) de uma cervejaria e soro de queijo ricota (RCW). O maior rendimento de CH_4 foi observado nos biodigestores (RCW1, RCW2 e RCW3) alimentados com DB em adição ao RCW. Esses resultados foram geralmente coerentes com as comunidades bacterianas ativas observadas, afirmando a estabilidade do processo. A pesquisa revelou que o tipo de substrato adicionado no co-AD, a relação carbono/nitrogênio (C:N) e o nitrogênio amoniacal (NH3-N) foram os fatores mais influentes que explicaram muitas das variações da microbiota nos biodigestores (BRE1, BRE2 e BRE3) alimentado com DB com resíduos do ETE de uma cervejaria. Este estudo demonstrou que existe um bom potencial para o uso do RCW na produção de biogás e sua posterior conversão em energia. Essas descobertas podem fornecer algumas informações fundamentais e técnicas para o co-tratamento de resíduos derivados de indústrias em instalações com BA centralizadas, de forma sustentável com alta capacidade de processo e recuperação de metano.

Palavras-chaves: Co-digestão anaeróbia; Rendimento de metano; Produção de biogás; Dejeto bovino leiteiro; Resíduos; Microrganismos.

ABSTRACT

Anaerobic biodigestion (AB) figures as a sustainable alternative to avoid discharge of cattle manure (CM) in the environment, which results in biogas and biofertilizer. The anaerobic bioconversion of biomass to methane (CH_4) via AB requires a multi-step biological process, including microorganisms with distinct roles. Here, the dynamics of acidogenic bacterial populations by classical microbiology, as well as biogas productivity by gasometer and chromatography, in the anaerobic co-digestion (co-AD) process were studied. This paper presents a performance evaluation of co-AD systems for the production of biogas using CM together with wastes from the Sewage Treatment Station (STS) of a brewery and ricotta cheese whey (RCW). The highest CH_4 yield was observed in the biodigesters (RCW1, RCW2 and RCW3) fed with CM in addition to RCW. These results were generally coherent with the observed active bacterial communities affirming the stability of the process. The search revealed that the type of substrate added in co-AD, Carbon/Nitrogen (C:N) ratio and Ammonia Nitrogen (NH_3-N) were the most influential factors that explained many of the variations of the microbiota in the biodigesters (BRE1, BRE2 and BRE3) fed with CM in addition to wastes from the STS of a brewery. This study demonstrated that there is a good potential for the use of RCW in the production of biogas and its further conversion into energy. These findings could provide some fundamental and technical information for the co-treatment of industrial derived wastes in centralized AB facilities, in a sustainable manner with high process capacity and methane recovery.

Keywords: Anaerobic co-digestion; Methane yields; Biogas production; Dairy cattle manure; Wastes; Microorganisms.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION AND ACRONYMS

AB	Anaerobic Biodigestion
ANOVA	Analysis of Variance
ANVISA	National Health Surveillance Agency
BRE1	Wastes from the Sewage Treatment Station of a brewery with 20%
BRE2	Wastes from the Sewage Treatment Station of a brewery with 40%
BRE3	Wastes from the Sewage Treatment Station of a brewery with 80%
CFU	Colony-Forming Unit
CO-AD	Co-digestão Anaeróbia
CH_4	Methane
CM	Cattle Manure
C:N	Carbon:Nitrogen
CON	Biodigesters Control
CSL	Eirelli Environmental Laboratory
EMB	Eosin Methylene Blue Agar
HRT	Hydraulic Retention Time
IBGE	Brazilian Institute of Geography and Statistics
NH_3-N	Ammonia Nitrogen
RCW	Ricotta Cheese Whey
RCW1	Ricotta Cheese Whey with 20%
RCW2	Ricotta Cheese Whey with 40%
RCW3	Ricotta Cheese Whey with 80%
SIM	Sulfate, Indole and Motility
STS	Sewage Treatment Station

SUMMARY

1. INTRODUCTION

The intensive breeding of dairy cattle is expanding strongly worldwide (Mao et al. 2015). In Brazil, during the first trimester of 2019, milk procurement in the country reached 6.2 billion liters, representing a growth of 3% in relation to the production of the year 2018 (IBGE 2019). Intensive breeding of dairy cattle produces large amount of cattle manure (CM), which provides additional gains for the productive system. Due to the high proportion of biomass, the CM is an interesting raw material for biogas production in Anaerobic biodigestion (AB) process, which additionally reduces their pollutants load. AB is a natural and controlled process that occurs in the absence of oxygen, in which anaerobic microorganisms degrade organic matter, converting it mainly into biogas (MENDONÇA et al. 2017; CORREA et al. 2018).

According to Iwasaki et al. (2019), there is a huge variety of biodegradable organic compounds, resulting in the existence of microorganisms such as intestinal and environmental bacteria in CM. Understanding how diversity and dynamics of microbial communities in AB contribute to the stability of this system is a big challenge. Furthermore, the high adaptability of the microbial communities in AB is a key factor for process stability and productivity (ZEALAND et al. 2018).

The performance of microorganisms and biogas yield from AB may vary mainly in function to the quality of the residue added to the digester, the degree of dilution and the retention time. However, other factors may also contribute to a better process response, such as the addition of another substrate that complements the waste composition (HIDALGO and MARTIN--MARROQUÍN, 2014). The co-digestão anaeróbia (co-AD) process consists of the simultaneous treatment of two or more biodegradable substances by AB. It is considered a current technique being explored intensively due to the individual characteristics of the waste, that when associated can maximize the potential of biogas production, increasing the system efficiency (ZAMANZADEH et al. 2017).

The performance of AB is related to the bacterial populations diversity present in the biodigester, and acts in four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The microorganisms in a biodigester directly determine the performance of anaerobic reactors just as environmental changes. Associating the performance of the AB process and the bacterial populations at different stages of operation helps to effectively manage the anaerobic bioreactor (QIN et al. 2019). In this regard, of large quantities of CM in dairy cattle farming is frequently

considered as a reservoir for potentially pathogenic and antimicrobial resistant bacteria or also, reservoir of antibiotic resistance genes. (RESENDE et al. 2014)

Nowadays, different methodologies for treatment of industrial effluents are gaining an increasing attention. For instance, dairy effluent treatment (ROSA et al. 2014; ANTONELLI et al. 2017; GHASEMI et al. 2017) and effluents with high nitrogen load *(*THIEN et al. 2019) like the brewing industries are already implemented.

For the dairy effluent treatment, the biological processes are the most widely used due to their effluent composition, which are generally rich organic biodegradable load (SILVA, 2010). Among the various biological valorization technologies, AB of ricotta cheese whey (RCW) is proposed as a sustainable process which can exploit the high organic load from dairy industries for bioenergy production. Thus, the same process of AB can be applied for the treatment of brewery effluents, as the waste has a high concentration of organic load and high content of suspended solids, its composition includes the remaining sugars from the fermentation process that provide a fermentable substrate (CORSINO et al. 2016; ARANTES 2018).

In the present study we tested the versatility of wastes from the Sewage Treatment Station (STS) of a brewery and RCW, both wastes constituted of biodegradable materials and can be availed for the production of biogas for various purposes, associated with the co-AD process with CM. The anaerobic co-digestion is a well-known method for bioconversion of wastes for the past few decades in domestic as well as industrial sectors (BI et al. 2020; WEI et al. 2020). In order to make AB technologies more attractive and profitable for industries, co-AD of manure with industrial byproducts can increase methane yield. The microbiological analysis becomes an ally in the evaluation productivity process.

The objective of this work was to evaluate the effect of bacterial populations with biogas productivity during the full co-AD process adding as an alternative for the production of biogas two industrial residues with cattle manure in the biodigesters, supporting sustainable development.

2. MATERIALS AND METHODS

The CM used in the experiment to supply the biodigesters was collected in the milk production system of the José Henrique Bruschi Experimental Farm, located in the municipality of Coronel Pacheco, Minas Gerais, Brazil (21° 33′ 58″ S; 43° 15′ 21″ W and altitude de 445 m). The climate in the region is classified as tropical (Cwa) on the scale of Köppen and Geiger, with mean annual temperature of 22 °C, maximum of 35 °C, and minimum of 18 °C, and average yearly rainfall of 1516 mm. The climate data were obtained from an automatic weather station located 200 m from the experimental site.

The collected CM was preserved in properly closed drums and transported to Embrapa Dairy Cattle research unit (Embrapa Gado de Leite), in the municipality of Juiz de Fora, Minas Gerais state, Brazil. There, it was manually homogenized and the substrates were prepared from the dilution of bovine feces with washing water of the floors of the "*free stall*", up to total solids content of 6%. In this scenario, besides the CM, wastes from the STS of a brewery and RCW were collected in industries located in the region, and stored weekly to supply daily load. The pH of the RCW was corrected with 59 mL/L of calcium hydroxide ($Ca(OH)_2$) at 4.24% to maintain a near-neutral pH, between 6.5 and 7.0. According to tests performed with RCW of different initial pH values, this concentration was the one that was most suitable for pH correction of the residue.

2.1 Mixture of waste

For the daily supply of the seven biodigesters in the full co-AD phase, two types of mixtures were prepared as affluent. Three of the biodigesters RCW1, RCW2 and RCW3 were filled with CM mixed at concentrations of 20%, 40% and 80% of RCW, respectively. Another three biodigesters BRE1, BRE2 and BRE3 were fed with CM mixed at concentrations of 20%, 40% and 80% of brewery residue, respectively. One of the biodigesters control (CON) was only fed with CM, kept for better process control.

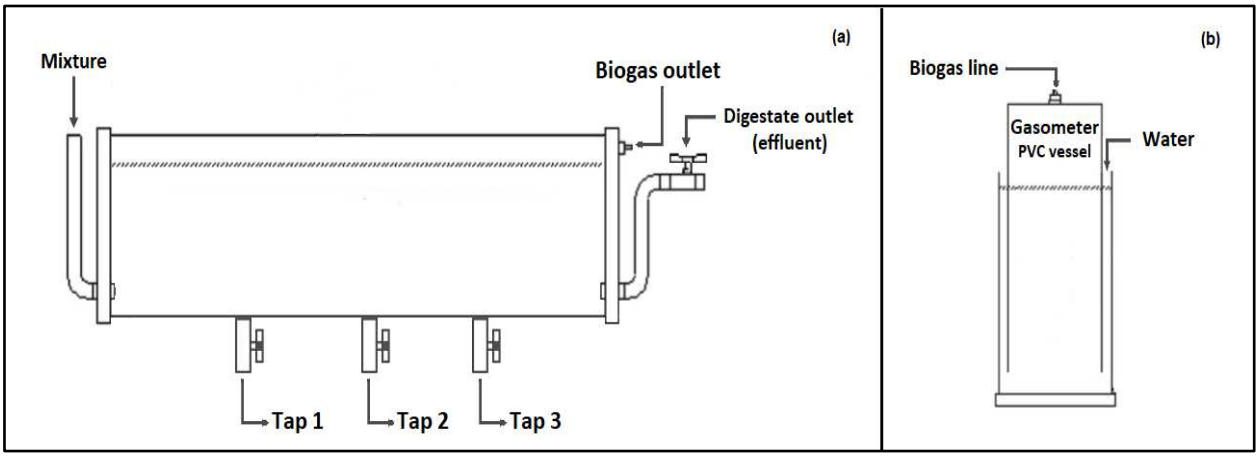
2.2 Biodigesters characterization

The present experiment was performed in seven cylindrical anaerobic biodigesters ~~a~~ in laboratory scale with a capacity of 60 L of substrate, designed as continuous feed reactors and operated under mesophilic conditions. All biodigesters were installed in the Embrapa Dairy Cattle.

The biodigester is divided into two parts: (i) a horizontal cylinder for the storage of the material in the AB process, with three fixed taps located at the bottom where the samples are collected; (ii) a vertical cylindrical tank, called gasometer. The complete biodigester functioning scheme is shown in Figure 1 a.

In the horizontal cylindrical tank, there are two ends, a main entrance where the supply of the mixtures of the affluent is carried out, and an outlet for the effluent disposal. The biodigesters have a piston flow system, also called *Plug-flow*. This system is unheated and unmixed. The mixture of affluent has continuous input at one end of the biodigester, passes through it and is discharged at the other end in the same sequence as it entered. The gases produced are reserved in the gasometer, connected by a properly sealed hose to absorb all the generated gas (Figure 1 b).

Figure 1 - Cylindrical continuous anaerobic biodigester, adapted image from RESENDE et al. (2016), details of gasometer



Source: Author

2.3 Outline of the experiment

The experiment began in November 2018 and ended in April 2019, 165 days total, divided into 3 phases: (i) the first phase lasted 15 days and resulted in the anaerobic establishment of the seven biodigesters exclusively supplied with CM; (ii) the second phase occurred to inoculum acclimatization of hydraulic retention time (HRT) of 30 days, in this phase each biodigester was specifically supplied with its mixture; and (iii) the third phase is called full co-AD, in each treatment there was a daily supply of the mixture for 120 days.

During the full co-AD phase, seven samples were collected of each of the treatments from the three taps located at the bottom of the biodigesters within a 15 days analysis interval, the samples were used for chemical physical and microbiological analysis. Through microbiological analysis two species of bacteria, *Escherichia coli* and *Pseudomonas aeruginosa,* representing the acidogenic phase were characterized and monitored. The two species of bacteria were selected and sub-cultivated through literature review according to other previous studies performed in Embrapa Dairy Cattle (RESENDE et al. 2014; FERNANDES 2016; MOURA 2017). In the pre-tests carried out with CM and the residues were found the bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, both presented easy isolation in Microbiological analysis performance of biodigesters. Quantification of the volume of biogas produced was measured on a graduated scale on the gasometers. To monitor the quality of the gas produced and the yield calculation, gas chromatography was performed.

2.4 Chemical physical analysis

Raw material biogas production depends on substrate concentration, pH, temperature and Carbon/Nitrogen (C:N) ratio (TUFANER and AVSAR, 2016). Temperature is one of the main factors for survival of microorganisms during AB process (JOHNSON 2017). Temperature is the important parameter for the microorganisms to grow based on the optimum requirement and to improve the biogas production. The ambient temperature was recorded daily, for 120 days, with the aid of a portable digital thermometer.

The pH value is one of the main operational factors which greatly affect the AB process most microorganisms prefer a neutral pH. The favorable range of pH to obtain maximal biogas

production in AB is 6.8 – 7.2 (APPELS et al. 2018). Thus, during the experiment time (120 days), routine monitoring of the analysis to measure the pH of effluents was performed.

During the time of the experiment, once every 15 days, elemental analyses were carried out to obtain the chemical physical characterization of effluents of each biodigester. A sample of effluent was collected in each biodigester and sent to *Eirelli Environmental Laboratory* – EPP (CSL). The following parameters were evaluated: total carbon, total nitrogen, C:N ratio and Ammonia Nitrogen (NH_3-N). At the same moment of the experiment, analysis of the composition of biogas was performed on each biodigester by gas chromatography in order to determine the composition of the gaseous products. In total, 19 analyses were taken every 7 days during the experiment time (120 days).

We used the Agilent 7820A Gas Chromatograph System and the EzChrom Elite interface software (COLLINS; BRAGA; BONATO, 1997). According to the adaptation of the method Collins; Braga; Bonato (1997) been used a split-splinter type 50:1 injector at a temperature of 120°C. The separation system consists of two columns: (i) an HP-Plot/Q 30m x 0.530 mm x 40.0 µm; (ii) the other HP-Molesieve 30m x 0.530mm x 25.0 µm, using H_2 as carrier gas at a flow of 8.3 mL/min. The detection system consists of: TCD Detector – conditions: 250°C heating; 25 mL/min reference flow; 0.5 mL/min complement flow (H_2); 8.8 mL/min column + constant complementation. FID Detector – conditions: 270°C heating; 15 mL/min flow H2; 350 mL/min synthetic air flow; 20 mL/min flow complement. Metanador at 375°C heating. The temperature of the oven is maintained at 55°C for 4.5 minutes, time required for the elution of the expected constituents. The calibration of the chromatograph is performed with reference standards, certified by Linde™ at methane (CH_4) concentrations: 5.05; 10.2; 14.7; 20.1 and CO_2: 20.2; 39.7; 58.3; 79.9.

2.5 Microbiological analysis

Samples of each tap in the biodigesters were collected in autoclaved glass vials and identified. Analyses of the samples were performed at the Rumen Microbiology Laboratory located at Embrapa Dairy Cattle, using classical microbiology method to the cultivable microorganisms.

Microbial analysis of the samples was performed on the same day "*on time*" of sample collection. The washed petri plates were sterilized by autoclaving (121 ºC, 15 psi) and oven dried

(171 ºC) for 60 minutes. The selective agars including Eosin Methylene Blue Agar (EMB) to count *Escherichia coli* and MacConkey Agar to count *Pseudomonas aeruginosa* were prepared and poured into sterile petri plates according to the manufacturer's instructions.

The cultivation medium EMB is a differential culture medium that inhibits the growth of Gram-positive bacteria and indicates if the bacteria ferment lactose or not, and is used as a medium for slightly selective differentiation for isolation and differentiation of gram-negative enteric bacilli. The colonies of *Escherichia coli* are easily identifiable by their metallic green coloring in the middle of EMB. MacConkey Agar is a culture medium intended to grow gram-negative bacteria and to indicate lactose fermentation. Bacterial colonies that ferment lactose make the medium light pink and bacteria that are not lactose fermenting make the medium light yellow. The colonies of *Pseudomonas aeruginosa* has a coloration ranging from colorless to green (ANVISA 2004).

Serial dilutions of the samples of 10^{-1}, 10^{-2} and 10^{-3} in saline (0,9%NaCl) were performed, the dilutions were fixed in these values to meet the objectives of the experiment. The dilutions were homogenized, and aliquots of 0,1 ml (100 µL) were seeded with the aid of a Drigalski loop in the aerobic selective culture media.

After inoculation, the plates were incubated an incubator for 24 hours at 37 ºC. According to Divya et al. 2015, the mesophilic process (around 35 ºC) often involves a more stable diversity of microorganisms compared to the thermophilic process (around 55 ºC).

After the incubation period, the petri dish with lowest dilution (between 20 and 200 colonies) was used to estimate by counting the colony-forming unit (CFU) of bacteria in the samples, with the help of the Phoenix® colony counter. The results were converted to CFU mL^{-1} and log CFU mL^{-1}.

2.6 Biochemical tests

Biochemical tests were performed on isolates for the identification of *Escherichia coli* and *Pseudomonas aeruginosa*. The colonies cultivated in the two-agar media have undergone analysis of Gram staining, catalase test, oxidase, SIM (Sulfate, Indole and Motility) and citrate to prove and confirm the isolated bacteria.

2.7 Statistical analysis

The statistical analyses were performed using the JMP 14.0 software. The differences in the means were evaluated through analysis of variance (ANOVA) followed by a comparison of means test (student's t). Prior the analyzes all data were checked for a normal distribution.

3. RESULTS

The present work resulted in the following results from the analysis of microbiological performance of biodigesters during co-AD process, methane productivity, temperature, pH and C:N ratio, e ammonia Nitrogen (NH3-N).

3.1 Microbiological performance of biodigesters during co-AD process

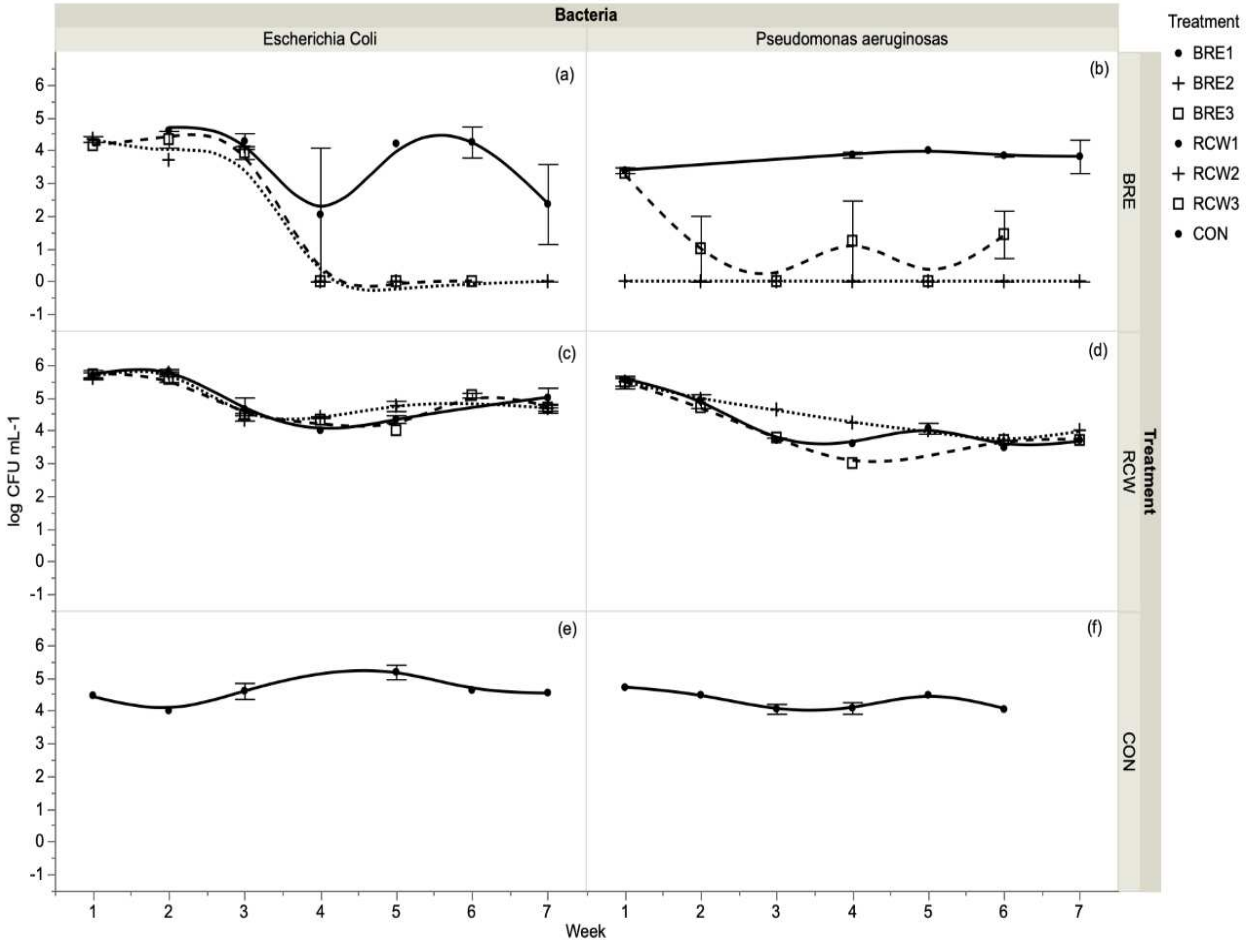
The biodiversity of microorganisms within a biodigester is of great significance due to the large contribution of microbial interactions in the production of biogas (MANYI-LOH et al. 2019). Generally speaking, the behavior of the two bacteria *Escherichia coli* and *Pseudomonas aeruginosa* in the biodigesters was different among the studied waste. There was no significant difference in the count of both bacteria in each tap. An average of the taps was performed in each biodigester in its respective treatment in order to follow the behavior during the experimental time.

In the biodigesters BRE2 and BRE3 from the third and fourth analysis showed no growth for the microorganisms (Figure 2 a-b). In the biodigester BRE3, the supply was ceased after the sixth week of analysis because it was no longer producing CH_4. It should be noted that in the biodigester BRE1 (Figure 2 a-b), up to the 120-day trial period, the two bacteria were present during all seven analyses performed although in a smaller number in the subsequent weeks than at the start of the experiment.

In contrast, the biodigesters RCW1, RCW2 and RCW3 showed better results and the two bacteria were present in all seven performed analyses (Figure 2 c-d). Over time in the three biodigesters there was a constant reduction of both bacteria of approximately 2 log CFU mL^{-1}. The mixture of the substrates of CM with RCW obtained a higher chance of containing all components that are important for microbial growth. Therefore, there was a stability in the process.

In the biodigester CON the analyzes performed maintained equivalent values of both bacteria throughout the experiment (Figure 2 e-f). There was no significant reduction in the count from first to last analysis.

Figure 2 - Microbiological performance of biodigesters during co-AD process. Mean value +/- standard deviation of viable microbial counts (log CFU mL-1) of the two-bacteria Escherichia coli and Pseudomonas aeruginosa during the experiment time



Source: Author

Note: (a) and (b) indicate biodigesters in the co-AD process with CM in addition to wastes from the Sewage Treatment Station (STS) of a brewery; (c) and (d) indicate biodigesters in the co-AD process with CM in addition to Ricotta cheese whey (RCW); (e) and (f) indicate biodigester control (CON).

3.2 Methane productivity

It has been observed that co-AD of CM and other waste may enhance biogas production, and lead to more stable biodigestion processes (GUILLAUME and LENDORMI 2015). Here, we also observed an increase on the CH_4 production efficiency when the RCW was used in co-AD with CM. However, a decrease on CH_4 production efficiency was observed by adding the STS of a brewery in a co-AD with CM.

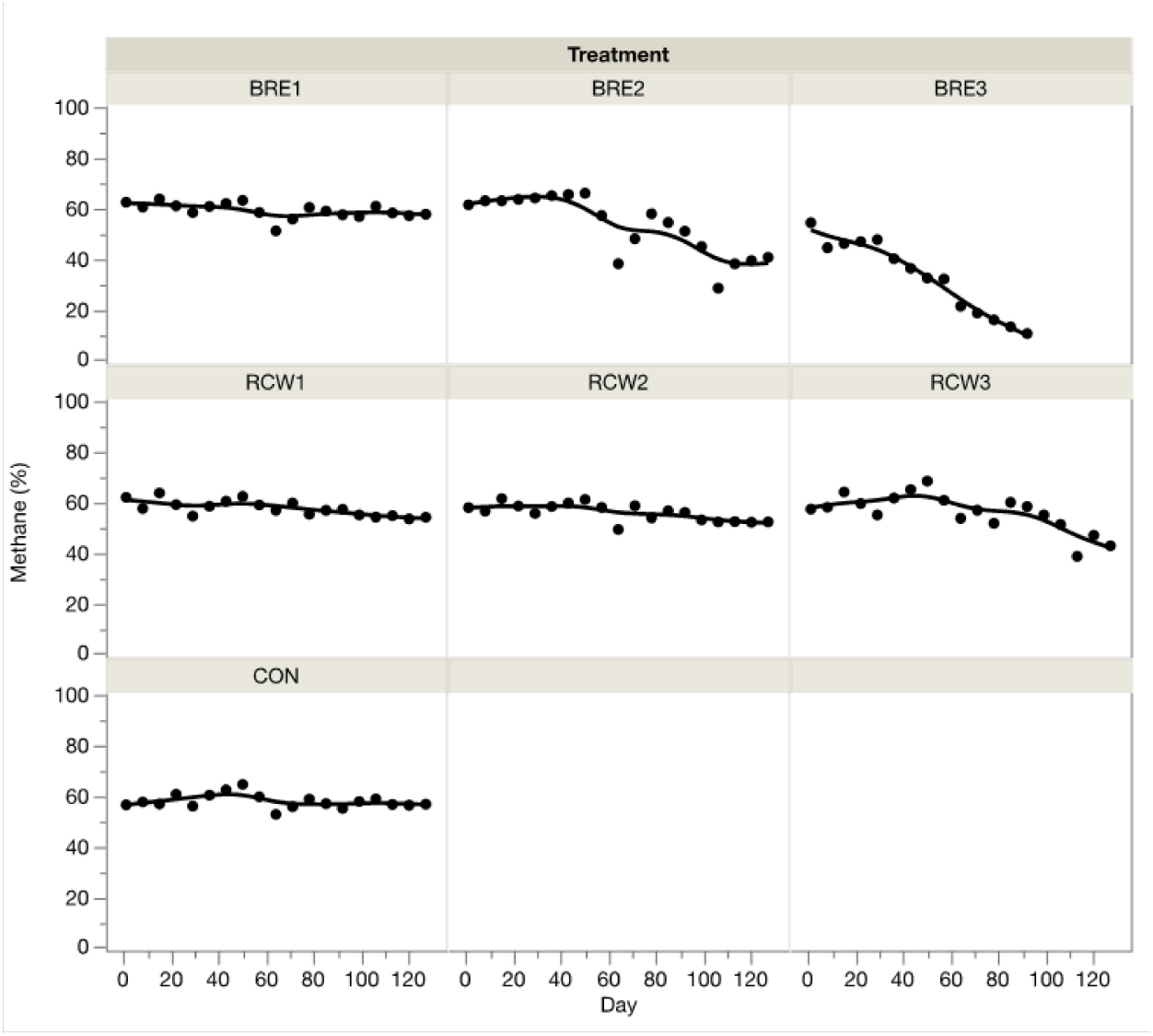
Comparing the efficiency of CH_4 production in biodigesters we obtained different results (Figure 3). The CH4 productivity for biodigesters RCW1, RCW2 and RCW3 during the 120 days of measurement showed a very low variation of values, thus maintaining a stable standard in the production of CH_4 in biodigestion process as well as the biodigester CON. Overall, both biodigesters showed high efficiency in CH_4 production.

Our results showed that the yields of biodigester CH_4 BRE1 during the 120 days of measurement maintained a stable production, presenting low variation of the measured values. However, CH_4 productivity in biodigesters BRE2 and BRE3 revealed a very different behavior presenting a high variation of the values, thus resulting in relatively low yields of CH_4 concentration. When we increase the concentration of wastes from STS of a brewery from 20% to 40% the biodigester BRE2 is infeasible with time, when we increase from 40% to 80% the biodigester BRE3 is completely infeasible.

Notably, the support limit of co-AD between the biodigesters BRE1, BRE2 and BRE3 during the experiment time was the biodigester BRE1 that had a mixture of 80% the CM and 20% of wastes from STS of a brewery.

Concerning productivity, the CH_4 (biogas), application of wastes from the STS of a brewery at concentrations above 20% in co-AD process for 120 days is not recommended, once a significant drop in productivity was observed. Significant differences were found ($P \leq 0.001$) between biodigesters (Table 1). Levels not connected by same letter are significantly different (Figure 3).

Figure 3 - CH4 yield in the co-AD biodigesters operated under different conditions (Treatment). The CH4 (%) was representing the variation of values of CH4 productivity



Source: Author

Table 1 - CH4 production in biodigesters, mean value, standard deviation and statistical test

Treatment	Methane		
	Average	Standard deviation	Statistical teste
RCW1	57.71	3.02	a,b
RCW2	56.11	3.41	a,b
RCW3	56.72	6.94	a,b
CON	57.96	2.81	a,b
BRE1	59.26	3.05	a
BRE2	53.81	11.74	b
BRE3	32.77	14.60	c

Source: Author

3.3 Temperature, pH and C:N ratio

Over the 120 days of hydraulic retention time, the co-AD biodigesters under different conditions (Treatment) operated in the ambient temperature range, between 18 and 26°C average of 22°C and temperatures inside the biodigester, between 14 and 33°C average of 25°C. Ambient temperature was in the mesophilic range.

The pH levels remained stable in the biodigesters in co-AD with CM and RCW with min-max value of: RCW1: 6,98-7,27; RCW2: 6,90-7,20; and RCW3: 6,93-7,17. However, the biodigesters in co-AD with CM and wastes from the STS of a brewery showed values outside the recommended standard, with min-max value of: BRE1: 7,06-7,58; BRE2: 6,95-7,89; and BRE3: 6,44-7,27 (Table 2). The active production of biogas depends on maintaining an optimum fermentation pH. Previous researches report that a low pH may induce an inhibition of methanogenesis, still reflecting on overload of organic matter that corroborates the inhibition of the bioprocess (ALKAYA and DEMIRER 2011; ZUO et al. 2013).

In the biodigesters RCW1, RCW2, RCW3, CON and BRE1 the C:N ratio was maintained in stable values during all the experimental period. Whereas the biodigesters BRE2 and BRE3 presented low values in C:N ratio during the experiment time. It is important to highlight BRE3 had extremely low C:N ratio after 80 days, and for that reason the BRE3 needed to be finalized after that period.

Table 2 - pH measured during the time of the experiment

Days	Treatment						
	RCW1	RCW2	RCW3	CON	BRE1	BRE2	BRE3
1	7.05	6.91	6.93	7.22	7.06	7.20	7.27
16	7.14	7.08	7.10	7.25	7.35	7.48	7.16
31	7.13	7.05	7.03	7.26	7.50	7.73	6.68
46	7.10	7.00	6.97	7.29	7.48	7.75	6.86
61	7.11	7.13	7.14	7.21	7.58	7.89	7.08
76	7.11	7.02	6.99	7.26	7.46	7.62	7.04
91	6.98	6.90	6.91	7.16	7.46	6.95	6.68
106	7.27	7.20	7.17	7.30	7.47	7.15	6.44
120	7.13	6.98	6.94	7.10	7.50	7.01	*
Average	7.11	7.03	7.02	7.23	7.43	7.42	6.90
Standard deviation	0.077	0.097	0.095	0.064	0.150	0.349	0.284

Source: Author

Note: * Biodigester BRE3 has been finalized

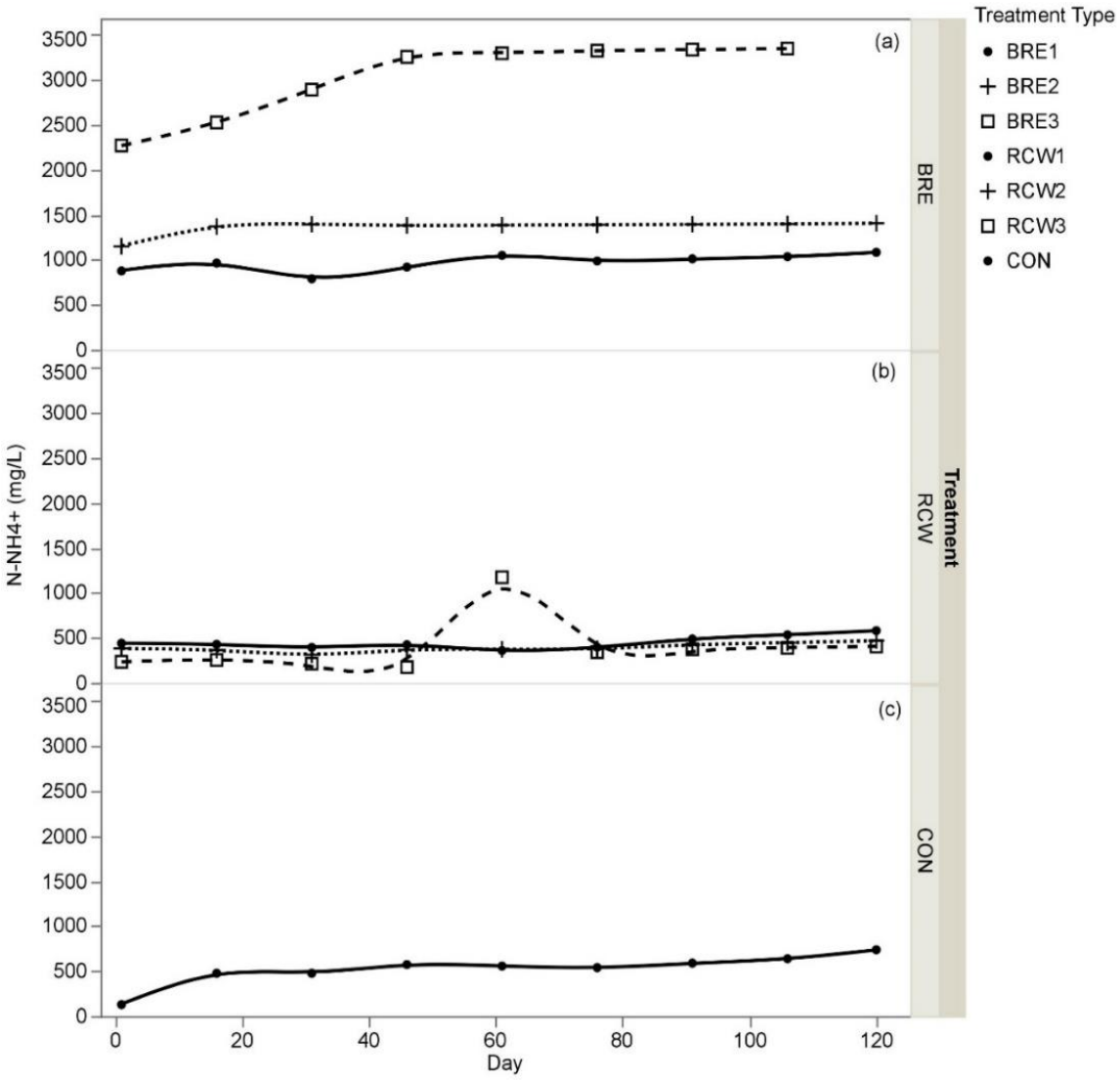
3.4 Ammonia Nitrogen (NH_3-N)

It is understood that while no growth of the microorganisms in the biodigesters BRE2 and BRE3 was verified, low CH_4 yield was provided. This agrees with the higher ammonia concentrations in the co-AD systems in biodigesters with wastes from the STS of a brewery (Figure 4 a). It was noted that the biodigesters BRE2 and BRE3 had an increasing trend in ammoniacal nitrogen concentration. The low performance of biodigesters in terms of microbial decay and CH_4 production can be partially explained by the inhibition of ammonia. Furthermore, in biodigester BRE1 the impacts of ammonia inhibition on the microbial population showed no significant change during the experiment time.

In the biodigesters RCW1, RCW2 and RCW3 (Figure 4 b), the concentration of NH_3-N during the experiment remained stable, with one exception of the biodigester RCW3 in which was observed an increase in 45-60 days, possibly justified due to a change in the receiving of the RCW wastes during these days.

In the biodigester CON, there should be an increase in the concentration of NH_3-N from the first days (Figure 4 c). However, the increase did not affect the production of CH_4 and the inhibition of microorganisms.

Figure 4 - Effect of ammonia accumulation during the experiment time. The trend in NH3-N concentration was presented in lines



Source: Author

4. DISCUSSION

The co-AD of different materials presents better results, using nutrients and bacterial diversities in various wastes to optimize the digestion process. When the substrates are mixed, they have higher chance to contain all components that are important for microbial growth (KARLSSON et al. 2014). Thus, co-AD may be a perspective option to improve the economic viability due to increased biogas production (PIÑAS et al. 2018). Protein-rich substrates are rich in energy and produce a relatively high amount of CH_4 in the biogas (WAGNER et al. 2013, KALLISTOVA et al. 2014).

The hydrolytic, acidogenic and acetogenic bacteria, together with methanogens, are the key players acting at specific phases of the AB process and depend on each other for proper functioning. During this investigation, the *Escherichia coli* and *Pseudomonas aeruginosa* acidogenic bacteria were chosen to indicate the microbial population involved in the fermentation of the co-AD process. These acidogenic bacteria play a primary role in producing major substrates such as hydrogen, carbon dioxide, acetate, and short-chain organic acids, for methanogenesis (KIM et al. 2010).

The mixture of carbon-rich substrates with nitrogen-rich by-products, such as CM and industrial waste can improve process stability, providing nutrients needed for microbial population and biogas production. According to Hagos et al. (2017), it is important when selecting the substrate and the proportion to be used in co-AD, stating that the C:N ratio is the main factor to increase process performance in biogas production. The imbalance of nutrient contents, associated with low or high C:N ratio, decreases the microorganism activity, it causes the instability, failure of the system and reduction of biogas production (ZESHAN et al. 2012, NAIK et al. 2014).

The biodigesters RCW1, RCW2 and RCW3 presented the best results for the dynamics of microorganisms, the bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were present throughout the experiment. Therefore, they had high efficiency and stability in CH_4 production. The co-AD of CM and RCW waste are an attractive option because CM can buffer the low pH of RCW and its rapid acidogenesis from readily fermentable sugars (LI et al. 2015). Continued studies have been reported on co-AD of dairy wastewater with CM in different operating conditions which confirmed that the biogas production is improved compared to single substrate feed (TOUMI et al. 2015). The concentration of NH_3-N in biodigesters RCW1, RCW2 and RCW3 remained relatively

stable during the experiment period. The C:N ratio presented ideal values to satisfy the process stability, and to give continuity to fermentation.

Similar results were observed by Treu et al. (2019), it was verified that the co-AD of the CM with wastes from dairy industries can reduce potential acidification incidents, which deteriorate the biomethanization process, where the microbial profile of the reactors was adequately correlated with the recorded biochemical parameters. These are the results in an even greater production of bioenergy.

According to our findings, the biodigesters BRE2 and BRE3 have demonstrated a failure in the co-AD process when the proportion of wastes from the STS of a brewery was ≥ 40%. The reason for bacterial non-growth states in both biodigester is explained due to the increase in NH_3-N. The brewery residue has significant concentrations of nitrogen and protein in its constitution. Existence of nitrogen in the feedstock is necessary for the synthesis of amino acids, proteins, and nucleic acids. However, an excess of nitrogen in the feedstocks can result in toxic effects to bacteria because of extreme ammonia formation. Therefore, a suitable amount of nitrogen is required to provide sufficient nutrients while avoiding ammonia toxicity (VINDIS et al. 2014; HAGOS et al. 2017).

The biodigesters BRE2 and BRE3 presented a lower C:N ratio during the time of the experiment. The substrates characterized by low C:N ratios result in accumulation of ammonia that are toxic to methanogenic bacteria, given the high buffer capacity that leads to microbial growth inhibition due to increased concentration of ammonia in the fermentation process (RABII et al. 2019). In addition, the lowest production of CH4 in these biodigesters is also related to the presence of higher concentrations of NH_3-N, potentially causing inhibition of the methanogenesis process. This is in agreement with the observation made by Vanwonterghem et al. (2014) that microbial group dynamics and CH4 production tend to respond poorly to changes of operational conditions.

Our results revealed that ammonia accumulation was the main parameter that shapes the bacterial community composition. Previous studies are mainly focused on ammonia inhibition effects on methanogens (SUN et al. 2016; HAGEN et al. 2017).

5. CONCLUSIONS

This study showed that the biodigesters in co-AD of CM with RCW had a better performance in bacterial populations diversity. The mixture of these two residues are advantageous in nutrition balance, microorganism enrichment, reduction in the accumulation of inhibitors, making the process possible and feasible. The permanence of the studied acidogenic bacteria was fundamental to the process stability as a result of high biogas production efficiency and CH_4 yield. In contrast, the performance of the biodigesters in anaerobic co-AD of CM with STS of a brewery demonstrated inefficiency in the process due to the increase in NH_3-N. However, the capacity to support brewery waste in a co-AD biodigester with CM is 20%. We concluded that a microbial diversity often is associated with an environment recalcitrant, subject to changes and stress, co-AD could potentially increase the robustness of the AB process.

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