FEDERAL UNIVERSITY OF JUIZ DE FORA
FACULTY OF PHARMACY AND BIOCHEMISTRY
GRADUATE PROGRAM SCIENCE AND TECHNOLOGY OF MILK AND DERIVATIVES

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Energy recovery from ricotta cheese whey co-digestion with bovine manure in anaerobic reactors

Juiz de Fora
2020

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Dissertation submitted to the Graduate Program Science and Technology of Milk and Derivatives of the Federal University of Juiz de Fora, of the requirements for the degree of Magister in Science and Technology of Milk and Derivatives.

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Juiz de Fora
2020

Ficha catalográfica elaborada através do programa de geração
automática da Biblioteca Universitária da UFJF,
com os dados fornecidos pelo(a) autor(a)

Santana, Larice Aparecida Rezende .
Energy recovery from ricotta cheese whey co-digestion with bovine manure in anaerobic reactors / Larice Aparecida Rezende Santana. -- 2020.
53 f. : il.

Orientador: Marcelo Henrique Otenio
Coorientadores: Jailton da Costa Carneiro, Henrique Vieira de Mendonça
Dissertação (mestrado profissional) - Universidade Federal de Juiz de Fora, Faculdade de Farmácia e Bioquímica. Programa de Pós-Graduação em Ciência e Tecnologia do Leite e Derivados, 2020.

1. Organic load. 2. Agroindustrial waste. 3. Bioenergy. I. Otenio, Marcelo Henrique , orient. II. Carneiro, Jailton da Costa , coorient. III. Mendonça, Henrique Vieira de , coorient. IV. Título.

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Dissertação apresentada ao Programa de Pós-graduação em Ciência e Tecnologia do Leite e Derivados da Universidade Federal de Juiz de Fora como requisito parcial à obtenção do título de Mestre em Ciência e Tecnologia do Leite e Derivados. Área de concentração: Ciência e Tecnologia do Leite e Derivados

Aprovada em 28 de fevereiro de 2020

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I dedicate this work to my parents and my husband who inspired, supported, encouraged and helped me.

ACKNOWLEDGMENTS

I thank God for the protection, blessing, strength and comfort for not giving up and continuing to fight for my goals.

To my family for the support, teachings and for always being present in my life.

To my advisor Marcelo Henrique Otenio and co-supervisors Jailton da Costa Carneiro and Henrique Vieira de Mendonça for their patience in difficult times, support, encouragement, teachings, dedication and trust.

To Vanessa Romário de Paula for her trust, support, encouragement and contributions.

To the *Conselho Nacional de Desenvolvimento Científico e Tecnológico Brazilian* (CNPq) (National Council for Science Technological Development), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) (Coordination for the Improvement of Higher Education Personnel), *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG) (Minas Gerais State Research Support Foundation) and *Empresa Brasileira de Pesquisa Agropecuária* (Embrapa) (Brazilian Agricultural Research Corporation) for the financial support received.

To Marlice Teixeira Ribeiro, Júnior César Fernandes, Ernando Ferreira Motta, Mario Baesso Tristão and Ricardo Jose de Medeiros Campos for the assistance and performance of the analyzes and all the interns who collaborated for the development of the project.

To Embrapa Gado de Leite drivers for transportation and assistance in collecting samples.

To the Federal University of Juiz de Fora, the Milk and Derivatives Science and Technology Program and its employees, for all the support and assistance.

To members of the bank for availability.

ABSTRACT

In this research, anaerobic reactors were tested for the treatment and energy recovery of industrial and cattle-raising waste. Four plug flow reactors operated with concentrations of 20% ricotta cheese whey and 80% bovine manure; 40% ricotta cheese whey and 60% bovine manure; 80% ricotta cheese whey and 20% bovine manure; and the control with 100% of bovine manure were tested in bench scale, at room temperature and hydraulic retention time of 30 days. The experiment was conducted for 106 days in fed batches. Biogas volumes between 17 to 27 L day^{-1} were produced, with an average CH_4 concentration of 57 (±1.9) to 60% (±1.44) with energy generation potential from 0.76 to 0.90 in kWh day^{-1}, values that demonstrate the potential of anaerobic co-digestion with ricotta cheese whey and bovine manure for biogas generation and a new alternative for renewable energy production. The average removals of biochemical oxygen demand and chemical oxygen demand were 51 (±13.15) to 78% (±12.00) and 40 (±9.44) to 69% (±20.71), of total solids and volatile solids of 36 (±5.14) to 54% (±11.34) and 44 (±12.53) to 69% (± 9.34), respectively. The pH values were always close to neutrality and alkalinity in ranges suitable for anaerobic co-digestion. The treatment process and recovery of these residues in an anaerobic reactor obtains satisfactory environmental results and can be considered promising.

Keywords: Organic load. Agroindustrial waste. Bioenergy.

RESUMO

Nesta pesquisa reatores anaeróbios foram testados para o tratamento e valorização energética de resíduos industrial e da bovinocultura. Quatro reatores *plug flow* operados com concentrações de 20% de soro de ricota e 80% de dejeto bovino; 40% de soro de ricota e 60% de dejeto bovino; 80% de soro de ricota e 20% de dejeto bovino; e o controle com 100% de dejeto bovino foram testados em escala de bancada, temperatura ambiente, tempo de retenção hidráulico de 30 dias. O experimento foi conduzido por 106 dias em batelada alimentada. Foram produzidos volumes de biogás entre 17 a 27 L dia^{-1}, com concentração média de CH_4 de 57 (±1,9) to 60% (±1,44) com potencial para geração de 0,76 a 0,90 de energia em kWh dia^{-1} valores que demonstram o potencial da co-digestão anaeróbia de soro de ricota com dejeto bovino para geração de biogás e uma nova alternativa para a produção de energia renovável. As remoções médias de demanda bioquímica de oxigênio e demanda química de oxigênio foram de 51 (±13,15) a 78% (±12,00) e 40 (±9,44) a 69% (±20,71), de sólidos totais e sólidos voláteis de 36 (±5,14) a 54% (±11,34) e 44 (±12,53) a 69% (±9,34), respectivamente. Valores de pH estiveram sempre próximos à neutralidade e a alcalinidade em faixas propícias à co-digestão anaeróbia. O processo de tratamento e valorização desses resíduos em reator anaeróbio obtêm resultados ambientais satisfatórios e pode ser considerado promissor.

Palavras-chave: Carga orgânica. Resíduo agroindustrial. Bioenergia.

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LIST OF ABBREVIATIONS AND ACRONYMS

AC	Anaerobic co-digestion
APHA	American Public Health Association
ASBR	Sequential batch anaerobic reactor
BA	Biodigestão anaeróbia
BM	Bovine manure
BOD_5	Biochemical oxygen demand
CA	Codigestão anaeróbia
CFU	Colony Forming Units
CH_4	Methane
COD	Chemical oxygen demand
CO_2	Carbon dioxide
CSTR	Continuous stirred tank reactor
Embrapa	Brazilian Agricultural Research Corporation
EMB	Eosin Methylene Blue Agar
H_2	Hydrogen
HRT	Hydraulic retention time
NaCl	Cloreto de sódio
$N\text{-}NH_3$	Ammoniacal nitrogen
NO_2^-	Nitrite
NO_3^-	Nitrate
OTUs	Operational Taxonomic Units
RCW	Ricotta cheese whey
SIM	sulfate, indole and motility
TKN	Total Kjeldahl nitrogen
TRH	Tempo de retenção hidráulico
TS	Total solids
UASB	Up-flow anaerobic sludge blanket
VFA	Volatile fatty acids
VS	Volatile solids

SUMMARY

1 INTRODUCTION

With the growing demand for renewable energy and environmental pollution control, technologies for biogas production through anaerobic co-digestion (AC) have attracted the scientific community attention (BROWN; GÜTTLER; SHILTON, 2016). Management and recovery of agro-industrial waste through anaerobic processes represent a significant opportunity to combine waste treatment and renewable energy production (VALTA *et al.*, 2017). Dairy effluents represent a promising source of renewable energy and research is focused on energy production with better use of dairy waste (KASMI, 2018).

In developed and developing countries, industrial effluents are becoming useful sources for biogas production (CAROTA *et al.*, 2017). Among other effluents, Ricotta cheese whey (RCW) represents an industrial pollutant, derived from the production of ricotta, composed of 0.15 to 0.22% of proteins, 1 to 1.13% of salts, 4.8 to 5% of lactose, estimated biological oxygen demand (BOD) of 50 g L^{-1} and a chemical oxygen demand (COD) of 88 g L^{-1}. Although a protein concentration does not allow its use in processes that involve the valorization of this macromolecule, the lactose content can be explored in fermentation processes (RIZZOLO; CORTELLINO, 2017). However, AC is an appropriate strategy for the treatment of this waste, as it contains easily fermentable carbohydrates, or makes it a suitable substrate for this process (FLORES-MENDOZAA *et al.,* 2020).

Energy recovery from bovine manure (BM) is a reality in several countries (MIRANDA *et al.*, 2016). As livestock effluent is a source of methane (CH_4), which is a highly valued energy resource (MENDONÇA; OMETTO; OTENIO, 2017). Given the need to control such wastes in order to reduce their environmental impact, the application in AC with other organic wastes with complementary characteristics enhances the energetic valorization of the substrate through the biogas/biomethane production, besides obtaining a final effluent with potential to be used as a biofertilizer in agriculture (RAHEEM; HASSAN; SHAKOOR, 2016).

AC represents one of the sustainable ways of treating waste that contains organic matter that is difficult to degrade and recalcitrant, increases biodegradation, reduces environmental impacts and constitutes an energy resource of great potential (CORSINO *et al.*, 2017; XU *et al.*, 2018).

In this study, the objective was to add RCW as an alternative for the production of biogas in the AC with BM, in different proportions, using the treatment system composed of four plug-flow reactors, operated on a bank scale as well as to analyze the performance of the AC process, measuring the biogas and CH_4 rates and verifying the behavior and changes in the physical-chemical parameters.

2 MATERIALS AND METHODS

The analysis were performed at the Brazilian Agricultural Research Corporation, Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil. The physical-chemical and microbiological analysis were performed at the Rumen Microbiology Laboratory and the analysis of the biogas composition at the Chromatography Laboratory. Metagenomic analysis were performed in the genetics laboratory and in an outsorced laboratory.

2.1 CHARACTERISTICS OF THE EXPERIMENT CONDUCTION SITE

The experiment was conducted at the Brazilian Agricultural Research Corporation, Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil, in geographic coordinates, 21° 46 '55 "S; 53° 22' 10" W. The area is classified as Cwa according to Köppen & Geiger, with an average temperature of 20.1 °C, a maximum of 27.9 °C and a minimum of 11.2 °C, with 1.504 mm annual rainfall average and atmospheric pressure of 0.97 atm (CLIMATE-DATE.ORG, 2019; DEGEO, 2009).

2.2 SUBSTRATES AND INOCULUM

The substrates for the plug flow reactor experiment were BM and RCW (Table 1, Table 2).

Table 1 – Microbiological BM characterization

BM
Escherichia coli
Pseudomonas aeruginosa
Methanobrevibacter
Methanosphaera
Methanocorpusculum
Methanosaeta
Methanosarcina

Source: Elaborated by the author (2020).
Note: BM: bovine manure.

Table 2 – Physicochemical characterization of substrates

Parameters	**Unity**	**BM**	**RCW**
pH	-	6.25(0.43)	5.88(0.11)
Alkalinity	mg L^{-1}	2,791.00(316.34)	860.00(3.51)
TS		62,000(6,900)	52,200(200)
VS		54,400(3,700)	41,700(400)

Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; TS: total solids; VS: volatile solids. Values in brackets indicate standard deviation.

The BM consisted of raw manure semi-confined lactating Girolando mixed with and wastewater from free-stall floor cleaning in milk production system of the experimental farm of Embrapa Dairy Cattle, in Coronel Pacheco, MG, Brazil. The animals diet was concentrated (mixture of 60% corn grains, 36% soy grains, 3% mineral core and 1% urea), corn silage, pasture and mineral salt, with an adopted average of 3.5 kg, 20 kg, 40 kg and 150 g per day, respectively.

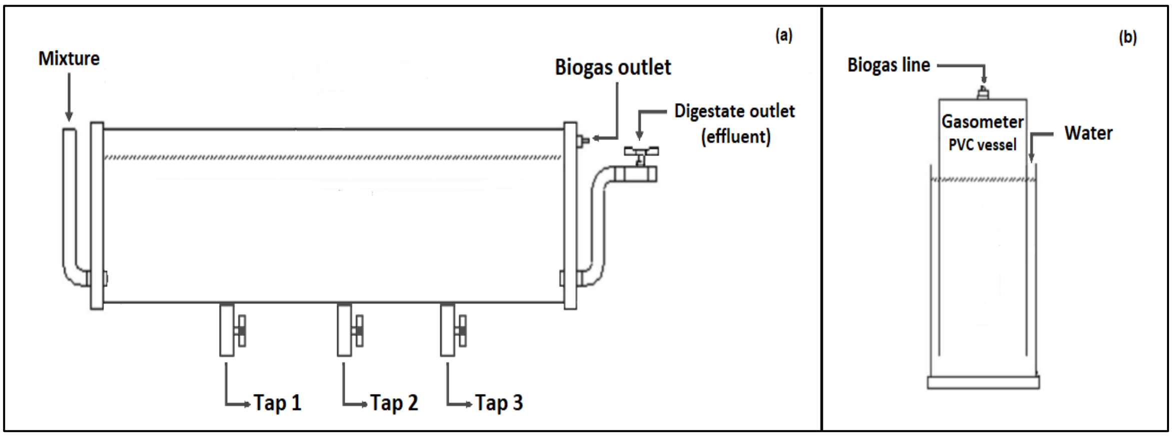
The BM was collected weekly and transported to Embrapa Dairy Cattle, in the city of Juiz de Fora, MG, Brazil. The mixture was sieved 3.7 mm mesh to homogenize the TS concentration in 6% (±2%). After the preparation of the BM related substrate, they were stored at 8 °C for later use in the daily supply of reactors. The BM itself was used as inoculum for the reactor start up.

The RCW was obtained weekly in a dairy company in the city of Juiz de Fora, MG, Brazil and transported to Embrapa Dairy Cattle, after stored at 8 °C. It was removed from refrigeration for daily use with bovine manure mixture and, when at room temperature, RCW pH was corrected between 6.5 and 7.0 with 59 mL of calcium hydroxide ($Ca(OH)_2$) at 4.24% for each 1 L of RCW. This concentration was sufficient for pH correction/increase, according to tests performed with different RCW initial pH values (SANTANA *et al.*, 2019).

2.3 REACTORS DESCRIPTION

Each reactor model used in this experiment were constructed from PVC tubes, composed by a 60 L fermentation chamber with 30 L individual gasometers, which worked in conjunction with the plastic hose-connected reactor (Figure 1). The gasometers were constructed using two PVC tubes, where one outer tube was filled with water and the second submerged in water to allow displacement of the gas produced in the fermentation chamber. The reactors were painted black to maximize their internal heating, placed on iron sports, operated outdoors at room temperature and grouped by side, so that the incidence of sunlight was homogeneous for all.

Figure 1 – Anaerobic reactor. (a) cylindrical anaerobic reactor. (b) details of gasometer



Source: Adapted from RESENDE *et al*. (2016).

2.4 TREATMENT DESCRIPTIONS

Four reactors were used for the AC essay (Table 3). The microbiological and physico-chemical characteristics of each mixture for station co-digestion presented on Table 4 and 5, respectively.

Table 3 – Mixture description per reaction unit

Reactor	**Treatment**
1	Control (100% BM)
2	20 % RCW + 80% BM
3	40% RCW + 60% BM
4	80% RCW + 20% BM

Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey.

Table 4 – Microbiological characteristic of the influents of each treatment e for co-digestion

20% RCW + 80% BM	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
Escherichia coli	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
Methanobacterium	*Methanobacterium*	*Methanobacterium*	*Methanobacterium*
Methanobrevibacter	*Methanobrevibacter*	*Methanobrevibacter*	*Methanobrevibacter*
Methanocorpusculum	*Methanocorpusculum*	*Methanocorpusculum*	*Methanocorpusculum*
Methanoculleus	*Methanoculleus*	*Methanoculleus*	*Methanoculleus*
Candidatus Methanoregula	*Candidatus Methanoregula*	*Candidatus Methanoregula*	*Candidatus Methanoregula*
Methanolinea	*Methanolinea*	*Methanolinea*	*Methanolinea*
Methanospirillum	*Methanospirillum*	*Methanospirillum*	*Methanospirillum*
Methanosaeta	*Methanosaeta*	*Methanosaeta*	*Methanosaeta*
Methanosarcina	*Methanosarcina*	*Methanosarcina*	*Methanosarcina*

Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey.

Table 5 – Physico-chemical characteristic of the influents of each treatment for co-digestion

Parameters	Unity	20% RCW + 80% BM	40% RCW+ 60% BM	80% RCW + 20% BM	Control
pH	-	6.00(0.50)	5.98(0.60)	5.85(0.53)	6.25(0.43)
Alkalinity	mgL^{-1}	2,199.00(299.00)	1,816.00(296.01)	1,501.00(318.59)	2,791.00(316.34)
TS		58,700(6,500)	52,500(6,200)	50,600(6,500)	62,000(6,900)
VS		48,400(2,900)	43,900(3,000)	40,500(3,600)	54,400(3,700)
COD		92,150(9,672)	81,117(189)	59,867(6,861)	97,433(571)
BOD_5		39,756(3,779)	37,200(14003)	27,367(3,172)	43,753(9,842)
TKN		470.00(352.04)	398.00(292.57)	382.00(382.37)	388.00(152.55)
$N\text{-}NH_3$		446.00(500.72)	353.00(382.63)	340.00(397.32)	339.00(289.33)
NO_3^-		32.00(20.21)	42.00(14.14)	38.00(21.21)	35.00(0.01)
NO_2^-		3.00(1.53)	4.00(0.35)	7.00(2.83)	5.00(2.47)

Source: Elaborated by the author (2020).

Notes: TS: total solids; VS: volatile solids; COD: chemical oxygen demand; BOD_5: biochemical oxygen demand; TKN: total Kjeldahl nitrogen ; $N\text{-}NH_3$: ammoniacal nitrogen; NO_3^-: nitrate; NO_2^-: nitrite. Values in brackets indicate standard deviation.

The operation of the reactor was carried out in three phases, the first being related to the start of the system and the other two to the operation of the unit.

Initially, all reactors were completely filled with BM, exclusively for the development of the inoculum, ensuring the growth and stabilization of colonizing microorganisms, which corresponds to the start up of the experiment (Phase 1). After the adaptation of the inoculum, verified with the concentration of CH_4 above 60%, the daily supply of 2L of the mixture and effluent outlet started (Phase 2) (MENDONÇA *et al.*, 2017). After 30 days of daily supply, the reactors operated in complete co-digestion (Phase 3). The experiment took 106 days in complete co-digestion at total with hydraulic retention time (HRT) of 30 days.

The treatment units operated in the ambient temperature range, between 18 and 26°C average of 22°C (± 2.02) and temperatures inside the reactor, between 14 and 33°C average of 25°C (± 2.32), these ranges that oscillated between the conditions of psychic (<20°C) and mesophilic (25 to 40°C) temperatures.

To prepare the mixtures, the following steps were followed: the substrates were measured separately in a glass beaker and mixed in a plastic beaker, both with a volume of 2 L, according to each treatment.

2.5 MICROBIOLOGICAL ANALYSIS

During the complete co-digestion phase, seven samples of effluents were collected from each treatment, for the characterization and monitoring of *Escherichia coli* and *Pseudomonas aeruginosa*, representing the acidogenic phase. As well as samples of substrates and influents.

To meet the objectives of the experiment, serial dilutions of samples from 10^{-1} to 10^{-3} were performed in 0.9% saline (NaCl), aliquots of 0.1 ml (100 µL) sown in selective culture medium, Eosin Methylene Blue Agar (EMB) for *Escherichia coli* and MacConkey Agar for *Pseudomonas aeruginosa*. The plates incubated in a bacteriological oven for 24 hours at 37ºC (APHA, 2017)

The quantification of microorganisms was performed from the direct count of Colony Forming Units (CFU) grown in the culture medium to contain between 30 and 300 CFU (APHA, 2017).

Biochemical tests were performed to identify *Escherichia coli* and *Pseudomonas aeruginosa*. The cultivated colonies were subjected to Gram stain analysis, catalase test, oxidase, SIM (sulfate, indole and motility) and citrate to prove and confirm the isolated bacteria (APHA, 2017).

2.6 METAGENOMIC ANALYSIS

The total of 11 samples was selected at different times, for better representation, prioritizing the selection of at least one sample/month of each reactor, using as a criterion the production of CH_4 associated with the temporal distribution to choose the co-digestion effluent samples complete. Two samples of substrate were collected, four of affluent and five of effluent.

DNA samples extracted were submit to a quality analysis through fluorescence quantification using Qubit® 3.0 Flurometer and Qubit^{TM} dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).

For amplification of polymorphic region (V4) of the 16S rRNA gene, PCR were conduct in triplicate using the oligonucleotides. The reactions were carry out in a Veriti^{TM} Thermal Cycler (Applied Biosystems, Foster, CA, USA). Then the amplification was confirm by agarose gel electrophoresis (2%). Amplification products were subject to purification steps using Agencourt AMPure XP beads (Beckman Coulter, High Wycombe, UK), according to manufacturer's protocol.

Subsequently, indexers were insert into common adapters required for generating clusters and sequencing the samples. The indexing reaction was perform following the protocol of Nextera XT Index Kit (Illumina, Inc., San Diego, CA, USA). Amplification reactions were conducted in Veriti^{TM} Thermal Cycler (Applied Biosystems, Foster, CA, USA). The created libraries were submit to the purification steps using Agencourt AMPure XP beads (Beckman Coulter, High Wycombe, UK), to remove small fragments of total population of molecules and remains of primers.

After this step, the quantification was performed by Real Time PCR using Kapa Library Quantification Kit Illumina GA Universal - KK4824 (Kapa Biosystems, Wilmington, MA, USA), according to manufacturer's recommendations. An equimolar DNA pool was generate by normalizing all samples to 3nM, to perform the sequencing, which was conducted using Illumina MiSeq platform (Illumina, San Diego, CA, USA) and MiSeq Reagent - V2 Micro 300 cycles Kit (Illumina, San Diego, CA, USA).

Bioinformatics analysis was performed on QIIME2 platform version 2019.7 (BOLYEN *et al*., 2019). The sequences were filter by quality and grouped into Operational Taxonomic Units (OTUs) using 97% of identity between them.

2.7 PHYSICAL-CHEMICAL ANALYSIS

Sixteen samples at total were collected at the entrance (affluent) and at the exit (effluent) of each reactor weekly and analyzed in duplicate (results expressed every 15 days), obtaining eight samples/repetition per month. The sampling period took place from December 2018 to March 2019 (Phase 3). The pH was assessed by Tecnal pHmeter model Tec-3MP. Alkalinity ($CaCO_3$) was analyzed by titration with 1N sulfuric acid solution until the pH reached 4.3. To quantify the total solids (TS), the samples were incubated in an oven at 105°C, cooled in a desiccator and the dry weight was quantified. For volatile solids (VS) the same samples were sent to the muffle furnace (575°C), cooled in a desiccator and the ash weight was quantified, which was subtracted from the dry weight. The determination of biochemical oxygen demand (BOD_5) occurred by iodometry and chemical oxygen demand (COD) by the colorimetric method with closed reflux, with the spectrophotometer operating in the range of 420 nm and 600 nm. To determine ammoniacal nitrogen ($N\text{-}NH_3$), nitrite (NO_2^-) and nitrate (NO_3^-) an optical emission spectrophotometer was used. The measurements were performed in accordance with the standard methods (APHA, 2017).

The CH_4 concentrations contained in the biogas were determined by gas chromatography, during phases 2 and 3 of the experiment, using the Agilent Technologies chromatograph, model 7.820A, consisting of HP-Plot / Q columns 30m x 0.530 mm x 40.0 µm and HP-Molesieve 30m x 0.530mm x 25.0 µm, with hydrogen (H_2) as the carrier gas (COLLINS; BRAGA; BONATO, 1997).

The ambient temperatures and inside the digesters were determined by a U12 4-External Channel Outdoor / Industrial Data Logger (Part # U12-008) together with the HOBOware software. The measurement was provided in degrees Celsius (°C) over a 12-hour time interval, with data collection of every 15 days.

2.8 NORMALIZATION OF THE VOLUME OF BIOGAS

The biogas measurements were carried out every day, twice a day, at the same time, in the morning, before the daily supply and in the afternoon, during all phases of the experiment.

The volume of the measured biogas was normalized to standard conditions (0°C and 1,013 bar), according to the ideal gas equation of Gay-Lussac (Equation 1).

$$V_0 = \frac{V_1 . P_1 \ . T_0}{P_0 \ . T_1} \qquad (1)$$

Where V_0 was the corrected biogas volume (m^3), P_0 the corrected biogas pressure, to 1 atm, T_0 the biogas corrected temperature (273K), V_1 the gas volume in the gasometer, P_1 the biogas pressure in the reading and T_1 the biogas temperature (K) at the time of reading.

The gas temperature inside the gasometer was monitored in °C by a long-stemmed digital thermometer HI 93510N (HANNA).

2.9 THEORETICAL POTENTIAL FOR ENERGY PRODUCTION

The energy production potential was calculated based on the energy content of CH_4 using 0.222 kWh $mol^{-1}CH_4$ factor (PFLUGER *et al.*, 2020).

The number of moles of CH_4 was calculated according to its concentration in the biogas (% p/v) and based on the that 1 mole of CH_4 contains a molar mass of 16 g.

3 RESULTS AND DISCUSSION

The experiment was characterized by monitoring physical-chemical and microbiological parameters of biomass and effluents to control the process and establish its efficiency.

3.1 BIOMASS

The composition of different biomasses is one of the parameters used to determine the suitability of a substrate for the production of biogas.

The performance of the AC depends on the functions and interactions of the microorganisms. However, a diversity of these microorganisms depends on the type of organic matter applied to the reactor (FLORES-MENDOZAA *et al.*, 2020).

The relationship between biomass and microorganisms is a factor that depends on the type and composition of the substrate and inoculum. The inoculum provides microorganisms capable of digesting organic matter, accelerating the initial biodegradation process. The characteristics of the inoculum influence the existence and initial activity of microorganisms and the different adaptations of the substrates (HOLLIGER *et al.*, 2016). The increase in the inoculum concentration increases the amount of methanogenic bacteria in the reactor, limiting the accumulation of volatile fatty acids (VFA) (SHI *et al.*, 2014).

In the present study acidogenic bacteria and methanogenic archaeas were identified in the substrates (Table 1).

Acidogenic bacteria were important in the co-digestion process, as they helped to convert organic matter into energy in the form of CH_4, by converting the substrate into acetate, the main precursor of CH_4 in the metabolic pathway (MANYI-LOH *et al.*, 2019). As well as the methanogenic acetoclastic and hydrogenotrophic archaeas, for converting acetate to CH_4, and H_2 and CO_2 to CH_4, respectively (JI; LIU; CONRAD, 2018). These bacteria are important in anaerobic reactors, at about 70% of the CH_4 produced in these systems is the result of acetate degradation (DARWIN; CORD-RUWISCH, 2019).

The increase in methanogenic bacteria, such as alkalinity, contributes to pH stabilization, reduces microbial inhibitions and improves CH_4 production. However, acidification is one of the challenges in the process (SHI *et al.*, 2014).

The acidity and low alkalinity of the RCW, were on average 5.88 (± 0.11) and 860 (±3.51) mg $CaCO_3$ L^{-1}, respectively, would make anaerobic digestion harder (Table 2) (BROWN; GÜTTLER; SHILTON, 2016). Alkalization to avoid acidification and impracticability of the process is essential (DIAMANTIS *et al.*, 2014).

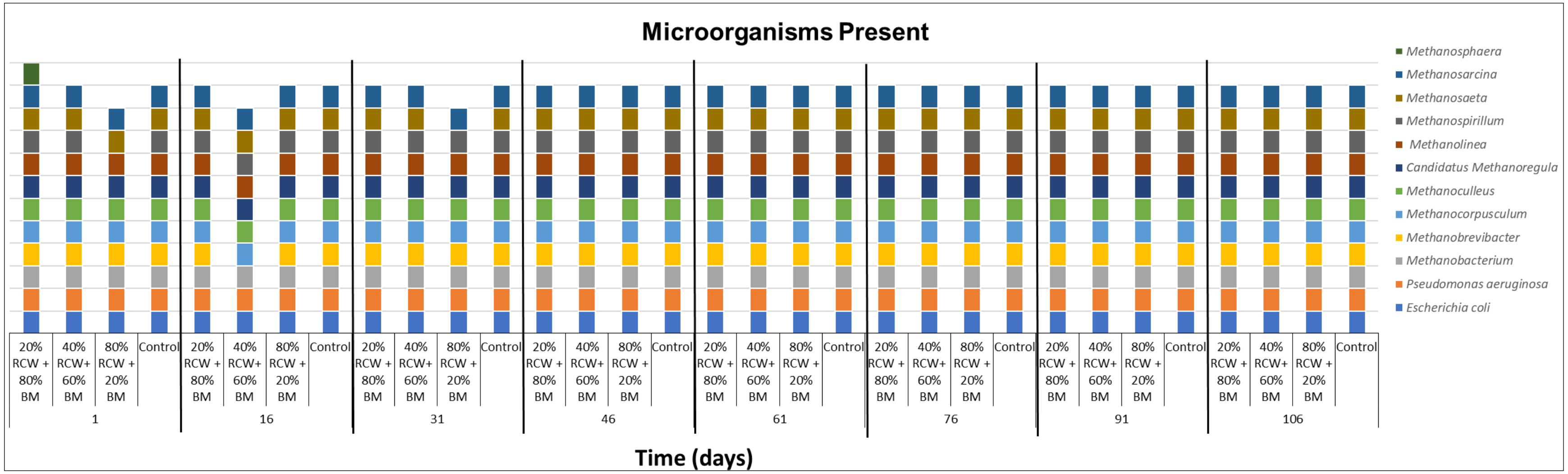
In this research, the TS values of the BM determined as ideal for the stability of the 30 day HRT process, were on average 62,000 (±6,900) mg L^{-1}, lower than that determined in reactors, with fasting at 80,000 mg L^{-1} TS and the same HRT used in the present study. While the VS values were 54400 (± 3700) mg L^{-1}, ranging from 49,200 to 65,100 mg L^{-1} the records under study and measuring as physical-chemical properties of bovine manure, were lower, varying between 61,600 to 69,500 mg L^{-1} (WANG *et al.*, 2019).

The composition of the RCW depends on factors such as type of milk and conditions of production and generation of industrial waste. Regarding their characteristics, the TS and VS were from 52,200 (±200) and 41,700 (±400), respectively.

3.2 MICROBIAL COMMUNITY

The diversity of the microbial community in the AC process in the different treatments showed less presence of *Methanosphaera* (Figure 2).

Figure 2 – Microbiological characteristic of the effluents from each treatment in the anaerobic co-digestion process



Source: Elaborated by the author (2020).

Notes: BM: bovine manure; RCW: ricotta cheese whey.

This methanogenic archaea uses H_2 to reduce methanol to CH_4 through the methylotrophic pathway and its reduced population can lead to a decrease in hydrogen consumption, inhibiting VFA conversion (LV *et al.*, 2019). However, *Methanosarcina* is generalist, it can form CH_4 by three metabolic pathways, acetoclastic, hydrogenotrophic and methylotrophic, from H_2, CO_2, methanol, methylamine and acetate (ROCHELEAU *et al.*, 1999). This bacterium remained present in all reactors during all days of operation, which cannot cause an increase in the volume of VFA, does not affect the pH of the medium and microbial inhibition (ZHANG *et al.*, 2014).

Studies carried out on the composition of the methanogenic community in BM have identified very low amounts of *Methanosphaera*, close to what was verified in the present research (GUO *et al.*, 2020; JIN *et al.*, 2017; LIU *et al.*, 2018). Indicating that microbial diversity can be attributed to several factors, such as animal breeds, diet sources and composition (DONG *et al.*, 2019).

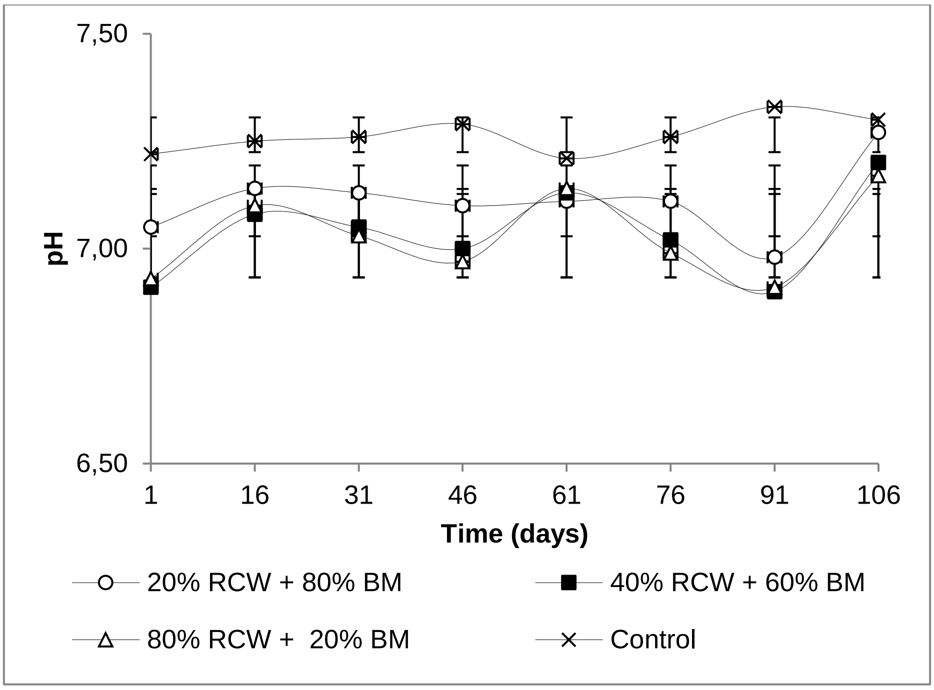
Among the other eight methanogenic bacteria identified, six produced CH_4 from reduced CO_2 and two from reduced acetic acid. However, the production of CH_4 depended on the production of acetic acid and H_2 and these on the conversion of organic compounds to VFA. These microorganisms depend on the substrate provided by the acetogens, which are dependent on the acidogens, which demonstrates that the metabolic routes and products generated are dependent on the balance between the metabolites in the process (KLEEREBEZEM; LOOSDRECHT, 2010).

In this research, the presence of acidogenic and methanogenic bacteria throughout the experiment characterized the smooth functioning of the process, through the dynamic balance between microbial groups, which favored the production of biogas, which may be related to the addition of the inoculum at the beginning of the process, as recorded by Mukhuba *et al.* (2020).

3.3 pH AND ALKALINITY

The pH influences the growth and performance of bacteria present in the AC, which can vary in pH between 4 and 8.5 (SHI *et al.*, 2014). In this research, the pH remained between 6.9 and 7.3, close to neutrality, or demonstrated that there were no accumulation of VFA, as they were converted into CO_2 and CH_4, preventing the decrease in pH and the instability of the process, or the which favored the decomposition of organic matter (Figure 3) (WAINAINA *et al.*, 2019; YUAN *et al.*, 2019).

Figure 3 – pH values of effluents from each treatment in the anaerobic co-digestion process



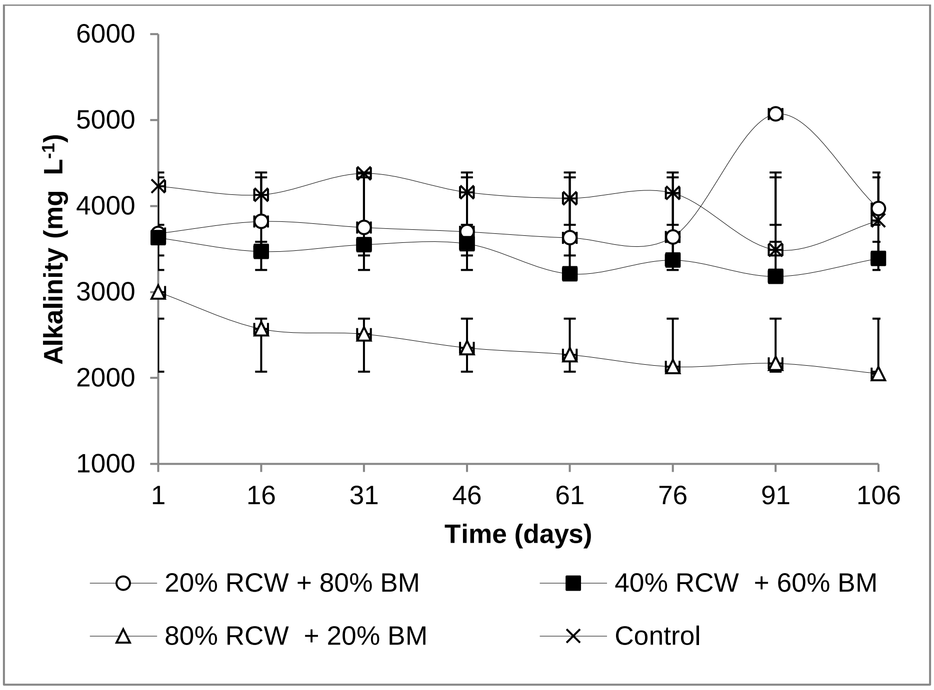
Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

However, changes in pH and presence of acidogenic bacteria in all reactors during all days of operation may indicate that there were no major changes in the metabolic pathway, not affecting the ability to produce acids from the fermentation of carbohydrates and the number of microorganisms present in reactors (DARWIN; CORD-RUWISCH, 2019). Although the ideal pH for acidogenesis was between 5.5 to 6.5 and the pH change during the fermentation process could vary the rate of microbial growth (GRESES; TOMÁS-PEJÓ; GÓNZALEZ-FERNÁNDEZ, 2020; SHI *et al.*, 2014).

The pH values between 6.8 to 7.2 favored the growth of methanogenic bacteria, without interrupting the production of CH_4. The high methanogenic population and high alkalinity can contribute to stabilize the pH by improving CH_4 production (PANIZIO *et al.*, 2020; ZHANG *et al.*, 2014).

In this study the alkalinity maintained from 2,050 to 5,070 mg L^{-1} the alkalinity supplementation may have been due to the solubilization of the CO_2 formed due to the degradation of organic matter, which due to the alkaline pH, the chemical balance of CO_2 changed to bicarbonate (dissociation of carbon dioxide in the bicarbonate ion) (Figure 4) (CHEAH *et al.*, 2019; PARRALEJO *et al.*, 2019) . As consequence, greater alkalinity returns to the system (KUNZ; MUKHTAR, 2016).

Figure 4 – Alkalinity values of effluents from each treatment in the anaerobic co-digestion process



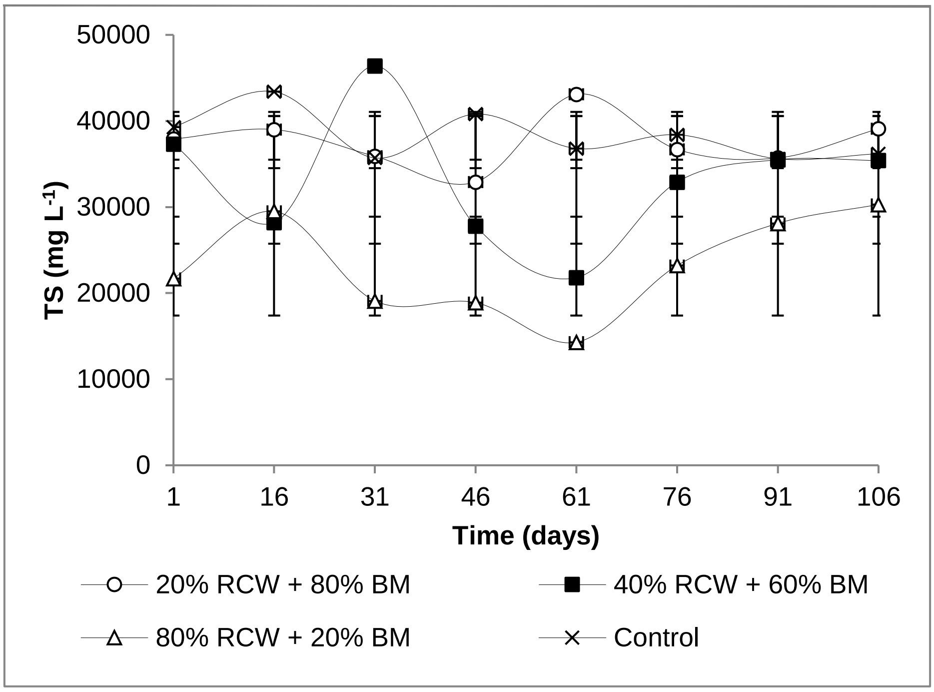
Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

Alkalinity is a control parameter to operate reactors under ideal pH conditions for the production of VFA, however, alkalinity above 2500 mg L^{-1} and little pH variation favored the occurrence of a relevant buffer effect in anaerobic reactors (CHEAH *et al.*, 2019; PARRALEJO *et al.*, 2019). In addition, it was able to avoid high pH, which indicated the production of alkalizing substances that neutralized the acids produced, and maintained the appropriate levels for system performance (CHEAH *et al.*, 2019).

3.4 TOTAL AND VOLATILE SOLIDS

The TS values of the affluents were on average 58,700 (±6,500), 52,500 (±6,200), 50,600 (±6,500) and 62,000 (± 6,900) mg L^{-1} (Table 5) and of the effluents from 14,300 to 46,400 mg L^{-1}, in reactors operated with 20, 40 e 80% of RCW and control, respectively (Figure 5).

Figure 5 – Total solids values of effluents from each treatment in the anaerobic co-digestion process

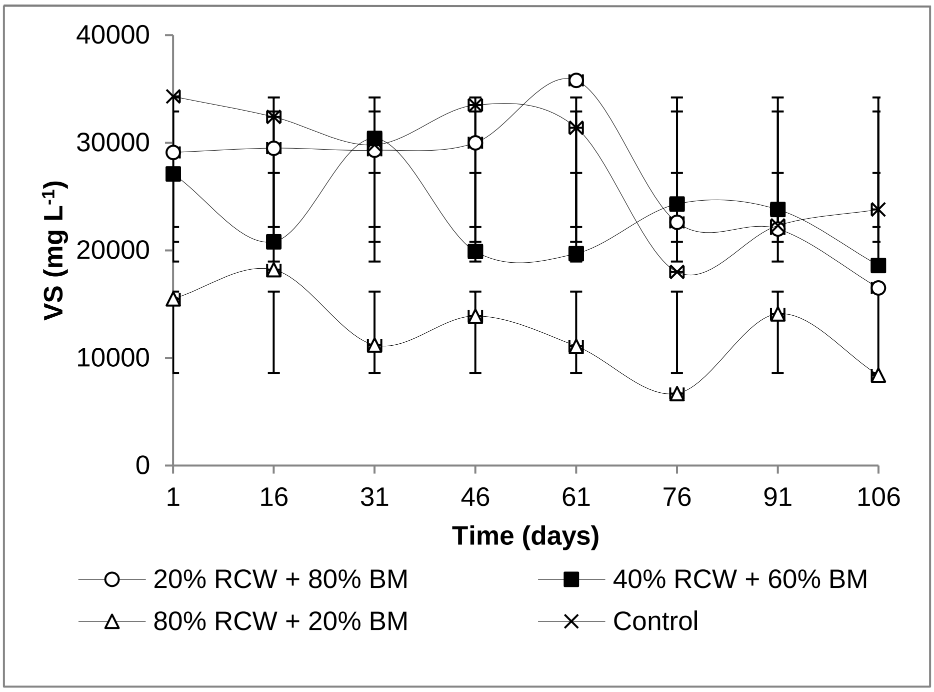


Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

For mesophilic operation of plug flow reactors, a range of TS between 10 and 14% is recommended (WILKIE, 2005). On the other hand, when reactors are operated at room temperature, lower percentages of TS are required, due to the difficult degradation of these compounds, which can lead to a decrease in removal efficiency (MENDONÇA; OMETTO; OTENIO, 2017).

The VS changed due to changes in the concentrations of the treatments, mainly in those with 80% RCW, resulting in greater variability in the reactors output load (Figure 6).

Figure 6 – Volatile solids values of effluents from each treatment in the anaerobic co-digestion process



Source: Elaborated by the author (2020).

Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The removal average day^{-1} of TS and VS were 27 to 72% and 26 to 83%, respectively (Table 6).

Table 6 – Daily removal: total and volatile solids from each treatment in the anaerobic co-digestion process

(continue)

Removal TS (%)				
Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
1	35.00	29.00	57.00	37.00
16	34.00	46.00	42.00	30.00
31	39.00	47.00	62.00	42.00
46	44.00	47.00	63.00	34.00
61	27.00	58.00	72.00	41.00
76	38.00	37.00	54.00	38.00
91	39.00	32.00	44.00	43.00
106	33.00	33.00	40.00	42.00
Av	36.00	37.00	54.00	38.00
Max	44.00	58.00	72.00	43.00
Min	27.00	12.00	40.00	30.00
SD	±5.14	±10.05	±11.34	±4.48

Removal VS (%)				
Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
1	40.00	38.00	62.00	37.00
16	39.00	53.00	55.00	40.00
31	39.00	31.00	72.00	45.00
46	38.00	55.00	66.00	38.00
61	26.00	55.00	73.00	42.00
76	53.00	45.00	83.00	67.00
91	55.00	46.00	65.00	59.00
106	66.00	58.00	79.00	56.00

Table 6 – Daily removal: total and volatile solids from each treatment in the anaerobic co-digestion process

(conclusion)

Removal VS (%)				
Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
Av	44.00	47.00	69.00	48.00
Max	66.00	58.00	83.00	67.00
Min	26.00	31.00	55.00	37.00
SD	±12.53	±9.38.00	±9.34	±11.07

Source: Elaborated by the author (2020).
Notes: Av: average; SD: standard deviation of 106 days in operation; BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The increase in the organic load changed the capacity of the reactor to promote degradation, considering that the VS are made up of organic compounds with different rates of degradation and that the removal solids is associated with the generation capacity and quality of biogas generated by microorganisms.

In the present research, due to the greater biodegradability of the RCW, a removal of the VS increased with the addition of its proportion in the mixtures, with a greater removal in the reactor with 80% of the RCW. In Spain, a study carried out in Cantabria, with AC of milk and bovine milk in the CSTR reactor operated at 35°C, obtained 56.2 and 69.9% of VS, for proportions of 15 and 85% of whey, respectively, at a 80-day HRT (RICO; MUÑOZ; RICO, 2015). In Tunis, Tunisia, a CSTR reactor operated in batch at 35°C in 20 day HRT with AC of whey and bovine milk at different selected levels quickly recovered SV (88.6%) without treatment with 80% effluent dairy and 20% bovine waste (JIHEN *et al.*, 2015).

The values of TS and VS removal verified in the control reactor varied from 30 to 33% and 37 to 67%, respectively, with higher removal efficiencies for TS and VS in the treatments with 80% RCW, at pH 7.14 and 6.99, with CH_4 production of 65 and 59%, demonstrating that the co-digestion increased the removal of solids, promoting greater degradation of organic matter. Sequential up-flow anaerobic sludge blanket (UASB) reactors of 20 and 40 L operated with bovine manure in the proportions of 500 and 800 g of manure to 1 L of water, at room temperature, between 20 and

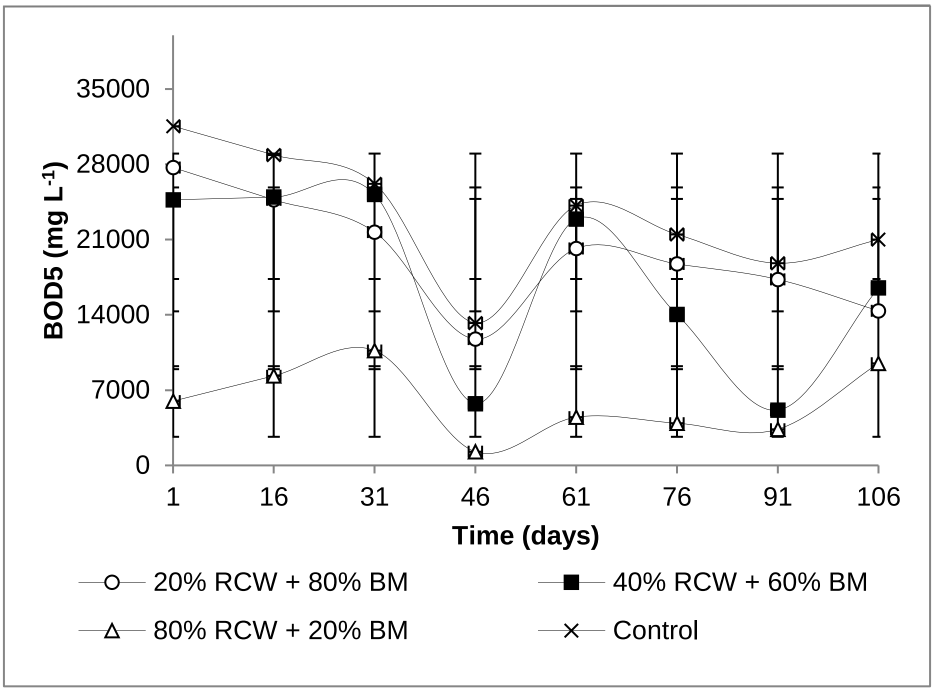
30°C, with HRTs of 7.5 and 12 days, respectively, obtained TS and VS removal greater than 71% (MONTOYA *et al.*, 2017). Plug flow reactors, due to their hydrodynamic characteristics, tend to have less TS and VS removal than UASB.

3.5 BOD_5 AND COD REMOVAL

The organic loads applied to the reactor, affluent BOD_5 and COD concentrations are shown in Table 5. Wide variations in BOD_5 and COD concentration were observed, with values average between 27,367 (±3,172.37) to 43,753 (±9,842.90) and 59,867 (±6,861.91) to 97,433 (±5,711.68) mg L^{-1}, respectively. This is because, in the collections carried out during the experiment, operational difficulties occurred, such as different batches of milk, heterogeneous generation of residues (manure and urine), type and form of feed provided for livestock and washing time of the corral by the operators. Such operating conditions reflected directly on the values of the applied organic loads.

In the present work, the BOD_5 and COD of the effluents varied from 1,275 to 31,550 mg L^{-1} and 9,125 to 71,850 mg L^{-1}, maintaining the stability of the process (Figure 7, Figure 8).

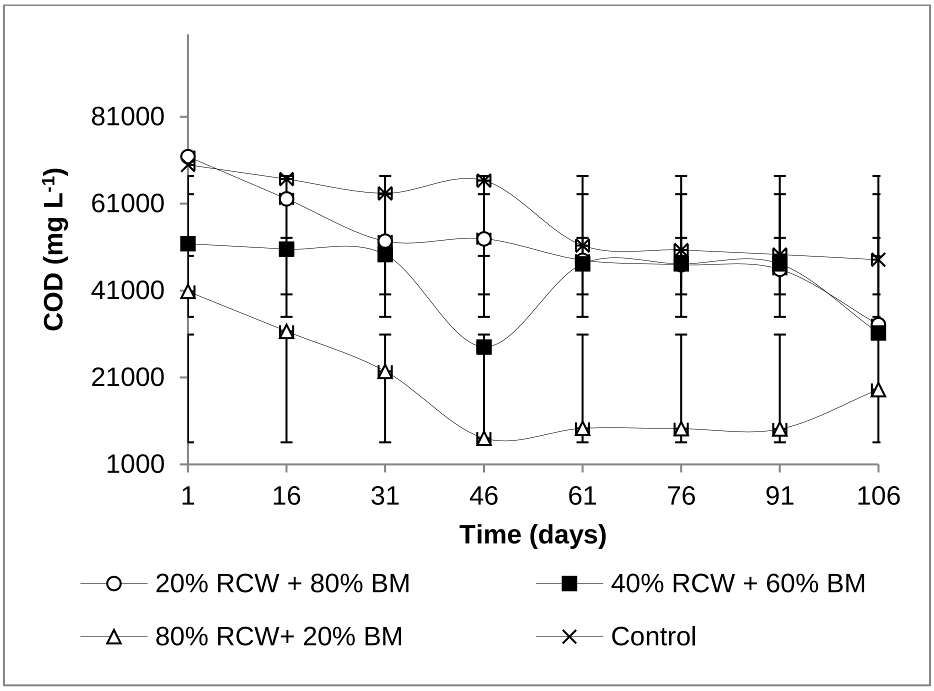
Figure 7 – Biological oxygen demand of effluents from each treatment in the anaerobic co-digestion process



Source: Elaborated by the author (2020).

Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

Figure 8 – Chemical oxygen demand of effluents from each treatment in the anaerobic co-digestion process



Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The RCW contain a high concentration of COD and are easy to digest, however the excess of these residues without BM can lead to process failure (BROWN; GÜTTLER; SHILTON, 2016). What did not occur in this research when operating a reactor with 80% RCW and 20% BM, due to the BM ability to supply nutrients that maintains the balanced buffering capacity and the stable digestion process.

The reactors removed 22 to 88% of COD and 30 to 95% of BOD_5 (Table 7).

Table 7 – Daily removal: Chemical oxygen demand and Biological oxygen demand from each treatment in the anaerobic co-digestion process

(continue)

Removal COD (%)				
Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
1	22.00	36.00	32.00	28.00
16	33.00	38.00	47.00	32.00
31	43.00	39.00	63.00	35.00
46	43.00	65.00	88.00	32.00
61	48.00	42.00	84.00	47.00
76	49.00	42.00	85.00	48.00
91	50.00	42.00	85.00	49.00
106	64.00	61.00	70.00	51.00
Av	44.00	46.00	69.00	40.00
Max	64.00	61.00	88.00	51.00
Min	22.00	36.00	32.00	28.00
SD	±12.51	±11.21	±20.71	±9.44
Removal BOD_5 (%)				
Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
1	30.00	34.00	78.00	28.00
16	38.00	33.00	70.00	34.00
31	45.00	32.00	61.00	40.00
46	70.00	85.00	95.00	70.00
61	49.00	38.00	84.00	45.00
76	53.00	62.00	86.00	51.00
91	56.00	86.00	88.00	57.00
106	64.00	56.00	65.00	52.00

Table 7 – Daily removal: Chemical oxygen demand and Biological oxygen demand from each treatment in the anaerobic co-digestion process

(conclusion)

Removal BOD_5 (%)

Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
Av	51.00	53.00	78.00	47.00
Max	70.00	86.00	95.00	70.00
Min	30.00	32.00	61.00	28.00
SD	±13.15	±22.72	±12.00	±13.33

Source: Elaborated by the author (2020).

Notes: Av: average; SD: standard deviation of 106 days in operation; BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The greatest removal efficiency was in the reactor with 80% RCW, demonstrating that the storage of the RCW at room temperature did not affect the performance of the AC. As in a research that operated a 10L completely agitated reactor coupled to a laboratory-scale cross-flow tubular ultrafiltration membrane module with whey, with supplemented alkalinity, in mesophilic conditions for 169 days and achieved removal above 90% (DERELI *et al.*, 2019).

In the present research the increase in applied organic loads provided greater efficiency in the removal of COD, without prejudice to methanogenesis, showing that the stability of the microbial community was followed by the stability of the reactors performance (WIRTH; REZA; MUMME, 2015).

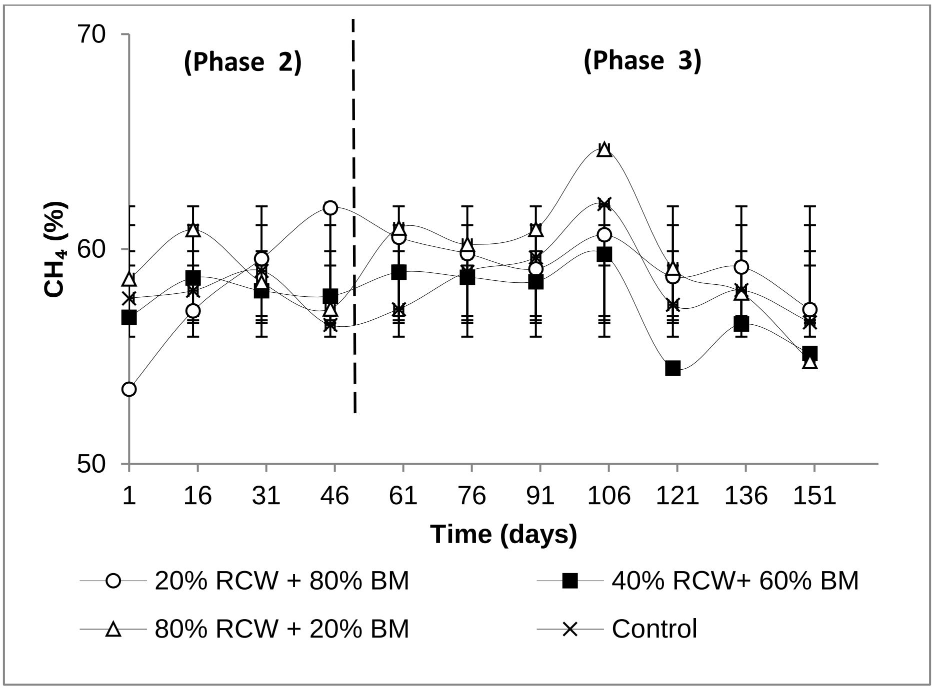
The reactor control showed less removal of COD, with a minimum removal of 28% and a maximum of 51%. The AC of BM with recalcitrant residues with complementary characteristics increases the removal of COD, as already reported in another study when operating a solar bioreactor from palm mill effluent co-digested with BM at 35 ° C for 24 days that obtained less removal of COD in the reactor with 100% BM, which was 15% (KHALID *et al.*, 2019).

According to the legal norms for the disposal of agroindustrial effluents in watercourses established by the local legislation of Minas Gerais, COPAM/CERH-MG Joint Normative n° 1, of May 5, 2008 the treatment efficiency of at least 75% in the removal of BOD, the final effluent could be discarded in watercourses (COPAM, 2008). In the present study, treatment with 80% RCW showed an average of 78% (± 12) BOD_5 removal, on the other hand, the removals verified throughout the experiment at the exit of the other reactors can still be harmful to watercourses with less flow. Very high rates of BOD_5 removal (78%) were also achieved when operating pilot scale anaerobic digesters with BM and cheese whey at 35°C for 56 days (COMINO; ROSSO; RIGGIO, 2009). If this process is adopted in full scale, post treatment will always be recommended to complement the removal of COD and BOD_5.

3.6 BIOGAS PRODUCTION AND ENERGY RECOVERY

The biogas produced during AC had a maximum CH_4 value of 62% and a minimum of 54% (Figure 9).

Figure 9 – Methane production from each treatment during the phases of the experimente



Source: Elaborated by the author (2020).

Notes: BM: bovine manure; RCW: ricotta cheese whey; Phase 2: adaptation of the inoculum; Phase 3: anaerobic co-digestion; Day1: first day of phase 2 of operation.

According to what is described in literature, anaerobic reactors operated with BM, the concentration of CH_4 can vary from 55 to 65% (TANJIL *et al.*, 2019). In the present work, the CH_4 yield was associated with the high organic load and biodegradability of RCW, which is a potential co-substrate for the generation of biogas and CH_4 (RICO; MUÑOZ; RICO, 2015). The AC with biodegradable substrates accelerates the production of microbial enzymes, which assist in the degradation of the recalcitrant residue, enabling the CH_4 production (VIVEKANAND *et al.*, 2018).

Average yields in CH_4 production volume from 1.96 to 2.44 m^3 $month^{-1}$ were recorded, presenting lower standard deviation of (±0.45) (Table 8).

Table 8 – Volumetric methane production (m^3. $month^{-1}$) from each treatment in the anaerobic co-digestion process

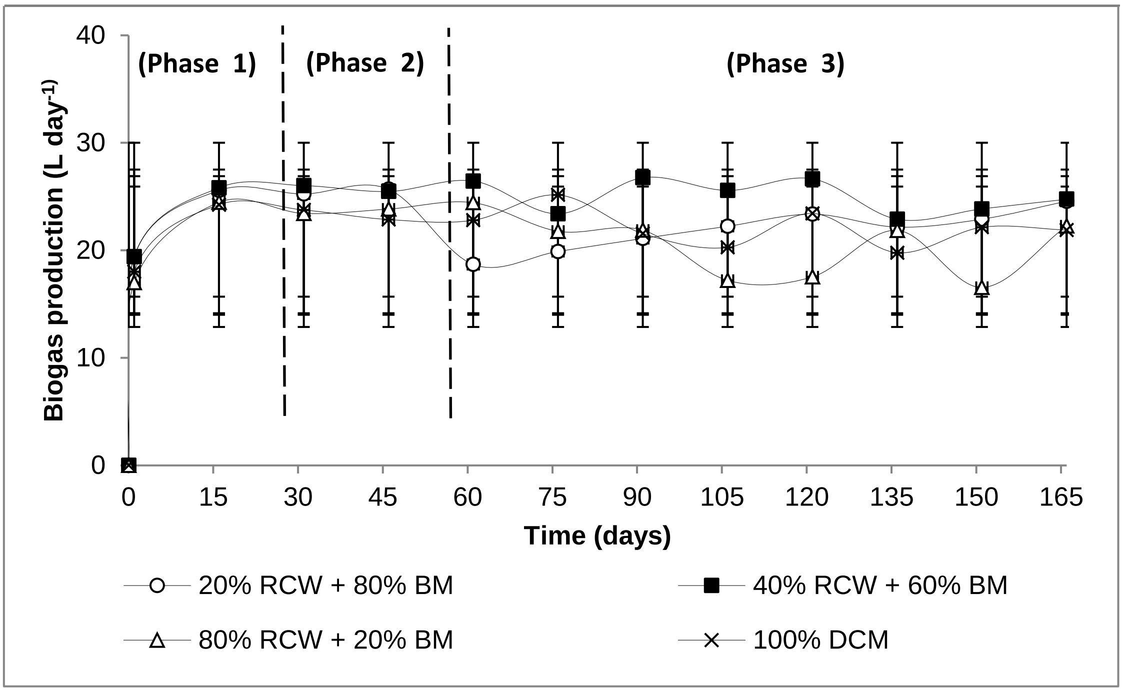
Days	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
1	1.30	1.63	2.59	1.34
30	2.48	1.78	2.16	1.73
60	2.60	2.49	3.02	2.01
90	3.27	2.39	2.69	2.62
106	0.65	1.53	1.75	2.32
Av	2.06	1.96	2.44	2.00
Max	3.27	2.49	3.02	2.62
Min	0.65	1.53	1.75	1.34
SD	1.06	0.45	0.50	0.50

Source: Elaborated by the author (2020).
Notes: Av: average; SD: standard deviation of 106 days in operation; BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

Lower CH_4 volumetric production values were recorded on the last day of the AC process. The verified reduction can be useful in the physiological stress of biomass due to the low availability of energy contained in the substrate (MENDONÇA; OMETTO; OTENIO, 2017). Developing RCW biodegradability with the values of volumetric production of CH_4 in the reactor of the present research can be considered promising.

The volumetric production of biogas in the present study varied between 17 and 27 L day^{-1} (Figure 10).

Figure 10 – Biogas production from each treatment during the phases of the experiment



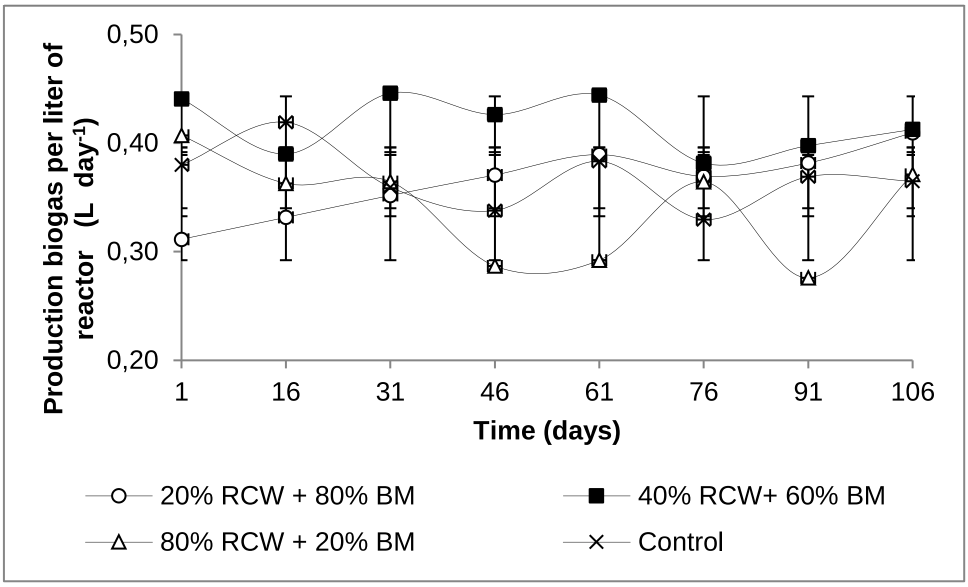
Source: Elaborated by the author (2020).

Notes: BM: bovine manure; RCW: ricotta cheese whey; Phase 1: start up of the experiment Phase 2: adaptation of the inoculum; Phase 3: anaerobic co-digestion.

The difference in the biogas production of the samples may be due to the different proportions of feed applied to the reactor. The increasement in the applied organic load results in an increasement in the production of biogas (MONTES *et al.*, 2019). Dairy effluents used as a co-substrate in AC system improve the production of biogas due to positive synergisms established in the digestion process (BROWN; GÜTTLER; SHILTON, 2016). Substrates rich in carbohydrates accelerate the process of degradation by hydrolysis and methanogenic bacteria cannot degrade fatty acids to the same extent that they are formed, with the accumulation of these acids (KAINTHOLA; KALAMDHAD; GOUD, 2019). Co-digestion with other substrates that contain recalcitrant compounds increases the production of biogas by slowing down the reaction speed in the initial stages of the process (BROWN; GÜTTLER; SHILTON, 2016). A research conducted to investigate the production of biogas by co-digesting bovine manure with acid whey obtained a yield between 0.33 and 0.49 m^3 kg^{-1} day^{-1} (VIVEKANAND *et al.*, 2018).

The reactors produced 0.45 L of biogas per liter of reactor (Figure 11).

Figure 11 – Biogas per liter of reactor production from each treatment in the anaerobic co-digestion process



Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

In China, a plug flow reactor produced 1.08 L of biogas per liter of reactor (70% CH_4) at an HRT of 18 days and a temperature close to 28°C (DONG; ZHANG; DIAO, 2019). This reactor received loads of 159,050 mg L^{-1} COD, 1.5 to 3.0 times higher than those applied in the present work, which reflected in higher biogas production and CH_4 concentration.

Regarding the potential for energy generation 1 m^3 of biogas would have the potential to generate 6.4 kWh (NASCIMENTO *et al.*, 2017). In this research, we estimated that the average of CH_4 contents measured throughout the survey would enable an energy generation of 0.76 to 0.9 kWh day^{-1} (Table 9).

Table 9 – Estimation of energy production potential (kWh.day $^{-1}$)

Days	20% RCW + 80% BM	40% RCW + 60% BM	80% RCW + 20% BM	Control
1	0.86	0.80	0.79	0.78
16	0.84	0.82	0.85	0.79
31	0.83	0.81	0.84	0.82
46	0.82	0.81	0.85	0.83
61	0.84	0.83	0.90	0.86
76	0.82	0.76	0.82	0.80
91	0.82	0.78	0.80	0.81
106	0.79	0.77	0.76	0.79
Av	0.83	0.80	0.83	0.81
Max	0.86	0.83	0.90	0.86
Min	0.79	0.76	0.76	0.78
SD	0.02	0.03	0.04	0.03

Source: Elaborated by the author (2020).

Notes: Av: average; SD: standard deviation of 106 days in operation; BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The variation in electricity generated was attributed to the oscillation of the application rate of organic load regarding the number of animals variation confined in the respective periods and the milk quality used in the manufacture of ricotta cheese, from which the RCW is derived.

This research shows promising results in AC of RCW with BM, as well as its potential for biogas generation and a new alternative for renewable energy generation (Table 10).

Table 10 – Accumulated production of reactors during 106 days of anaerobic co-digestion

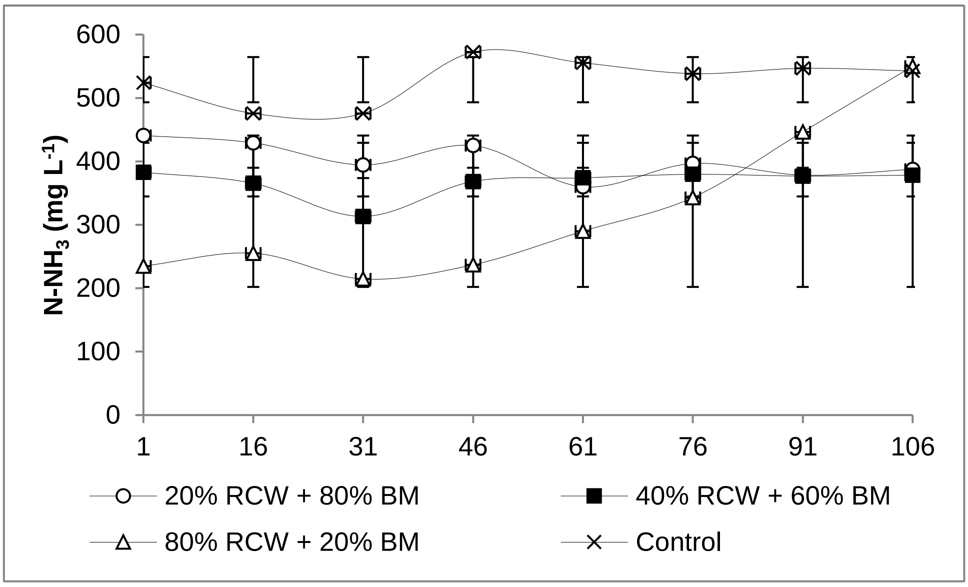
Production	**Unity**	**20% RCW + 80% BM**	**40% RCW + 60% BM**	**80% RCW + 20% BM**	**Control**
Biogas volume	L	175.00	200.00	164.00	177.00
Recovered energy	kWh	6.62	6.38	6.60	6.47

Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey.

3.7 PROPORTIONS OF NITROGEN FRACTIONS

The variations in the concentration of N-NH_3 in the influents and effluents were 128 to 1024 mg L^{-1} and 128 to 572 mg L^{-1} (Figure 12).

Figure 12 – Ammoniacal nitrogen of effluents from each treatment in the anaerobic co-digestion process



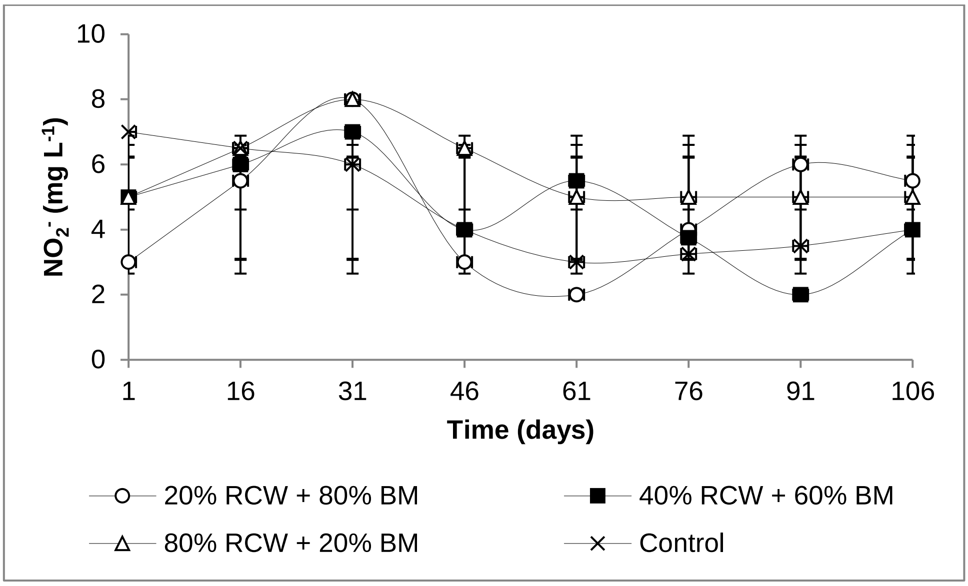
Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The concentrations of $N\text{-}NH_3$ increased during anaerobic digestion, indicating that the ammonification process degraded amino acids and proteins. This increase is intensified in corral washing waters due to the presence of relevant concentrations of organic matter (MENDONÇA; OMETTO; OTENIO, 2017). Although there was nitrogen concentration increase, this did not cause toxicity to microorganisms. In anaerobic reactors due to nitrogen concentration relevance in BM, ammonia tends to accumulate, resulting microbial activity inhibition in the process (BERTIN *et al.*, 2013). Over 150 mg L^{-1}, some authors consider the effect of $N\text{-}NH_3$ to be harmful to anaerobic systems, however, in the present study, the reactors operated satisfactorily even with values 1.4 to 3.8 times higher.

At lower concentrations, ammonia can be beneficial to CH_4 production, but toxic at higher doses, in the dissociated form found in alkaline pH (ANGELIDAKI; ELLEGAARD, 2003). In our results, the biogas productivity and CH_4 yield indicated that the process worked at high concentrations of ammonia. In reactors operated at room temperature methanogenic bacteria are more resistant to a high ammonia load, due to the lower concentration of free ammonia (WANG; ZHANG; ANGELIDAKI, 2016).

The NO_2^- was detected in low concentrations at the outlet of the reactors between 2 to 8 mg L^{-1}, with no inhibition of NO_2^- oxidizing bacteria, due to the accumulation of free nitrous acid (Figure 13).

Figure 13 – Nitrite of effluents from each treatment in the anaerobic co-digestion process

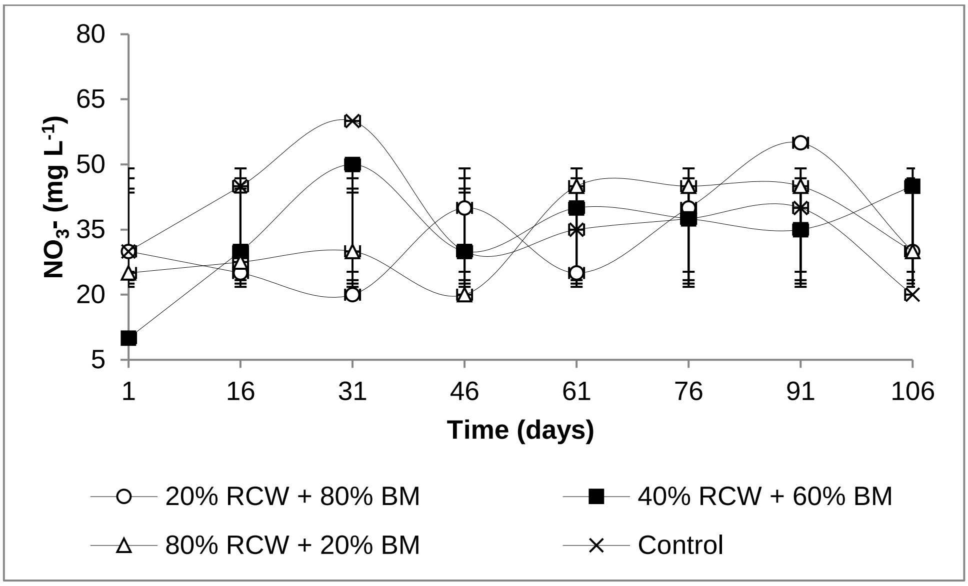


Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

As NO_2^- is an intermediate and unstable compound, generally the concentrations found in the effluents are small (SANTAELLA *et al.*, 2009). In low concentrations, NO_2^- can be an intermediate compound in the reduction of nitrate (SEPEHRI; SARRAFZADEH, 2019).

At the end of the co-digestion process, the NO_3^- concentration was from 20 to 60 mg L^{-1} (Figure 14).

Figure 14 – Nitrate of effluents from each treatment in the anaerobic co-digestion process



Source: Elaborated by the author (2020).
Notes: BM: bovine manure; RCW: ricotta cheese whey; Day 1: first day of operation phase 3.

The NO_3^- concentration between 20 and 50 mg L^{-1} are permissible for irrigation without causing potential impacts to the soil (NOUKEU *et al.*, 2016). In the present study, the nitrate concentration exceeded the one recommended by the author, by a 10 mg L^{-1} of only.

4 CONCLUSION

The reactors reached CH_4 volumetric production from 0.65 to 3.27 m^3 $month^{-1}$, with biogas production between 24 to 27 L day^{-1}, with an average from 57 (±1.9) to 60% (±1.44) of CH_4, which provided generation of energy from 0.80 to 0.83 kWh.day^{-1}. The addition of RCW did not decrease the energy production, however the AC with RCW and BM were viable for biogas production and effective in the treatment of this wastes. The AC between these residues can solve the environmental problems faced by dairy products and increase energy production via AB.

The digestion of organic matter and volatile solids occurred without the accumulation of volatile fatty acids or pH and alkalinity misalignments in the reactor.

The correction of the RCW pH provided stability of the methanogenic process, even in periods of lower temperature.

The total average daily efficiencies verified in the removal of BOD_5 and COD were from 47 to 78% and from 40 to 69%, of ST and SV from 36 to 54% and 44 to 69%.

The use of biomass from BM and RCW to produce biogas diversifies the energy matrix with renewable energy, supporting sustainable development.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION - APHA. **Standard Methods for the Examination of Water and Wastewater**. 23. ed. Washington: *[s.n.]*, 2017. 1–6160 p.

ANGELIDAKI, I.; ELLEGAARD, L. Codigestion of manure and organic wastes in centralized biogas plants. **Applied Biochemistry and Biotechnology,** [*s.l.*], v. 109, p. 95–105 , apr. 2003. DOI: http://dx.doi.org/10.1385/ABAB:109:1-3:95.

BERTIN, Lorenzo *et al.* Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. **Bioresource Technology,** New York, v. 128, p. 779–783 , jan. 2013. DOI: http://dx.doi.org/10.1016/j.biortech.2012.10.118.

BOLYEN, Evan *et al*. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. **Nature Biotechnology**, [*S.l.*], v. 37, p. 852-857, aug. 2019.

BROWN, N; GÜTTLER, J; SHILTON, A. Overcoming the challenges of full scale anaerobic co-digestion of casein whey. **Renewable Energy,** [*s.l.*], v. 96, p. 425–432, oct. 2016. DOI: https://doi.org/10.1016/j.renene.2016.04.044.

CAROTA, Eleonora *et al.* A sustainable use of Ricotta Cheese Whey for microbial biodiesel production. **Science of the Total Environment,** Amsterdam, v. 584, n. 15, p. 554–560, apr. 2017.DOI: http://dx.doi.org/10.1016/j.scitotenv.2017.01.068.

CHEAH, Yen Keong *et al.* Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH. **Environmental Science and Pollution Research,** [*s.l.*], v. 26, p. 35509–35522, may. 2019. DOI: https://doi.org/10.1007/s11356-019-05394-6.

CLIMATE-DATA.ORG. **Clima**: Juiz de Fora. Juiz de Fora. Available in: https://pt.climate-data.org/america-do-sul/brasil/minas-gerais/juiz-de-fora-5957/. Access in: 14. oct. 2019.

COLLINS, Carol H.; BRAGA, Gilberto L.; BONATO, Pierina R. **Introdução a métodos cromatográficos**. 7. ed. Campinas: Unicamp, 1997. 279 p

COMINO, Elena; ROSSO, Maurizio; RIGGIO, Vincenzo. Development of a pilot scale anaerobic digester for biogas production from cow manure and whey mix. **Bioresource Technology,** New York, v. 100, n. 21, p. 5072–5078, nov. 2009. DOI: http://dx.doi.org/10.1016/j.biortech.2009.05.059.

COPAM/CERH-MG. Deliberação Normativa Conjunta COPAM/CERH-MG n.1, de 05 de maio de 2008. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. **Diário do Executivo,** 13 may. 2008.

CORSINO, Santo Fabio *et al.* Effect of Extended Famine Conditions on Aerobic

Granular Sludge Stability in the Treatment of Brewery Wastewater. **Bioresource Technology,** New York, v. 226, p. 150-157, feb. 2017. DOI: http://dx.doi.org/10.1016/j.biortech.2016.12.026.

DARWIN; CORD-RUWISCH, Ralf. Thermodynamics of anaerobic digestion : mechanism of suppression on biogas production during acidogenesis. **INMATEH - Agricultural Engineering,** [*s.l.*], v. 57, n. 1, p. 287–296 , jan. 2019.

DEGEO - ICH/UFJF, 2009. Laboratório de Climatologia Geográfica e Análise Ambiental. LAB. **Climatologia**: clima de juiz de fora. Clima de Juiz de Fora. 2009. Available in: https://www.pjf.mg.gov.br/cidade/anuario_2009/basededados/PDF/1%20Hist.%20e%20Geografia/1%203%20Climatologia/1.3.1.pdf. Access in: 14 oct. 2019.

DERELI, Recep Kaan *et al.* Treatment of cheese whey by a cross-flow anaerobic membrane bioreactor: Biological and filtration performance. **Environmental Research,** [*s.l.*], v. 168, p. 109–117 , jan. 2019. DOI: https://doi.org/10.1016/j.envres.2018.09.021.

DIAMANTIS, Vasileios I *et al.* Two-stage CSTR – UASB digestion enables superior and alkali addition-free cheese whey treatment. **Biochemical Engineering Journal,** Amsterdam, v. 84, p. 45–52 , mar. 2014. DOI: http://dx.doi.org/10.1016/j.bej.2014.01.001.

DONG, Lifeng; ZHANG, Tingting; DIAO, Qiyu. Effect of dietary supplementation of moringa oleifera on the production performance and fecal methanogenic community of lactating dairy cows. **Animals,** [*s.l.*], v. 9, n. 5, p. 1–12 , may. 2019. DOI: 10.3390/ani9050262.

DONG, Lili *et al.* Reflux of acidizing fluid for enchancing biomethane production from cattle manure in plug flow reactor. **Bioresource Technology,** New York, v. 284, p. 248–255 , jul. 2019. DOI: https://doi.org/10.1016/j.biortech.2019.03.092.

FLORES-MENDOZA, Ana . *et al.* Methanogenesis of raw cheese whey: pH and substrate-inoculum ratio evaluation at mesophyll temperature range. **Chemical Technology and Biotechnology,** [*s.l.*], v. 95, p. 1946–1952 , feb. 2020. DOI: https://doi.org/10.1002/jctb.6391.

GRESES, Silvia; TOMÁS-PEJÓ, Elia; GÓNZALEZ-FERNÁNDEZ, Cristina. Agroindustrial waste as a resource for volatile fatty acids production via anaerobic fermentation. **Bioresource Technology,** New York, v. 297, p.1-7, feb. 2020.DOI: https://doi.org/10.1016/j.biortech.2019.122486.

GUO, Guang *et al.* Effects of livestock manure properties and temperature on the methanogen community composition and methane production during storage. **Environmental Technology,** [*s.l.*], v. 41, n. 2, p. 131–140 , jan. 2020. DOI: https://doi.org/10.1080/09593330.2018.1491640.

HOLLIGER, Christof *et al.* Towards a standardization of biomethane potential tests. **Water Science and Technology,** [*s.l.*], v. 74, n. 11, p. 2515–2522 , dec. 2016. DOI: https://doi.org/10.2166/wst.2016.336.

JI, Yang; LIU, Pengfei; CONRAD, Ralf. Response of fermenting bacterial and methanogenic archaeal communities in paddy soil to progressing rice straw degradation. **Soil Biology and Biochemistry,** [*s.l.*], v. 124, p. 70–80 , sep. 2018. doi: https://doi.org/10.1016/j.soilbio.2018.05.029.

JIHEN, Toumi *et al.* Microbial ecology overview during anaerobic codigestion of dairy wastewater and cattle manure and use in agriculture of obtained bio-fertilisers. **Bioresource Technology**, New York, v. 198, p. 141–149 , dec. 2015. DOI: http://dx.doi.org/10.1016/j.biortech.2015.09.004.

JIN, Dingxing *et al.* Effects of dietary supplementation of active dried yeast on fecal methanogenic archaea diversity in dairy cows. **Anaerobe,** London, v. 44, p. 78–86 , apr. 2017. DOI: http://dx.doi.org/10.1016/j.anaerobe.2017.02.007.

KAINTHOLA, Jyoti; KALAMDHAD, Ajay S; GOUD, Vaibhav V. A review on enhanced biogas production from anaerobic digestion of lignocellulosic biomass by different enhancement techniques. **Process Biochemistry journal,** [*s.l.*], v. 84, p. 81–90, sep. 2019. DOI: https://doi.org/10.1016/j.procbio.2019.05.023.

KASMI, Miriam. Biological Processes as Promoting Way for Both Treatment and Valorization of Dairy Industry Effluents. **Waste and Biomass Valorization,** [*s.l.*], v. 9, n. 2, p. 195–209, dec. 2018. DOI: 10.1007/s12649-016-9795-7.

KHALID, Zaied Bin *et al.* Application of solar assisted bioreactor for biogas production from palm oil mill effluent co-digested with cattle manure. **Environmental Technology and Innovation,** [*s.l.*], v. 16, p. 1-10. nov. 2019.DOI: https://doi.org/10.1016/j.eti.2019.100446.

KLEEREBEZEM, Robbert; LOOSDRECHT, Mark C.M. A generalized method for thermodynamic state analysis of environmental systems. **Critical Reviews in Environmental Science and Technology,** [*s. l.*], v. 40, n. 1, p. 1–54 , jan. 2010. DOI: https://doi.org/10.1080/10643380802000974.

KUNZ, Airton; MUKHTAR, Saqib. Hydrophobic membrane technology for ammonia extraction from wastewaters. **Engenharia Agricola**, [*s. l.*], v. 36, n. 2, p. 377–386 , mar. 2016. DOI: https://doi.org/10.1590/1809-4430-Eng.Agric.v36n2p377-386/2016.

LIU, Chong *et al.* Physicochemical characteristics of stored cattle manure affect methane emissions by inducing divergence of methanogens that have different interactions with bacteria. **Agriculture, Ecosystems and Environment**, [*s. l.*], v. 253, p. 38–47 , feb. 2018. DOI:https://doi.org/10.1016/j.agee.2017.10.020.

LV, Zuopeng *et al.* Effect of one step temperature increment from mesophilic to thermophilic anaerobic digestion on the linked pattern between bacterial and methanogenic communities. **Bioresource Technology**, [*s.l.*], v. 292 , nov. 2019. DOI:https://doi.org/10.1016/j.biortech.2019.121968

MANYI-LOH, Christy *et al.* Characterizing bacteria and methanogens in a balloon-type digester fed with dairy cattle manure for anaerobic mono-digestion. **Polish Journal of Environmental Studies**, [*s.l.*], v. 28, n. 3, p. 1287–1293 , 2019. DOI: https://doi.org/10.15244/pjoes/76180.

MENDONÇA, Henrique Vieira De *et al.* Bioenergy recovery from cattle wastewater in an UASB-AF hybrid reactor. **Water Science & Technology**, [*s.l.*], v. 76, n. 9, p. 2268–2279 , nov. 2017. DOI: https://doi.org/10.2166/wst.2017.325**.**

MENDONÇA, Henrique Vieira ; OMETTO, Jean Pierre Henry Balbaud; OTENIO, Marcelo Henrique. Production of Energy and Biofertilizer from Cattle Wastewater in Farms with Intensive Cattle Breeding. **Water, Air, & Soil Pollution,** [*s.l.*], v. 228, n. 72, p. 1–14 , jan. 2017.DOI: http://dx.doi.org/10.1007/s11270-017-3264-1.

MIRANDA, Nicole *et al.* Meta-analysis of methane yields from anaerobic digestion of dairy cattle manure. **Biomass and Bioenergy**, [*s.l.*], v. 86, p. 65–75 , mar. 2016. DOI: https://doi.org/10.1016/j.biombioe.2016.01.012.

MONTES, Jesús A. *et al.* Biogas production from the liquid waste of distilled gin production: Optimization of UASB reactor performance with increasing organic loading rate for co-digestion with swine wastewater. **Bioresource Technology**, [*s.l.*], v. 274, p. 43–47 , feb. 2019. DOI: https://doi.org/10.1016/j.biortech.2018.11.060.

MONTOYA, A. C.V. *et al.* Dairy manure wastewater in serial UASB reactors for energy recovery and potential effluent reuse. **Brazilian Journal of Chemical Engineering**, [*s.l.*], v. 34, n. 4, p. 971–983 , out. 2017. DOI: https://doi.org/10.1590/0104-6632.20170344s20160297.

MUKHUBA, Mashudu *et al.* Metagenomic insights into the microbial community and biogas production pattern during anaerobic digestion of cow dung and mixed food waste. **Journal of Chemical Technology and Biotechnology**, [*s.l.*], v. 95, p. 151–162 , jan. 2020. DOI: https://doi.org/10.1002/jctb.6217.

NASCIMENTO, Kennedy Luiz Souza *et al.* Geração de energia elétrica e viabilidade técnico-econômica de um biodigestor no setor hospitalar. **PUBVET**, [*s.l.*], v. 11, n. 12, p. 1263–1273 , dec. 2017. DOI: 10.22256/pubvet.v11n12.1263-1273.

NOUKEU, N. A. *et al.* Characterization of effluent from food processing industries and stillage treatment trial with Eichhornia crassipes (Mart.) and Panicum maximum (Jacq.). **Water Resources and Industry,** [*s.l.*], v. 16, p. 1-18 , dec. 2016. DOI: https://doi.org/10.1016/j.wri.2016.07.001.

PANIZIO, Roberta Mota *et al.* Potential of Biogas Production in Anaerobic Co - digestion of Opuntia ficus - indica and Slaughterhouse Wastes. **Waste and Biomass Valorization**, [*s.l.*], v. 11, n. 9, p. 4639 - 4647, sep. 2020. DOI: https://doi.org/10.1007/s12649-019-00835-2.

PARRALEJO, A I *et al.* Small scale biogas production with animal excrement and agricultural residues. **Industrial Crops & Products,** [*s.l.*], v. 131, p. 307–314 , may 2019. DOI: https://doi.org/10.1016/j.indcrop.2019.01.059.

PFLUGER, Andrew *et al.* Energy-generating potential of anaerobically enhanced primary treatment of domestic wastewater using multiple-compartment bioreactors. **Environmental Science: Water Research & Technology,** [*s.l.*], v. 6, n.1, p. 117–131 , jan. 2020. DOI: http://dx.doi.org/10.1039/C9EW00526A.

RAHEEM, Abdur; HASSAN, Mohammad Yusri; SHAKOOR, Rabia. Bioenergy from anaerobic digestion in Pakistan: Potential, development and prospects. **Renewable and Sustainable Energy Reviews,** [*s.l.*], v. 59, p. 264–275 , jun. 2016. DOI: https://doi.org/10.1016/j.rser.2016.01.010.

RESENDE, Juliana Alves *et al*. Seasonal Variation on Microbial Community and Methane Production during Anaerobic Digestion of Cattle Manure in Brazi. **Physiology And Biotechnology**, Juiz de Fora, v. 71, n. 3, p. 735-746, apr. 2016. DOI: https://doi.org/10.1007/s00248-015-0647-y.

RICO, Carlos; MUÑOZ, Noelia; RICO, José Luis. Anaerobic co-digestion of cheese whey and the screened liquid fraction of dairy manure in a single continuously stirred tank reactor process: Limits in co-substrate ratios and organic loading rate. **Bioresource Technology,** [*s.l.*], v. 189, p. 327–333 , aug. 2015. DOI: https://doi.org/10.1016/j.biortech.2015.04.032.

RIZZOLO, Anna; CORTELLINO, Giovanna. Ricotta Cheese Whey-Fruit-Based Beverages: Pasteurization Effects on Antioxidant Composition and Color. **Beverages,** [*s.l.*], v. 3, n. 1, p. 1–16 , mar. 2017. DOI: https://doi.org/10.3390/beverages3010015.

ROCHELEAU, Sylvie *et al.* Differentiation of Methanosaeta concilii and Methanosarcina barkeri in Anaerobic Mesophilic Granular Sludge by Fluorescent In Situ Hybridization and Confocal Scanning Laser Microscopy. **APPLIED AND ENVIRONMENTAL MICROBIOLOGY,** [*s.l.*], v. 65, n. 5, p. 2222–2229 , 1999. DOI: https://doi.org/10.1128/AEM.65.5.2222-2229.1999.

SANTAELLA, Sandra Tédde *et al.* Tratamento de efluentes de refinaria de petróleo em reatores com Aspergillus niger. **Engenharia Sanitaria e Ambiental,** [*s.l.*], v. 14, n. 1, p. 139–148 , 2009. DOI: https://doi.org/10.1590/S1413-41522009000100015.

SANTANA, Larice Aparecida Rezende *et al*. Soro de ricota para biodigestão e produção de biogás. In: CONGRESSO NACIONAL DE LATICÍNIOS, 32., 2019, Juiz de Fora. **Anais [...] .** Belo Horizonte: Epamig, july 16th to 18th, 2019. p. 34-34.

SEPEHRI, Arsalan; SARRAFZADEH, Mohammad-Hossein. Activity enhancement of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria in activated sludge process: metabolite reduction and CO2 mitigation intensification process. **Applied Water Science,** [*s.l.*], v. 9, n. 5, p. 1–12 , jul. 2019. DOI: https://doi.org/10.1007/s13201-019-1017-6.

SHI, Jian *et al.* Effects of microbial and non-microbial factors of liquid anaerobic digestion effluent as inoculum on solid-state anaerobic digestion of corn stover. **Bioresource Technology,** [*s.l.*], v. 157, p. 188–196 ,apr. 2014. DOI: http://dx.doi.org/10.1016/j.biortech.2014.01.089.

TANJIL, Hossain Al *et al.* Biogas as an Alternative Energy Source in Rural Areas and Public Awareness: A Case Study in Jessore District. **Journal of Energy and Natural Resources,** [*s.l.*], v. 8, n. 1, p. 12–17 , mar. 2019. DOI: https://doi.org/10.11648/j.jenr.20190801.13.

VALTA, K. *et al.* Current Treatment Technologies of Cheese Whey and Wastewater by Greek Cheese Manufacturing Units and Potential Valorisation Opportunities. **Waste Biomass Valor,** [*s.l.*], v. 8, n. 5, p. 1649 –1663, jul. 2017. DOI: https://doi.org/10.1007/s12649-017-9862-8.

VIVEKANAND, Vivekanand *et al.* Synergistic effects of anaerobic co-digestion of whey , manure and fish ensilage. **Bioresource Technology,** [*s.l.*],v. 249, p. 35-41, feb. 2018. DOI: https://doi.org/10.1016/j.biortech.2017.09.169.

WAINAINA, Steven *et al.* Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. **Bioengineered**, [*s.l.*],v. 10, n. 1, p. 437–458 , oct. 2019. DOI: https://doi.org/10.1080/21655979.2019.1673937.

WANG, Han; ZHANG, Yifeng; ANGELIDAKI, Irini. Ammonia inhibition on hydrogen enriched anaerobic digestion of manure under mesophilic and thermophilic conditions. **Water Research,** [*s.l.*], v. 105, p. 314–319 , nov. 2016. DOI: http://dx.doi.org/10.1016/j.watres.2016.09.006.

WANG, Hui *et al.* Physical properties of dairy manure pre- and post-anaerobic digestion. **Applied Sciences (Switzerland),** [*s.l.*], v. 9, n. 13, p. 1-10, jul. 2019. DOI: https://doi.org/10.3390/app9132703.

WILKIE, Ann C.. Anaerobic digestion of dairy manure: Design and process considerations. In: DAIRY MANURE MANAGEMENT: TREATMENT HANDLING, AND COMMUNITY RELATIONS, 2005, Ithaca. **Dairy manure conference.** Ithaca: Natural Resource Agriculture And Engineering Service, mar. 2005. v. 176, p. 301-312.

WIRTH, Benjamin; REZA, Toufiq; MUMME, Jan. Influence of digestion temperature and organic loading rate on the continuous anaerobic treatment of process liquor from hydrothermal carbonization of sewage sludge. **Bioresource Technology,** [*s.l.*], v. 198, p. 215–222 , dec. 2015. DOI: https://doi.org/10.1016/j.biortech.2015.09.022.

XU, Rong *et al.* A critical review on the interaction of substrate nutrient balance and microbial community structure and function in anaerobic co-digestion. **Bioresource Technology,** [*s.l.*], v. 247, p. 1119–1127 , jan. 2018. DOI: https://doi.org/10.1016/j.biortech.2017.09.095.

YUAN, Yayi *et al.* Advances in enhanced volatile fatty acid production from anaerobic fermentation of waste activated sludge. **Science of the Total Environment,** [*s.l.*], v. 694, p. 1-12 , dec. 2019. DOI: https://doi.org/10.1016/j.scitotenv.2019.133741.

ZHANG, Cunsheng *et al.* Reviewing the anaerobic digestion of food waste for biogas production. **Renewable and Sustainable Energy Reviews,** [*s.l.*], v. 38, p. 383–392, oct. 2014. DOI: https://doi.org/10.1016/j.rser.2014.05.038.