



# Biogas production and composition optimization in an anaerobic digester using cheese whey and swine manure as substrate

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**ABSTRACT.** The search for new sources of energy has intensified these days due to the environmental impacts caused by fossil fuels. The tripod composed of energy, food and water is the base of human existence. Food production implies the generation of organic waste and the need to manage it properly. The dairy and pig farming sectors have an essential role in the Brazilian economy, producing a large amount of waste. One energy and environmental alternative to treat this issue is anaerobic digestion. Here we aimed to optimize the production and composition of biogas obtained from cheese whey and swine manure. Batch-scale laboratory tests were performed on bench anaerobic digesters for 65 days with 6 triplicates loaded with different proportions of cheese whey and swine manure. The proportion of 50% cheese whey and 50% swine manure presented the highest biogas production and methane concentration (CH<sub>4</sub>).

**Keywords:** Dairy industry; pig farming; renewable energy.

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## Introduction

Agribusiness sectors, such as pig farming and dairy, have significant economic prominence in Brazil. Over the past 30 years, the dairy industry has undergone significant modernization, boosting development and production that reached an annual mark of 33,8 billion liters of milk in 2018, ranking third in the world (Instituto Brasileiro de Geografia e Estatística [IBGE], 2018). In 2018, pig production reached 3.97 million tons in the country, the fourth largest in the world, as well as exports, which also occupied this position with a total of 646 thousand tons (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2018).

However, these sectors generate large amounts of waste with great polluting potential due to the high organic loads, such as cheese whey and swine manure. Cheese whey is a by-product of cheese making, and it is separated by physical, enzymatic and biological actions. It is the aqueous part of milk, composed of water, lactose, proteins and minerals with a characteristic odor and an off-white color (Pagno, Baldasso, Tessaro, Flores, & Jong, 2009). Swine manure consists of liquid and solid fractions composed of organic matter, nutrient sand coliforms. Besides, they cause an unpleasant smell and the release of volatile compounds such as ammonia, volatile fatty acids, hydrogen sulfide and others, which harm human and animal health (Silva, França, & Oyamada, 2015).

Anaerobic digestion, a process of decomposition of organic matter that results in biogas and biofertilizer, presents itself as an efficient, clean and profitable alternative for the correct management of these effluents and residues (Fachagentur Nachwachsende Rohstoffe [FNR], 2010; Wandrey & Aivasidis, 1983).

Biogas comprises a mixture of gases such as methane, carbon dioxide, oxygen, nitrogen, water vapor, hydrogen sulfide and others in percentage that vary according to the substrate used, temperature, characteristics of the digester, among others (Bertinatto et al., 2017). It is colorless, odorless and highly flammable. Also, it pollutes the atmosphere less than other gases such as butane (Rasi, Veijanen, & Rintala, 2007; Konrad, Akwa, Koch, Lumi, & Tonetto, 2016). It can generate thermal energy, electric energy, cogeneration (electric and thermal energy) and trigeneration (cooling, heating and electricity) (Brizi et al., 2014; Capodaglio, Callegari, & Lopez, 2016; Gazda & Stanek, 2016). Moreover, the anaerobic process results in the formation of biofertilizer, a nutrient-rich compound that can replace the chemical fertilizers used in agriculture (Brahmaprakash & Sahu, 2012).

Biogas formation occurs in four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Salminen & Rintala, 2002). The occurrence of these steps depends on some conditions of the environment, such as temperature, pH, hydraulic retention time (HRT), C/N ratio, availability of nutrients and the presence of inhibitors such as the formation of volatile fatty acids (Prazeres, Carvalho, & Rivas 2012; Fernández, Cuetos, Martínez, & Gómez, 2015).

The anaerobic process can occur in three temperature ranges: psychrophilic (<25°C), mesophilic (35-40°C) and thermophilic (55-60°C) (FNR, 2010). The pH is also a factor that influences the anaerobic digestion and the ideal range for the development of methanogenic microorganisms is between 6.7 and 7.5 (Rajendran, Aslanzadeh, & Taherzadeh, 2012).

When two or more types of wastes are used as substrate, the process is called anaerobic co-digestion. In this case, one residue can complement the other by balancing the pH and supplying a possible nutrient deficiency. Anaerobic co-digestion optimizes the process as the unification of the particularities of each substrate allows for greater efficiency in biogas production, as well as lowering the costs of equipment, installations and management through the simultaneous treatment of waste (Wang, Yang, Feng, Ren, & Han, 2012).

The objective of this research was to evaluate, on a laboratory scale, the best conditions for the production and methane content of biogas obtained from anaerobic co-digestion of whey and swine manure.

## Material and methods

### Substrate

Cheese whey and swine manure are from a small agribusiness in the interior of the State of Rio de Janeiro (Brazil), located under UTM SIRGAS 2000 coordinate 24k 249512.33 mE and 7578185.38 mS.

The activities performed by the agroindustry are the production amounts of cheeses and the raising of swine in complete cycle.

### Characterization of effluents

The physical-chemical analysis of cheese whey and swine manure included the follow parameters: BOD, electrical conductivity (EC), total solids (TS), volatile solids (VS) and fixed solids (FS). The methods used were American Public Health Association (APHA, 1998) and Associação Brasileira de Normas Técnicas (ABNT, 1989), following the standard protocols.

### Laboratory scale tests

The tests occurred at the Biogas and Renewable Energy Laboratory (LABER) of the Fluminense Federal Institute, Campus Itaperuna, in the northwest of Rio de Janeiro State.

### Construction of bench biodigesters

Eighteen anaerobic digesters were built on a laboratory scale, composed by: (i) 5L polyethylene container for the anaerobic reactor; (ii) 2L container composed of a flexible PVC bag for biogas storage; (iii) 3L polyethylene bottle to store the water displaced with biogas production. All connections were adequately sealed and interconnected with a flexible PVC hose, as shown in Figure 1 below:

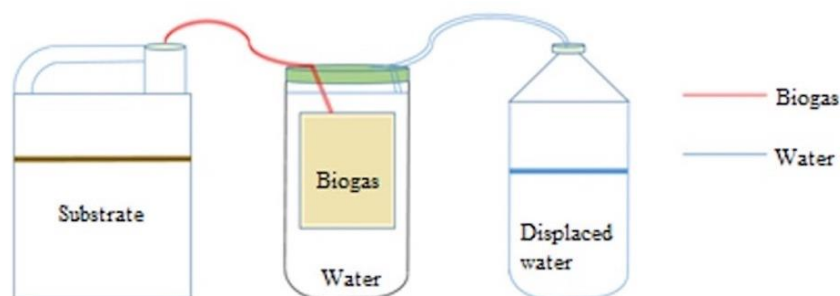


Figure 1. Schematic model of the anaerobic digesters built.

### Experimental design

Table 1 shows the loading of the eighteen anaerobic digesters (six triplicates) with different volume volume<sup>-1</sup> ( $v v^{-1}$ ) mixing ratios of cheese whey (CS) and swine manure (SM).

**Table 1.** Mixing ratios of the experiment.

Triplicates	Biodigestors identification	Substrates volume	Substrate percentage
1	B1A	Inoculum (1L)	
2	B1B	Inoculum (1L)	
3	B1C	Inoculum (1L)	
4	B2A	Inoculum (1L) + Swine manure (0.5L)	100% SM
5	B2B	Inoculum (1L) + Swine manure (0.5L)	100% SM
6	B2C	Inoculum (1L) + Swine manure (0.5L)	100% SM
7	B3A	Inoculum (1L) + Cheese whey (0.5L)	100% CS
8	B3B	Inoculum (1L) + Cheese whey (0.5L)	100% CS
9	B3C	Inoculum (1L) + Cheese whey (0.5L)	100% CS
10	B4A	Inoculum (1L) + Swine manure (0.25L) + Cheese whey (0.25L)	50% SM + 50% CS
11	B4B	Inoculum (1L) + Swine manure (0.25L) + Cheese whey (0.25L)	50% SM + 50% CS
12	B4C	Inoculum (1L) + Swine manure (0.25L) + Cheese whey (0.25L)	50% SM + 50% CS
13	B5A	Inoculum (1L) + Swine manure (0.375L) + Cheese whey (0.125L)	75% SM + 25% CS
14	B5B	Inoculum (1L) + Swine manure (0.375L) + Cheese whey (0.125L)	75% SM + 25% CS
15	B5C	Inoculum (1L) + Swine manure (0.375L) + Cheese whey (0.125L)	75% SM + 25% CS
16	B6A	Inoculum (1L) + Swine manure (0.125L) + Cheese whey (0.375L)	25% SM + 75% CS
17	B6B	Inoculum (1L) + Swine manure (0.125L) + Cheese whey (0.375L)	25% SM + 75% CS
18	B6C	Inoculum (1L) + Swine manure (0.125L) + Cheese whey (0.375L)	25% SM + 75% CS

The inoculum used in the experiment was from an operating anaerobic digester. The digesters operated in batches at room temperature with a 65-day HRT.

### Analysis of biogas production and composition

Biogas production was measured once a week during the 10 weeks. The measurement of the volume of biogas produced occurred by measuring the volume of water displaced. The methane content was measured using a brand Sewerin gas analyzer model Multitec 545.

## Results and discussion

### Physico-chemical characterization of effluents

Table 2 presents the data concerning the characterization of cheese whey compared with values found in the literature.

**Table 2.** Values of some physico-chemical parameters of cheese whey.

	Values obtained	Literature values
pH	4.12	4.90 (Venetsaneas, Antonopoulou, Stamatelatou, Kornaros, & Lyberatos, 2009) 4.95 (Diamantis et al., 2014) 3.90 (Maragkaki et al., 2018)
BOD (mg L <sup>-1</sup> )	31,800	27,000–60,000 (Prazeres et al., 2012) 30,000–50,000 (Gelegenis, Georgakakis, Angelidaki, & Mavris, 2007)
Electrical conductivity (mS cm <sup>-1</sup> )	36.51	4.69 (Diamantis et al., 2014)
Total solids (g L <sup>-1</sup> )	57.62	71.66 (Jasko, Skripsts, Dubrovskis, Zabarovskis, & Kotelencs, 2011) 57.80 (Bertin, Grilli, Spagni, & Fava, 2013) 65.93 (Ghaly, 1997)
Volatile solids (g L <sup>-1</sup> )	53.30	65.88 (Jasko et al., 2011) 52.80 (Bertin et al., 2013) 47.28 (Ghaly, 1997)
Fixed solids (g L <sup>-1</sup> )	4.32	18.65 (Ghaly, 1997)

The results obtained showed that there is a proximity between the values obtained and the values found in the literature for pH. The value found for BOD is consistent with the values described by Prazeres et al. (2012) and Gelegenis et al. (2007). The electrical conductivity was far above the one found in the literature. This fact can be explained by the regional methods of cheese production that uses different amounts of sodium chloride. The values found for total solids and volatile solids were consistent with the results obtained by Bertin et al. (2013). Whereas, the result obtained for the fixed solids was well below that found by Ghaly (1997), possibly due to the different cheese production methods used.

Table 3 presents the data regarding the characterization of swine manure compared to values found in the literature.

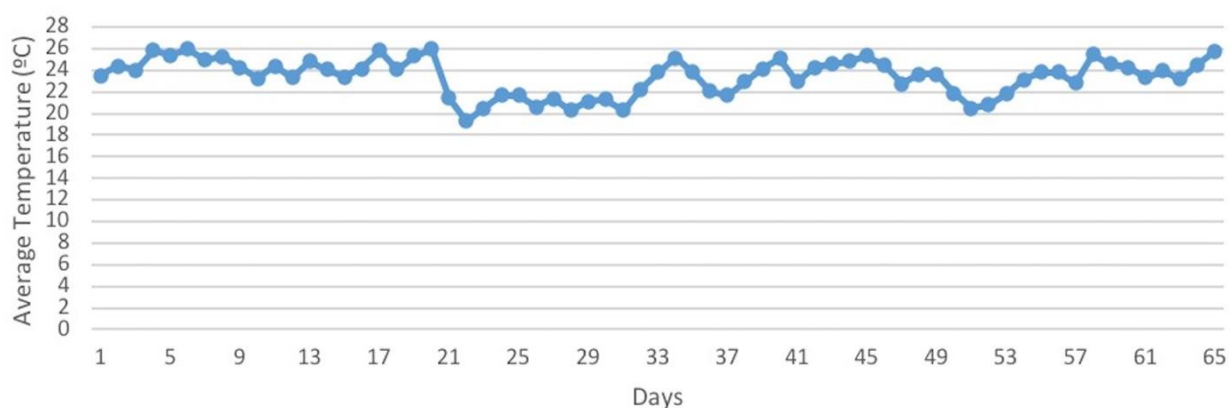
**Table 3.** Values of some physico-chemical parameters of swine manure.

	Values obtained	Literature values
pH	7.06	7.62 (Wu, Yao, & Zhu, 2010) 8.05 (Kafle, Kim, & Sung, 2012) 7.08 (Xia, Massé, McAllister, Beaulieu, & Ungerfeld, 2012)
BOD (mg L <sup>-1</sup> )	>40,000	3,650 (Deng et al., 2012) 13,390 (Yang et al., 2015)
Electrical conductivity (mS cm <sup>-1</sup> )	9.33	6.99 (Dal Bosco, Cosmann, Sbizzaro, Taiatele Junior, & Silva, 2015)
Total solids (%)	27.89	7.21 (Córdoba, Fernández, & Santalla, 2016) 27.43 (Xiu, Zhang, & Shahbazi, 2009)
Volatile solids (%)	94.81	86.39 (Córdoba et al., 2016) 86.60 (Xiu et al., 2009)
Fixed solids (%)	5.19	12.30 (Xiu et al., 2009)

The above values indicated a coherence between the data obtained and those found in the literature for pH and electrical conductivity. The BOD was above the values found by Deng et al. (2012) and Yang et al. (2015). This value may vary according to the content of the substrate used, so characteristics such as the percentage of the solid and liquid fraction, as well as if the swine manure was fresh or not, will influence the amount of organic matter and consequently the BOD. The values of total solids and volatile solids in this paper were according to the data obtained by Xiu et al. (2009). The value of fixed solids was below that found in the literature. Xiu et al. (2009) performed a comparison between fresh swine manure, manure taken from a deep shallow pit, and it was found that fresh swine manure had higher values of volatile solids and lower values of fixed solids compared to other manure. According to Orrico Junior, Orrico and Lucas Júnior (2011), animal diet interferes directly in the anaerobic process, in which diets with large amounts of bulky feed lead to the formation of fibrous material delaying the production of biogas and decreasing the efficiency of the process.

### Average ambient temperature

Figure 2 shows the average local temperature during the 65 days of the experiment.



**Figure 2.** Average local temperature during the experiment.

The anaerobic process in the digesters occurred under psychrophilic conditions considering the local average ambient temperature.

### Biogas production

Figure 3 shows the accumulated biogas production of the three anaerobic digesters loaded only with inoculum. The B1A, B1B and B1C anaerobic digesters were the control triplicate of the experiment. It was observed that from the first two weeks, B1B digester produced a higher volume of biogas.

Figure 4 presents the accumulated biogas production from three digesters loaded with inoculum and swine manure.

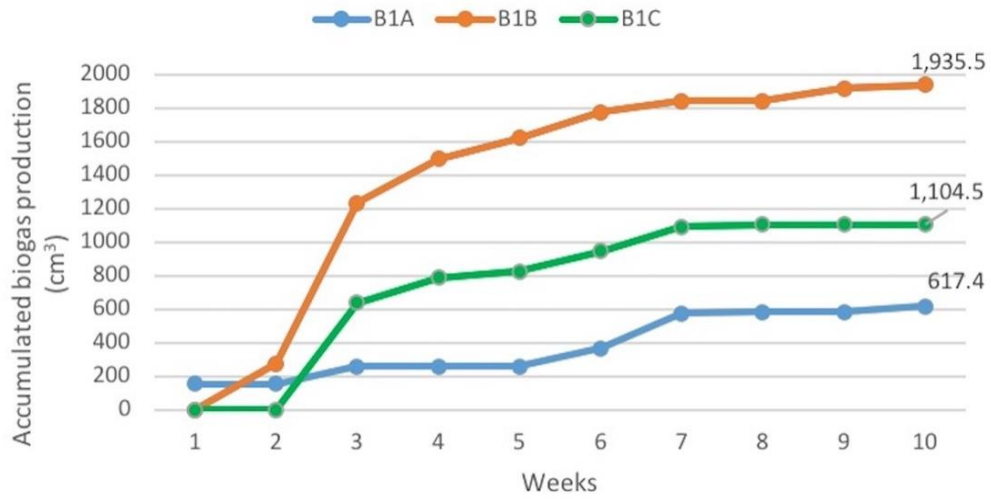


Figure 3. Accumulated production of biogas from the triplicates of digesters with 100% inoculum.

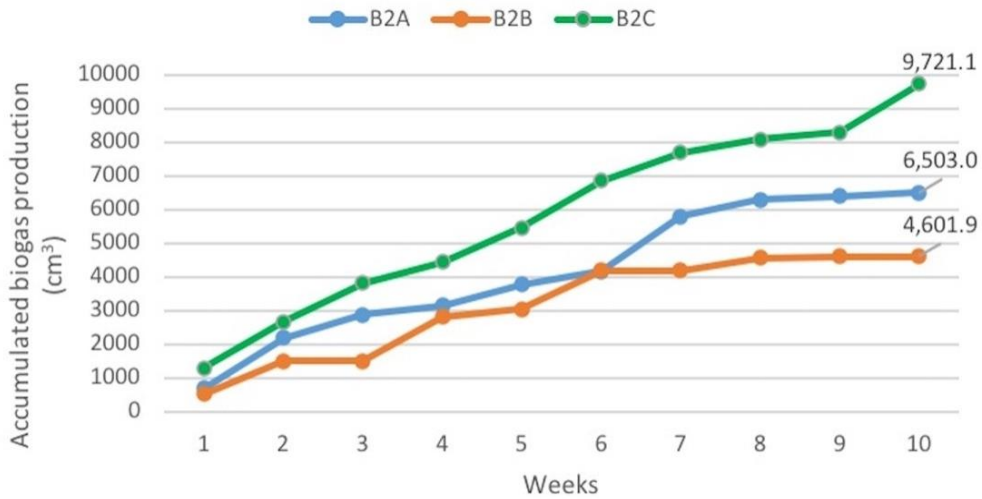


Figure 4. Accumulated production of biogas from the triplicates of digesters with 100% swine manure.

Biogas production until the second week was quite close in the three digesters. From the third week on production in the B2C, digester was higher, which may be associated with higher microbiological activity inside it.

Figure 5 presents the accumulated biogas production from three digesters loaded with inoculum and cheese whey.

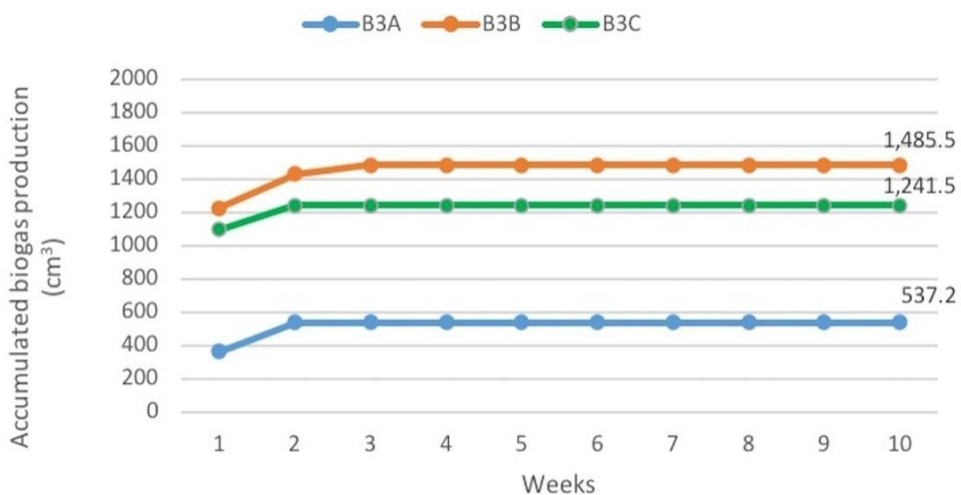


Figure 5. Accumulated production of biogas from the triplicates of digesters with 100% cheese whey.

From the second week until the end of the experiment, the digesters B3A and B3C did not produce more biogas. From the third week until the end of the experiment, the B3B digester did not produce biogas. The end of biogas production in digesters may be associated with the pH of the substrate.

Yan, Liao, and Lo (1988) treated cheese whey in a UASB type reactor, varying the organic load rates. With a rate of up to 28.8 g COD L<sup>-1</sup>, methane production was satisfactory; however, the increase in methane production, to 41.1 g COD L<sup>-1</sup>, caused the process instability by the formation of acids that resulted in a decrease in methane production.

Ghaly (1997) found that anaerobic digestion of cheese whey was not feasible when the pH was not controlled as acidification in the process stages resulted in a small production of biogas with low methane content and removal of COD and volatile solids. By controlling the pH in methanogenesis, all these rates were improved, especially when the temperature was 35°C (mesophilic conditions) with the pH control.

Figure 6 presents the accumulated biogas production from three digesters loaded with inoculum, 50% of swine manure and 50% of cheese whey.

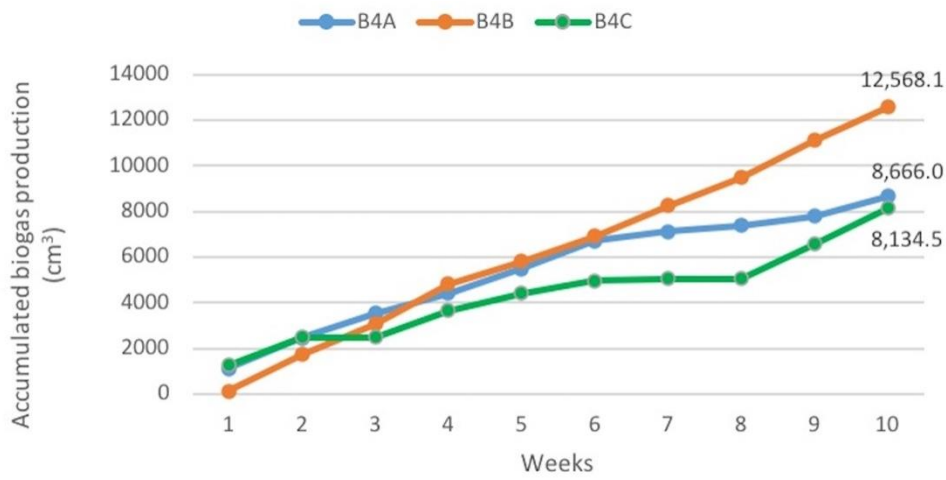


Figure 6. Accumulated production of biogas from the triplicates of digesters with 50% swine manure + 50% cheese whey.

An increase in biogas production was observed throughout the experiment. This behavior can be associated with the anaerobic co-digestion of cheese whey and swine manure in which one residue supplied the lack of micro and macronutrients from the other, optimizing and maximizing the production of biogas.

Figure 7 presents the accumulated biogas production from three digesters loaded with inoculum, 75% of swine manure and 25% of cheese whey.

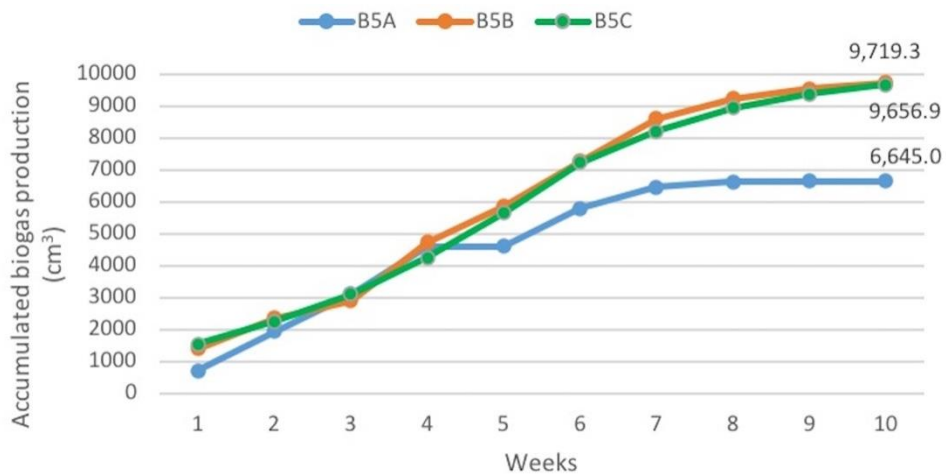


Figure 7. Accumulated production of biogas from the triplicates of digesters with 75% swine manure + 25% cheese whey.

Biogas production in B5B and B5C digesters was similar during the 10 weeks of the experiment. In digester B5A, the production of biogas increased during the first 7 weeks and from then on remained almost unchanged.

Figure 8 presents the accumulated biogas production from three digesters loaded with inoculum, 25% of swine manure and 75% of cheese whey.



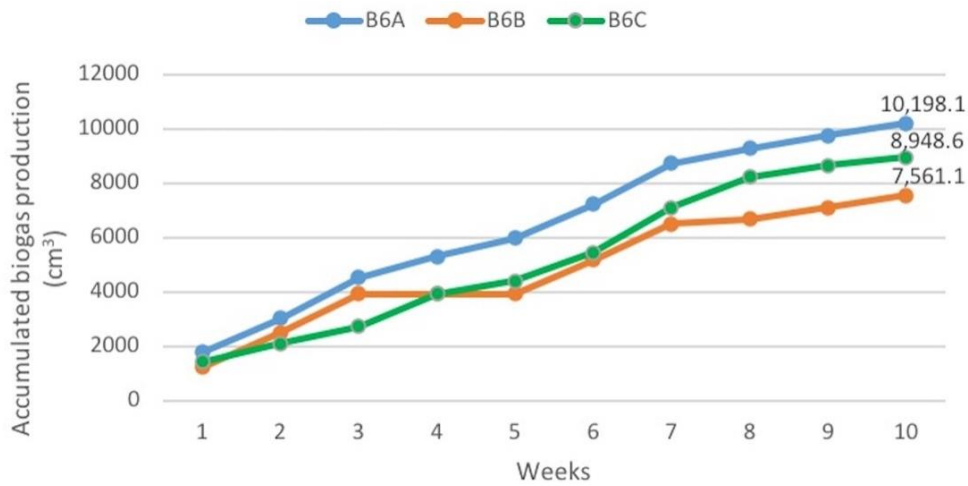


Figure 8. Accumulated production of biogas from the triplicates of digesters with 25% swine manure + 75% cheese whey.

The B6A digester showed a continuous increase in biogas production during the 10 weeks the experiment lasted. In the B6B digester production stopped between the third and fifth weeks and increased again from then until the end. The B6C digester showed continuous growth in production, but less than B6A.

Figure 9 presents the accumulated average biogas production for all triplicates: B1, B2, B3, B4, B5 e B6.

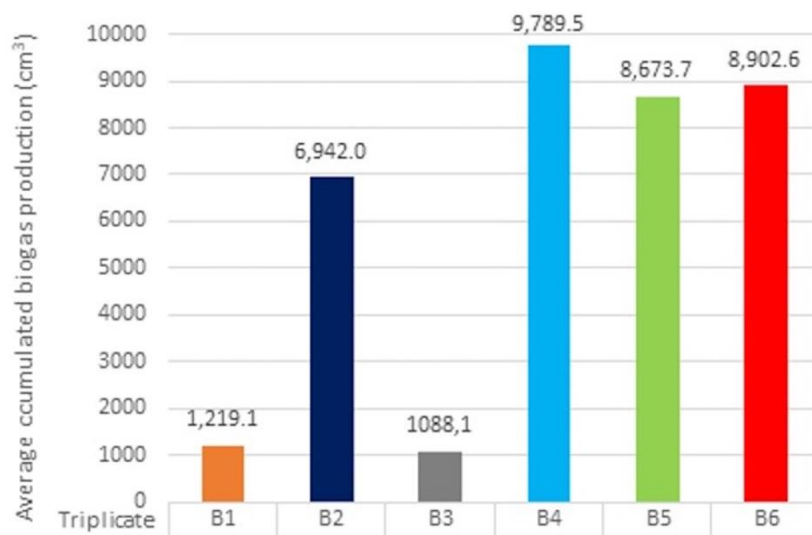


Figure 9. Average accumulated biogas production of all triplicates.

The highest accumulated production of biogas occurred for the proportion of whey and swine manure of 50:50 ( $v v^{-1}$ ), with an average of 9,789.5  $cm^3$  during the ten weeks of the experiment. The triplicate of digesters B2 (only swine manure) had an accumulated production higher than B3 (only cheese whey), which may have been influenced by the pH of the residues. The higher accumulated production of biogas in the triplicates B4, B5 and B6 (co-digestion) may be associated with pH balance, improvement in the C/N ratio and higher availability of micro and macronutrients for microorganisms.

### Methane content analysis of the biogas produced

Figure 10 shows the methane ( $CH_4$ ) content in biogas produced in B1A, B1B and B1C digesters during the ten weeks of the experiment.

The concentration of  $CH_4$  in the control triplicate (loaded with inoculum only) was irregular. Some readings do not identify the presence of the gas. The highest content identified was 25.6% in the B1B digester.

Figure 11 shows the methane ( $CH_4$ ) content in biogas produced in B2A, B2B and B2C digesters during the ten weeks of the experiment.

The biogas produced in the B2A digester showed a concentration of  $CH_4$  in all readings showing a sharp drop in the ninth and tenth week. The methane content of the biogas produced in the B2B digester varied

greatly over the weeks, being zero in the third and tenth week. The digester B3B showed more regular CH<sub>4</sub> content during the experiment reaching a concentration of 77.0 % in the tenth week.

Figure 12 shows the methane (CH<sub>4</sub>) content in biogas produced in digesters B3A, B3B and B3C during the ten weeks of the experiment.

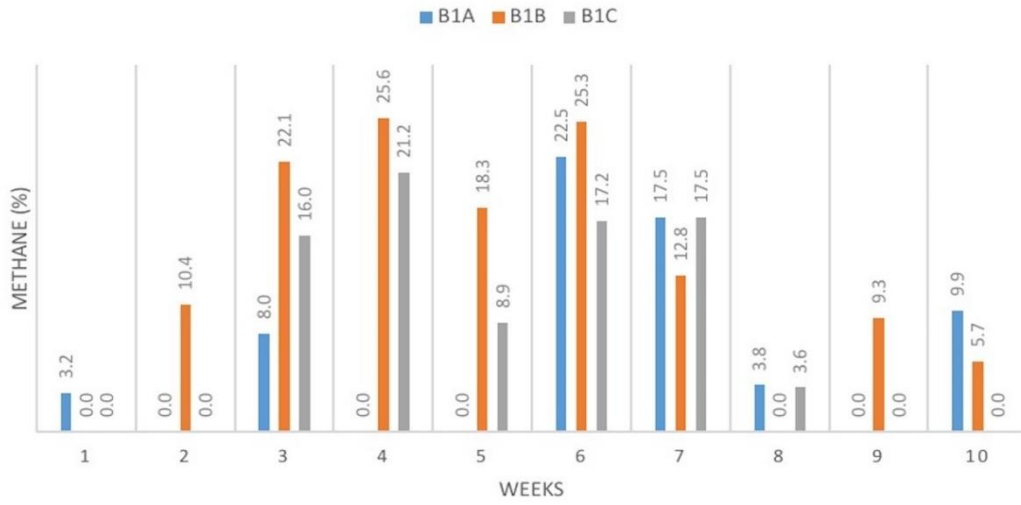


Figure 10. Methane content in biogas produced in B1A, B1B and B1C.

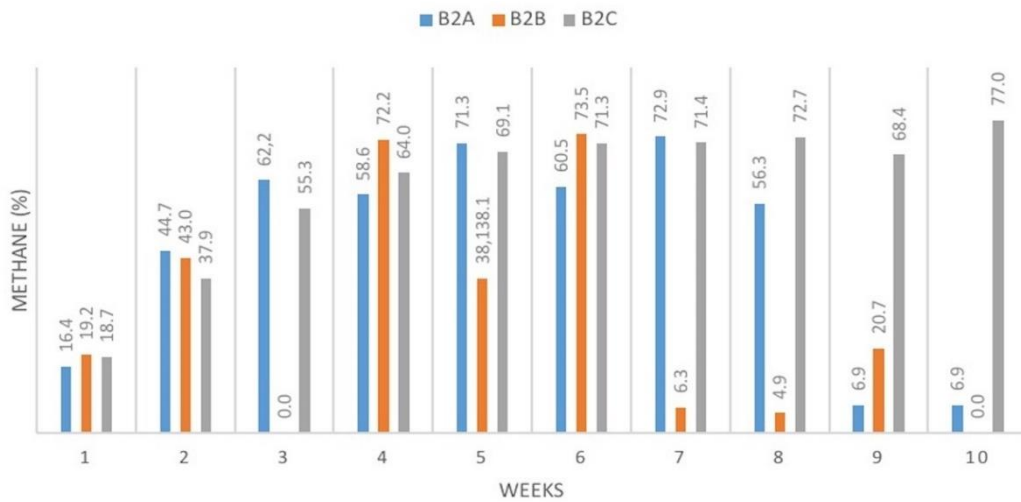


Figure 11. Methane content in biogas produced in B2A, B2B and B2C.

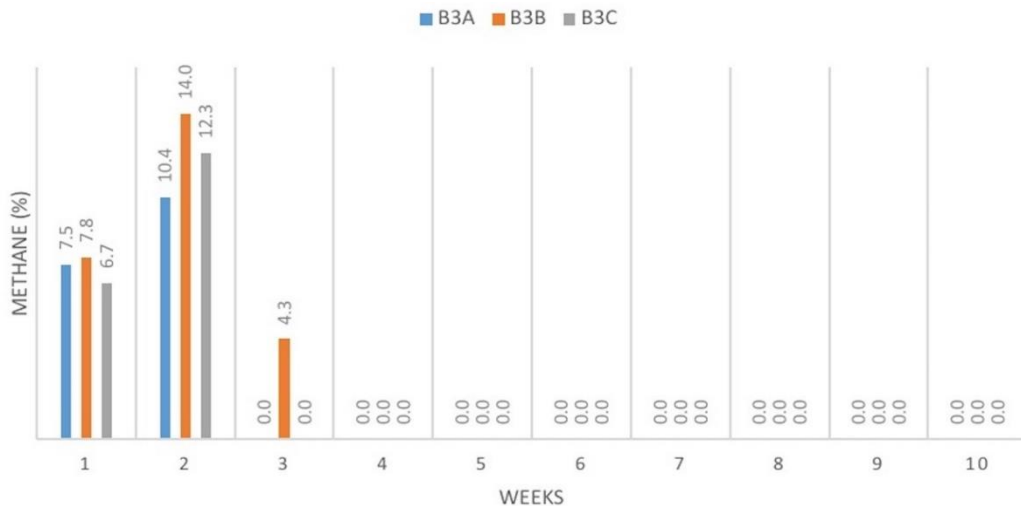


Figure 12. Methane content in biogas produced in B3A, B3B and B3C.



A concentration of CH<sub>4</sub> less than 15% was observed in the digesters of the triplicate in the first and second week. In the third week, only the digester B3B had a methane content equal to 4.3%. From the fourth week on no digester showed traces of CH<sub>4</sub>, as there was no production of biogas as discussed earlier.

Figure 13 shows the methane content in biogas produced in digesters B4A, B4B e B4C during the ten weeks of the experiment.

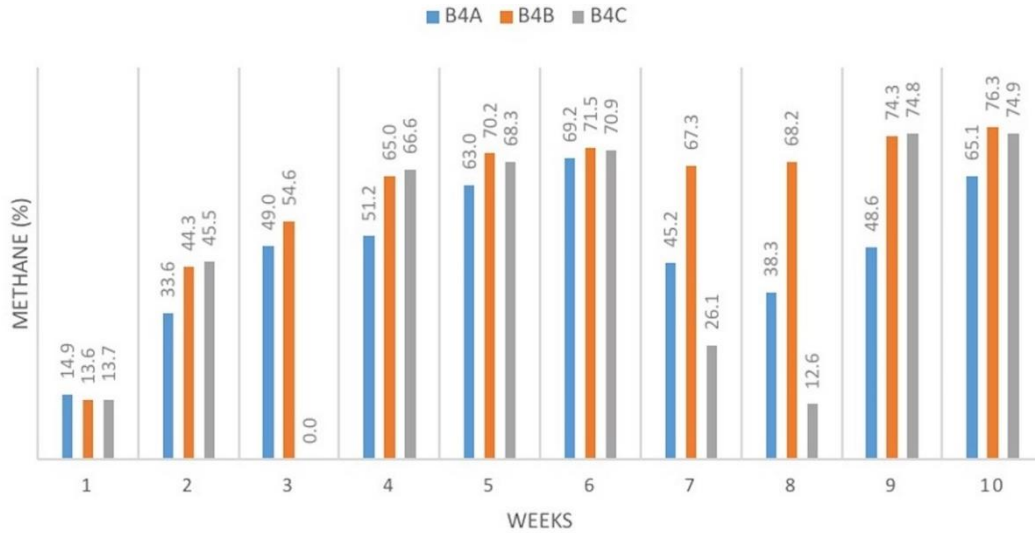


Figure 13. Methane content in biogas produced in B4A, B4B and B4C.

The digesters of this triplicate had a fairly regular CH<sub>4</sub> concentration, except for the B4C digester, which did not present CH<sub>4</sub> content in the third week because there was no biogas production. The maximum CH<sub>4</sub> content in digester B4A was 69.2%, in digester B4B, 76.3% and in digester B4C, 74.9%.

Figure 14 shows the methane (CH<sub>4</sub>) content in biogas produced in the digesters B5A, B5B and B5C during the ten weeks of the experiment.

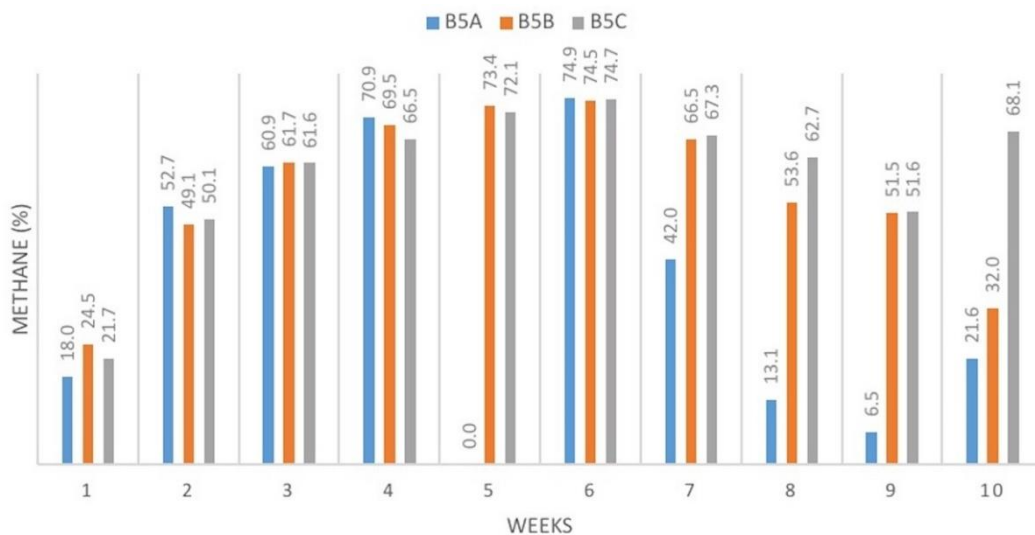
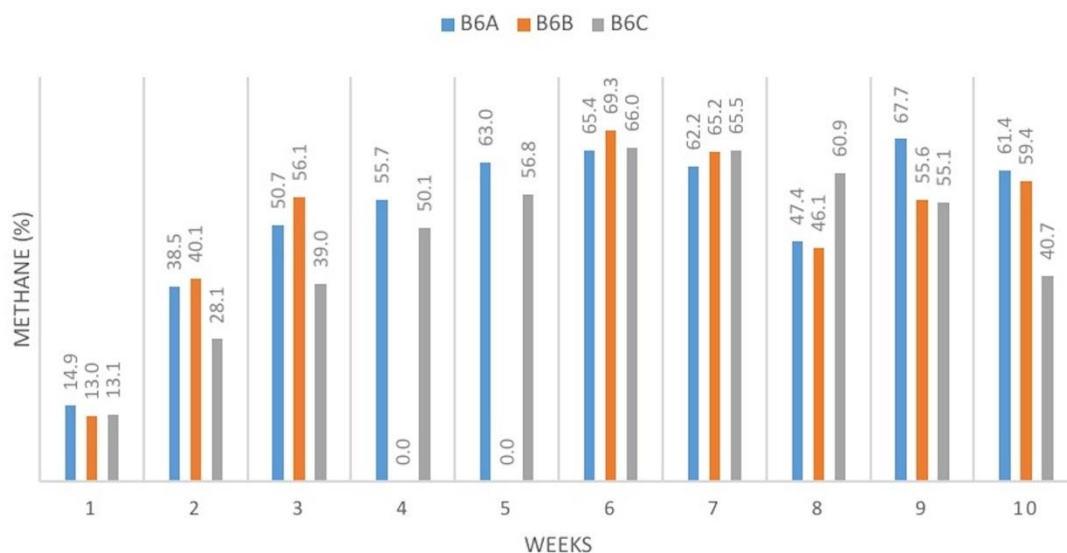


Figure 14. Methane content in biogas produced in B5A, B5B and B5C.

In the second, third and fourth weeks, the concentration of CH<sub>4</sub> in the digesters of the triplicate was very close. In digester B5A the CH<sub>4</sub> content was more irregular being zero in the fifth week due to the non-production of biogas, but equal to 74.9% in the sixth week (higher percentage of the triplicate).

Figure 15 shows the methane (CH<sub>4</sub>) content in biogas produced in digesters B6A, B6B and B6C during the ten weeks of the experiment.

The concentration of CH<sub>4</sub> in this triplicate was very irregular. The maximum content was obtained in digester B6B during the sixth week but did not produce biogas in the fourth and fifth weeks.



**Figure 15.** Methane content in biogas produced in B6A, B6B and B6C.

The triplicate that showed the best results about CH<sub>4</sub> concentration was with digesters B4A, B4B and B4C with a 50:50 (v v<sup>-1</sup>) proportion of cheese whey and swine manure.

The irregularity in biogas production and the CH<sub>4</sub> content in the triplicates can be explained by the different proportions of residues (cheese whey and swine manure) in the digesters and the respective parameter values that optimize and inhibit the anaerobic digestion process such as pH, the concentration of volatile fatty acids, C/N ratio.

Laboratory scale tests showed that anaerobic co-digestion of cheese whey and swine manure showed better results compared to anaerobic digestion of only one of these residues.

## Conclusion

Anaerobic co-digestion has advantages such as low operational and investment costs since it is possible to treat more than one waste simultaneously, in addition to the synergy between the wastes during the process.

The ratio of 50:50 (v v<sup>-1</sup>) cheese whey and swine manure presented higher accumulated production of biogas with an average of 9,789.5 cm<sup>3</sup> and a maximum CH<sub>4</sub> content of 76.3%.

It is necessary to carry out new studies with the control and monitoring of the fundamental parameters of anaerobic co-digestion to optimize and maximize production and increase the methane content in the biogas composition.

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