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ENERGY RECOVERY FROM THE ANAEROBIC CO-DIGESTION OF RICOTTA CHEESE WHEY AND DAIRY CATTLE MANURE

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Abstract

This study evaluated biogas production, methane concentration and yield, as well as the potential for energy production of anaerobic co-digestion of ricotta cheese whey and dairy cattle manure in 60 L plug flow reactors. Four reactors were operated: three with different proportions of ricotta cheese whey (20, 40, and 80%) and a control reactor containing only dairy cattle manure. The reactors were operated for 106 days, with a hydraulic retention time of 30 days. In all reactors, pH remained near neutrality, and alkalinity stayed above 2,500 mg L^{-1}. Notably, only the reactor with 80% ricotta cheese whey demonstrated more efficient removal of total solids, volatile solids, biochemical oxygen demand, and chemical oxygen demand, with average reductions of 54, 69, 69, and 78%, respectively. However, the reactor operated with 40% ricotta cheese whey produced the highest accumulated biogas volume, surpassing the 80% ricotta cheese whey reactor by 36 L, with a realistic extrapolation of electricity production estimated at 1.67 kWh m^{-3}. Although methane yields were close to theoretical maximum values and methane concentrations showed no significant differences between treatments, the results confirm the effectiveness of anaerobic co-digestion in the treating of agro-industrial waste. The 40% ricotta cheese whey and 60% dairy cattle manure ratio stands as a promising alternative for biogas production.

Keywords: Alternative energy. Bioprocesses. Dairy plant. Industrial waste. Renewable energy.

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1. Introduction

With the increasing demand for renewable energy sources and the urgent need to control environmental pollution, biogas production technologies through anaerobic co-digestion have gained significant attention in the scientific community (Zhao et al. 2024). Globally, solid and liquid waste generated by agribusiness is being recognized as valuable resources for biogas and biomethane production, contributing to the sustainable generation of bioenergy (da Silva et al. 2025).

Anaerobic biodigestion (AB) of agro-industrial waste emerges as an attractive solution, combining the efficient treatment of this waste with the production of renewable energy (Cozmuta et al. 2020). In particular, effluents from dairy plants and dairy cattle farming present great potential for renewable energy production (Luzzi et al. 2024; Silva-Gálvez et al. 2024; Tugume et al. 2024). Among the by-products generated by these industries, dairy whey stands out as one of the most challenging pollutants due to its high concentration of organic matter and eutrophic nutrients (El-Aidie and Khalifa 2024).

In Brazil, ricotta production has emerged as a key strategy for adding value to whey, a significant byproduct of cheese making. During this process, the proteins in the whey precipitate through thermal treatment at temperatures between 85 and 90 °C, resulting in an acidic whey (pH = 5.88) known as ricotta cheese whey (RCW), which stands out as one of the main byproducts of the dairy industry (Cozmuta et al. 2020).

Ricotta cheese whey has a specific composition, containing between 0.15 to 0.22% proteins, 1 to 1.13% salts, 4.8 to 5% lactose, 50 g L^{-1} of biochemical oxygen demand (BOD520), and 80 g L^{-1} of chemical oxygen demand (COD), in addition to a limited buffering capacity (Cozmuta et al. 2020; da Motta et al. 2022). The relatively low protein concentrations make RCW unsuitable for applications aimed at directly valorizing this macromolecule (Monti et al. 2018).

However, the significant lactose and organic matter content makes this byproduct a promising candidate for anaerobic fermentation processes (da Silva et al. 2021). This by-product can be used in the production of ethanol, lactobionic acid, RCW-based beverages, fruit juices, and fermented drinks, offering a viable pathway for the valorization of this by-product (de Giorgi et al. 2018; da Silva et al. 2021). Small and medium-sized dairy industries often face financial challenges in implementing these industrial processes for RCW valorization. Additionally, the quality of RCW may not always meet the required standards for these applications (Flores-Mendozaa et al. 2020).

Given these limitations, anaerobic co-digestion emerges as a sustainable and viable alternative for small and medium-sized dairy industries. The literature shows that anaerobic co-digestion not only provides an efficient treatment for dairy effluents, which are rich in easily fermentable carbohydrates, but also reduces the concentration of organic pollutants, minimizes environmental impacts, and enhances bioenergy production (Flores-Mendozaa et al. 2020). This approach represents an effective solution for waste management and the economic sustainability of these industries. Therefore, the study and application of anaerobic co-digestion with RCW can offer promising solutions for sustainable waste management and energy recovery (da Silva et al. 2021).

The literature highlights that the anaerobic co-digestion of RCW in combination with residues that have complementary characteristics, such as dairy cattle manure (DCM), not only optimizes the anaerobic co-digestion bioprocess but also improves treatment efficiency and maximizes energy recovery, offering a promising solution for sustainable waste treatment (da Silva et al. 2021). Dairy cattle manure is considered a valuable raw material due to its rich content of carbon, nitrogen, and other essential nutrients, such as sulfur, phosphorus, magnesium, and potassium, which are remnants of cattle diets (Zhu et al. 2021).

The inclusion of DCM in anaerobic co-digestion bioprocesses has been widely studied and shows great potential in biogas production, especially due to its significant methane (CH_4) content, making the bioprocess not only energy-efficient but also environmentally beneficial (da Silva et al. 2021). Several studies have explored the anaerobic co-digestion of DCM with various organic residues, such as brewery wastewater, corn straw, potato pulp, aloe vera peel, and other dairy residues, aiming to maximize biogas production (Xu et al. 2020; Chen et al. 2021; da Silva et al. 2021; Jaimes-Estévez et al. 2022; Yan et al. 2022).

In this context, the anaerobic co-digestion of RCW with DCM emerges as a viable and innovative alternative for the management of dairy waste, offering not only a suitable destination for RCW but also contributing to the improvement of biogas productivity. This approach not only helps mitigate the environmental challenges associated with the improper disposal of these residues but also promotes efficient energy recovery, aligning with the growing need for sustainable solutions in organic waste management.

The present study aimed to investigate the anaerobic co-digestion of RCW and DCM in different proportions, using four bench-scale plug flow reactors, each with a capacity of 60 L. The research focused on the detailed assessment of biogas production, including CH_4 concentration and yield, as well as the potential for energy generation. Additionally, variables related to bioprocess efficiency, such as organic matter reduction, solids removal, nutrient dynamics, and the behavior of pH and alkalinity throughout the bioprocess, were analyzed.

2. Material and Methods

Characteristics of the experiment study location

The experiment was conducted at the Brazilian Agricultural Research Corporation, Embrapa Dairy Cattle, located in Juiz de Fora, MG, Brazil, at geographic coordinates 21° 46'55 "S; 53° 22' 10" W. The local climate is classified as tropical (Cwa) on the Köppen & Geiger scale, with a mean annual temperature of 22 °C, ranging from 18 to 35 °C, and a mean annual rainfall of 1,516 mm. Climate data were provided by an automatic meteorological station located 1 km from the experiment site, at the Federal University of Juiz de Fora.

Feedstocks and inoculum

In this study, the substrates used were DCM and RCW. Dairy cattle manure was employed in the development and acclimation of the inoculum due to its composition, which provides essential substrates for the growth and development of anaerobic microorganisms, crucial for the anaerobic co-digestion bioprocess.

The dairy cattle manure used in this study was obtained from a mixture of fresh manure and wastewater from cleaning the confinement floors. This waste was collected from the milk production system of the experimental farm at Embrapa Dairy Cattle, located in Coronel, MG, Brazil. The cows' diet consisted of a concentrate mixture (composed of 60% corn grains, 36% soybeans, 3% mineral supplement, and 1% urea), corn silage, pasture, and mineral salt, provided daily in amounts of 3.5 kg, 20 kg, 40 kg, and 150 g, respectively. To prepare the DCM, the mixture was initially subjected to separation using a sieve with a 3.7 mm mesh size. This process resulted in a fraction with a total solids (TS) concentration of approximately 6% (± 2%). After separation, the sample was stored at 8 °C to maintain stability and preserve its physicochemical characteristics until analysis.

Ricotta whey was collected weekly from a dairy industry located in Juiz de Fora, MG, Brazil. After collection, the whey was transported to the Embrapa Dairy Cattle facilities and stored at 8 °C to preserve its physicochemical and microbiological properties until it was used in the anaerobic co-digestion bioprocess. To adjust the pH of the RCW to the desired range of 6.5 to 7.0, calcium hydroxide was used at a concentration of 4.24% (v/v)59 mL of this solution was added to the whey. The concentration of calcium hydroxide used was determined based on tests conducted on previous batches, which showed it to be effective for correcting and increasing the pH of the RCW within the target range. The effectiveness of the pH adjustment was monitored through regular measurements to ensure that the pH remained stable during storage and analysis.

Description of the reactors

The reactors used in this study were PVC tubes with a diameter of 200 mm, painted black to optimize the heating of the fermentation chamber. Each reactor had a volume of 60 L, was equipped with an individual 30 L gasometer. The gasometers were connected to the reactors via plastic hoses, which directed the generated biogas to the gasometers (Figure 1). The volume quantification was performed using the water displacement method.

The reactors operated in an outdoor environment at room temperature and were positioned horizontally on iron supports, arranged side by side. This setup ensured uniform exposure to sunlight across all reactors, providing homogeneous heating conditions.

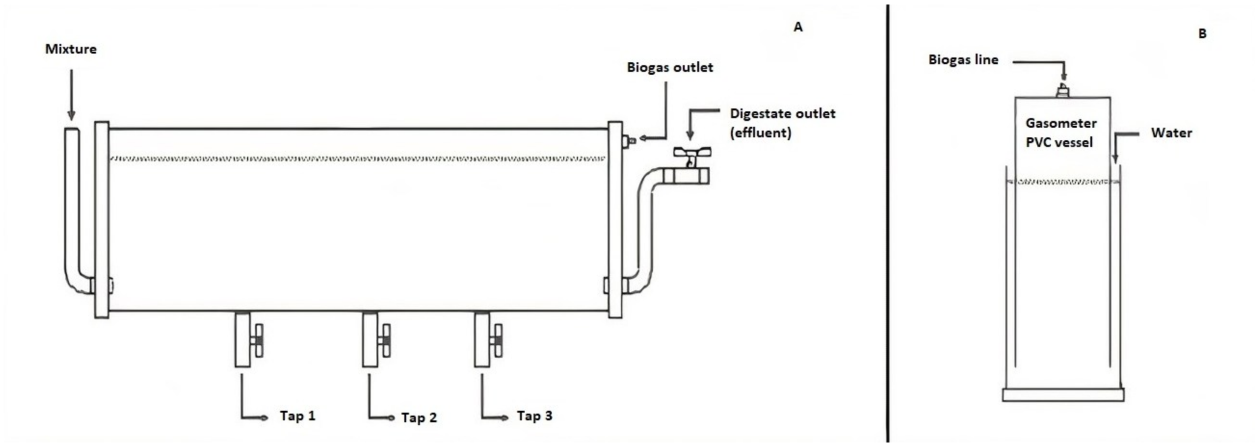

Figure 1. Anaerobic reactor. Cylindrical anaerobic reactor (A), Details of gasometer (B).

Descriptions of the treatments

In this study, four reactors were used, with three dedicated to the anaerobic co-digestion of DCM with RCW and one solely for the anaerobic digestion of DCM, which served as a control. Reactor 1 was used as a control, containing 100% DCM. Reactor 2 was operated with 20% RCW and 80% DCM. Reactor 3 was operated with 40% RCW and 60% DCM, while reactor 4 was operated with 80% RCW and 20% DCM. Each reactor had different physicochemical characteristics to meet the experimental conditions (Table 1).

Table 1. Physical-chemical characteristic of the influents of each treatment for co-digestion.

Parameters	Unity	20% RCW	40% RCW	80% RCW	Control
pH	-	6(0.50)	5.98(0.60)	5.85(0.53)	6.25(0.43)
Alkalinity	mg L^{-1}	2,199(299)	1,816(296.01)	1,501(318.59)	2,791(316.34)
TS	mg L^{-1}	58,700(6,500)	52,500(6200)	50,600(6,500)	62,000(6900)
VS	mg L^{-1}	48,400(2,900)	43,900(3000)	40,500(3,600)	54,400(3,700)
COD	mg L^{-1}	92,150(9,672.51)	81,117(189.29)	59,867(6,861.91)	97,433(571.68)
BOD5	mg L^{-1}	39,756(3,779.07)	37,200(14003.21)	27,367(3,172.37)	43,753(9,842.90)
NTK	mg L^{-1}	470(352.04)	398(292.57)	382(382.37)	388(152.55)
NO_3^-	mg L^{-1}	32(20.21)	42(14.14)	38(21.21)	35(0.01)
NO_2^-	mg L^{-1}	3(1.53)	4(0.35)	7(2.83)	5(2.47)
$N\text{-}NH_3$	mg L^{-1}	446(500.72)	353(382.63)	340(397.32)	339(289.33)

NTK Total Kjeldahl nitrogen. Values in brackets indicate standard deviation

The reactors were operated in three distinct phases: Phase I involved the start-up and stabilization of the system; Phase II consisted of adapting the inoculum to the experimental conditions; and Phase III was the operation of the anaerobic co-digestion bioprocess.

In Phase I, all reactors were initially fed with 60 L of DCM. After 15 days, the biogas concentration analysis indicated 60% CH_4, confirming the effectiveness of anaerobic digestion and the active presence of inoculum in the reactors. In Phase II, the reactors were gradually supplied with experimental mixtures of DCM and RCW in the specified proportions, with a daily feed of 2 L, to acclimate the inoculum. In Phase III, after 30 days of acclimatization, the reactors operated in full anaerobic co-digestion for 106 days, with a hydraulic retention time (HRT) of 30 days.

Reactors operated at ambient temperatures ranging from 18 to 26 °C (average 22 °C ± 2.02). Internal temperatures, monitored with a long-stem digital thermometer HI 93510N (HANNA), ranged from 14 to 33 °C (average 25 °C ± 2.32), leading to operation under both psychrophilic (below 20 °C) and mesophilic (25 - 40 °C) conditions.

Physical-chemical analyses

Physicochemical analyses of influent (input) and effluent (output) samples from each reactor were performed weekly, in duplicate, over 106 days during experimental phase III. All analytical procedures were conducted according to APHA standards (Collins, Braga, Bonato 1997). The pH was measured using a Tecnal Tec-3MP pH meter. Alkalinity was determined as $CaCO_3$, determined by titration with a 1 N sulfuric acid solution until reaching a pH of 4.3 (Collins, Braga, Bonato 1997).

For the quantification of TS, 2 g of the samples were oven-dried at 105 ± 2 °C until a constant weight was achieved. Subsequently, the samples were then calcined in a muffle furnace at 550 ± 50 °C for 30 minutes to determine the ash content, following APHA standards, sections 2540E.2 and 2540D.2. The ash weight was subtracted from the dry weight to calculate the volatile solids (VS) content (Collins, Braga, Bonato 1997).

The biochemical oxygen demand was determined by iodometry, while the COD was measured by the closed reflux colorimetric method, with readings taken using a spectrophotometer in the range of 420 nm to 600 nm. Ammonia nitrogen ($N\text{-}NH_3$) was quantified by optical emission spectrophotometry. All analytical procedures followed the standards established by APHA (Collins, Braga, Bonato 1997).

Methane concentrations in the biogas were determined weekly during experimental phases II and III using gas chromatography. The analyses were performed with an Agilent Technologies model 7.820A chromatograph, equipped with two specific columns: HP-Plot/Q (30 m x 0.530 mm x 40.0 µm) and HP-Molesieve (30 m x 0.530 mm x 25.0 µm). Hydrogen was used as the carrier gas. The biogas samples, collected in 25 mL glass syringes, were immediately analyzed to prevent any alteration in biogas composition. The CH_4 analysis results were expressed as a percentage, ensuring accuracy and reproducibility of the measurements (Collins, Braga, Bonato 1997).

Biogas volume normalization

The biogas volume was determined using a ruler attached to the gasometer to measure the displacement of the internal cylinder containing the biogas. The biogas volume was measured daily, always at the same time, before the refueling. Biogas production was normalized to standard conditions (0 °C and 1.013 bar) using the Gay-Lussac ideal gas equation (Equation 1).

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

Where, V_0 was the corrected biogas volume (m³), 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.

Statistical analyses

Significant differences between treatment means were assessed using Tukey's test with a 95% confidence level. Prior to conducting parametric tests, data normality was confirmed using the Shapiro-Wilk test with the PAST version 4.03 software (Hammer et al. 2001).

3. Results

Biomass

Regarding the composition of RCW, the TS and VS values were 52,200 ± 200 and 41,700 ± 400 mg L^{-1}, respectively. Acidity and alkalinity were measured at 5.88 ± 0.11 and 860 ± 3.51 mg L^{-1}, respectively (Table 2).

For dairy cattle manure, the TS and VS values were 62,000 ± 6,900 mg L^{-1}, ranging from 51,100 to 68,000 mg L^{-1}, and 54,400 ± 3,700 mg L^{-1}, ranging from 49,200 to 65,100 mg L^{-1}, respectively (Table 2).

Table 2. Physical-chemical characterization of feedstocks.

Parameters	Unity	DCM	RCW
pH	-	6.25$_{(0.43)}$	5.88$_{(0.11)}$
Alkalinity	mg L^{-1}	2,791$_{(316.34)}$	860$_{(3.51)}$
TS	mg L^{-1}	62,000$_{(6,900)}$	52,200$_{(200)}$
VS	mg L^{-1}	54,400$_{(3,700)}$	41,700$_{(400)}$

Values in brackets indicate standard deviation

pH and alkalinity

During the anaerobic co-digestion bioprocess, pH values ranged from 6.9 to 7.3 (Figure 2A), with no significant differences between the treatments. The lowest values were observed in the reactor containing 80% RCW. Alkalinity measurements remained within the ideal range to ensure stability in the anaerobic co-digestion process (Figure 2B).

Total and volatile solids

The total solids and VS values in the effluent ranged from 14,300 to 46,400 mg L^{-1} and from 6,700 to 35,800 mg L^{-1}, respectively, across all reactors (Figure 2C and D). The average removals of TS and VS ranged from 36 to 54% and from 44 to 69%, respectively (Table 3). The reactor operated with 80% RCW exhibited the highest VS removal, showing a significant difference compared to the other reactors.

Removal of Biochemical and Chemical Oxygen Demand

In this study, effluent BOD_5^{20} and COD values ranged from 1,275 to 31,550 mg L^{-1} and from 9,125 to 71,850 mg L^{-1}, respectively, with no destabilization of the fermentation process due to fluctuations in the applied organic load (Figure 2E and F). During anaerobic co-digestion, the average COD removal ranged from 40 to 69% and the average BOD_5^{20} removal ranged from 47 to 78% (Table 3). The reactor with 80% RCW achieved the highest removal rates for both COD and BOD_5^{20}. Significant differences were observed only in COD and BOD_5^{20} removals in the treatment with 80% RCW, while no significant differences were found among the other reactors, with removal rates ranging from 28 to 51%.

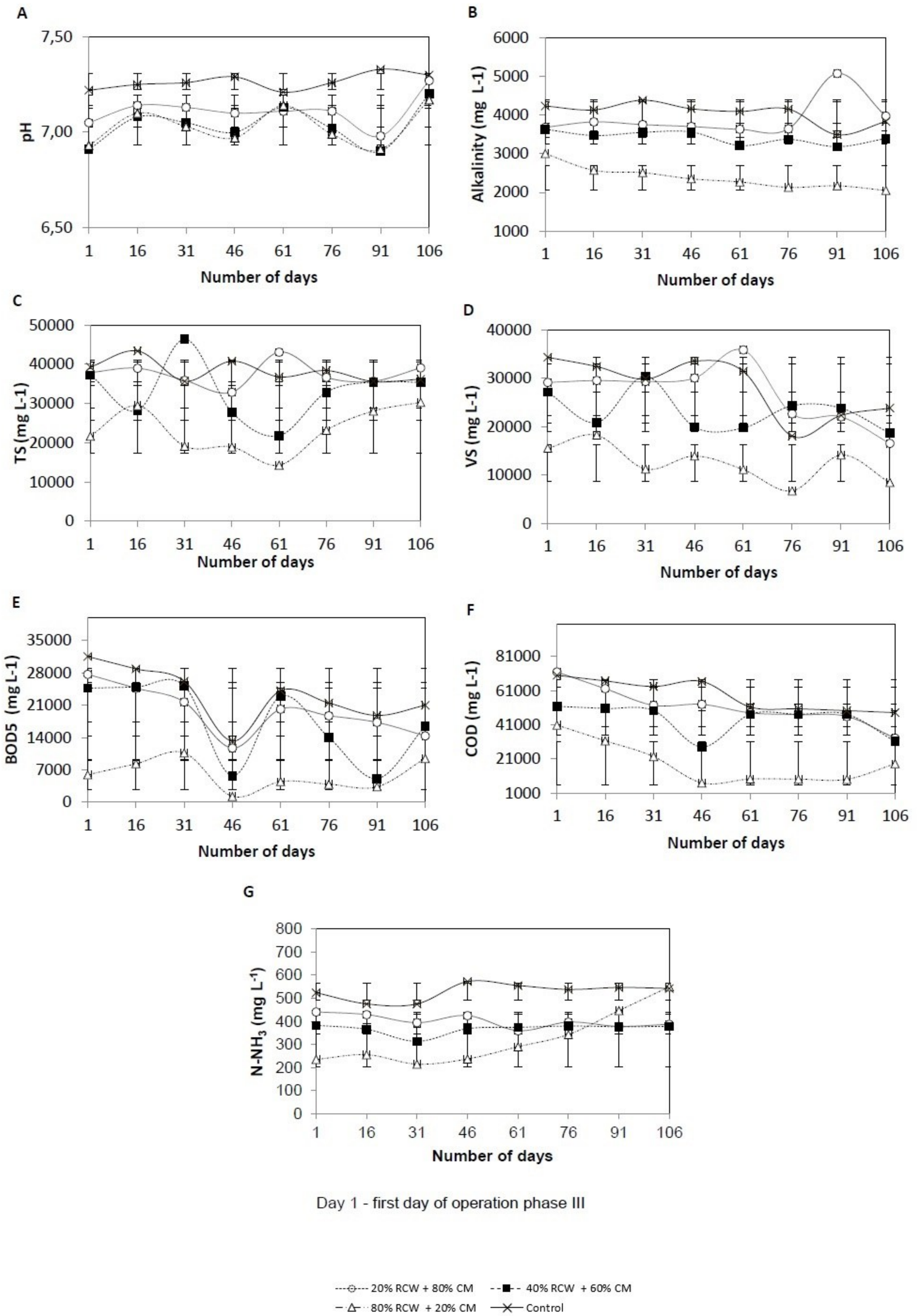

Figure 2. Physico-chemical characteristic of the effluents during anaerobic co-digestion. Values of pH (A), values of alkalinity (B), values of TS (C), values of VS (D), values of BOD (E), values of COD (F) and values of $N\text{-}NH_3$ (G).

Table 3. Daily removal: total/volatile solids and COD/BOD_5^{20} from each treatment in the anaerobic co-digestion process.

Removal TS and VS (%)								
Days	**20% RCW**		**40% RCW**		**80% RCW**		**Control**	
1	35	40	29	38	57	62	37	37
16	34	39	46	53	42	55	30	40
31	39	39	47	31	62	72	42	45
46	44	38	47	55	63	66	34	38
61	27	26	58	55	72	73	41	42
76	38	53	37	45	54	83	38	67
91	39	55	32	46	44	65	43	59
106	33	66	33	58	40	79	42	56
Av	36	44	37	47	54	69	38	48
Max	44	66	58	58	72	83	43	67
Min	27	26	12	31	40	55	30	37
SD	±5.14	±12.53	±10.05	±9.38	±11.34	±9.34	±4.48	±11.07
Removal COD and BOD_5^{20} (%)								
Days	**20% RCW**		**40% RCW**		**80% RCW**		**Control**	
1	22	30	36	34	32	78	28	28
16	33	38	38	33	47	70	32	34
31	43	45	39	32	63	61	35	40
46	43	70	65	85	88	95	32	70
61	48	49	42	38	84	84	47	45
76	49	53	42	62	85	86	48	51
91	50	56	42	86	85	88	49	57
106	64	64	61	56	70	65	51	52
Av	44	51	46	53	69	78	40	47
Max	64	70	61	86	88	95	51	70
Min	22	30	36	32	32	61	28	28
SD	±12.51	±13.15	±11.21	±22.72	±20.71	±12.00	±9.44	±13.33

Av average, *SD* standard deviation: 106 days in operation
Day 1 - first day of operation phase III

Biogas production

During experimental phase III, the volumetric biogas production in the reactor containing 20% RCW was 22 ± 1.91 L day^{-1}, with values ranging from 18 to 25 L day^{-1}. In the reactor with 40% RCW, the average production was 25 ± 1.54 L day^{-1}, with values between 23 and 27 L day^{-1}. The reactor that received 80% RCW had an average production of 20 ± 2.91 L day^{-1}, with variations between 17 and 24 L day^{-1}. The control reactor produced 22 ± 1.72 L day^{-1}, with values ranging from 20 to 25 L day^{-1} (Figure 3B). No significant differences were observed between the different treatments regarding biogas production.

The maximum biogas production per liter of reactor was 0.41, 0.45, 0.41, and 0.42 L biogas L $reactor^{-1}$ day^{-1} for the treatments with 20, 40, and 80% RCW, and control, respectively. The average productions were 0.36 ± 0.03, 0.42 ± 0.03, 0.34 ± 0.05, and 0.37 ± 0.03 L biogas L $reactor^{-1}$ day^{-1}. No significant differences were observed between the treatments (Figure 3A).

The accumulated biogas volumes were 175, 200, 164, and 177 L for the reactors with 20, 40, and 80% RCW and control, respectively. The reactor operated with 40% RCW showed an average biogas production 5 L day^{-1} higher than that of the reactor with 80% RCW, resulting in an accumulated increase of 36 L of biogas during Experimental Phase III.

The methane concentration during anaerobic co-digestion in the reactor containing 20% RCW was 60 ± 1.44%, with a maximum of 62% and a minimum of 57%. In the reactor containing 40% RCW, the average CH_4 concentration was 57 ± 1.90%, with a maximum of 60% and a minimum of 54%. In the reactor that received 80% RCW, the average CH_4 concentration was 59 ± 2.96%, with a maximum of 61% and a minimum of 55%. In the control reactor, this value reached 58 ± 1.89%, with a maximum of 62% and a minimum of

56%. In this study, CH_4 concentrations ranged from 54 to 62%. No significant differences were recorded between the treatments in terms of CH_4 production (Figure 3C).

The volumetric CH_4 production among the treatments ranged from 1.96 to 2.44 m³ month⁻¹ (Figure 3D). In terms of CH_4 production yield per kg of COD removed, values were recorded as 0.28, 0.30, and 0.25 m³ CH_4 kg COD removed^{-1} in the treatments containing 20, 40 and 80% RCW, and 0.27 m³ CH_4 kg COD removed^{-1} in the control reactor.

This study observed a realistic projection of energy production at 1.67 kWh m^{-3} of biogas produced when 40% RCW was added.

Ammonia nitrogen

The N-NH_3 concentrations in the effluents in the reactors containing 20, 40 and 80% RCW and control were 402 ± 27.81, 367 ± 22.60, 321 ± 119.36 and 529 ± 35.62 mg L^{-1} (Figure 2G). Between treatments, observed no significant differences.

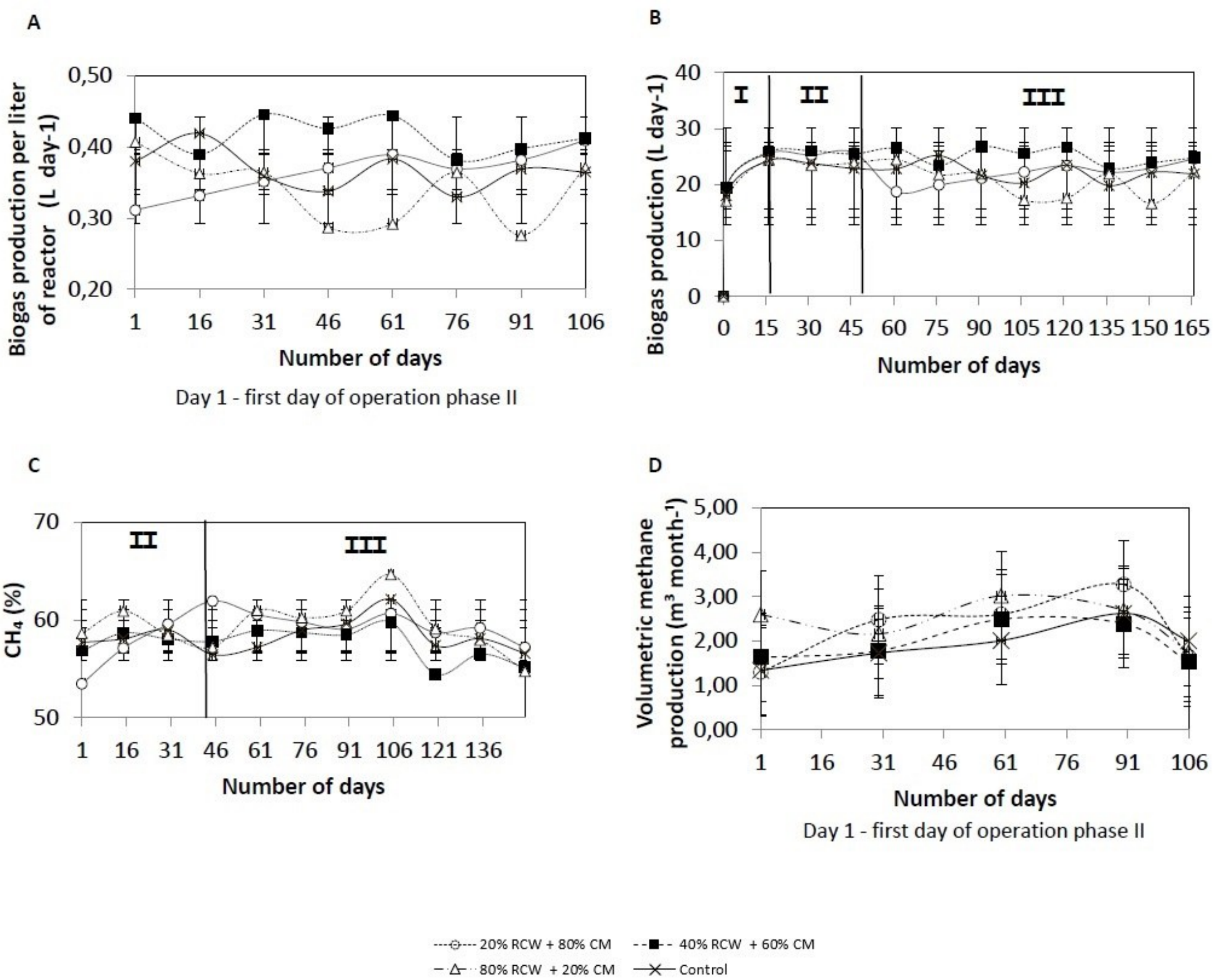

Figure 3. Biogas production during the phases of the experiment. Biogas per liter of reactor production (A), Biogas production (B), CH_4 production (C) and volumetric methane production (D).

4. Discussion

Biomass

To maintain process stability, the biodegradability of the biomass and the organic load were crucial parameters in the anaerobic co-digestion process and biogas production. This stability may be associated with the use of DCM as a complementary residue in the anaerobic co-digestion of RCW. The literature indicates that DCM, when applied to reactors, contributes to maintaining the fermentation process, stimulating initial biodegradation processes, and ensuring anaerobic stability (da Silva et al. 2021).

The stability observed in this study was similar to that reported by Flores-Mendozaa et al. (2020), who evaluated the potential of raw cheese whey as a by-product to ensure reactor stability and improve CH_4 production. These findings suggest that biomass derived from dairy residues, such as RCW, can be efficiently treated by anaerobic co-digestion under slightly alkaline pH conditions, resulting in improved CH_4 production and yield, while maintaining adequate operational conditions.

pH and alkalinity

The alkalinization of RCW was crucial for preventing acidification and ensuring the stability of anaerobic co-digestion. The stability of this bioprocess was also directly associated with maintaining an alkalinity above 2,500 mg L^{-1}, which resulted in a significant increase in CH_4 yield (Cheah et al. 2019). Studies indicate that alkalinity values below this level should be strictly avoided, as they can trigger harmful imbalances in the bioprocess, such as the inhibition of methanogenic activity and the consequent decrease in biogas production (Elazhar et al. 2022).

Reactor operated with 80% RCW emerged as the maximum viable concentration for application in anaerobic co-digestion with DCM, as they maintained alkalinity values close to the minimum required to ensure bioprocess stability and optimize CH_4 production over time. This condition was particularly evident when compared to reactors operated with 20 and 40% RCW, which exhibited alkalinity values significantly above the minimum required (Figure 2B). These results indicate that the RCW concentration should be carefully adjusted to promote a favorable environment for methanogenic activity, ensuring bioprocess stability and optimizing CH_4 production.

Total and volatile solids

The wide variation observed in TS and VS values in the effluents indicates the effectiveness of the anaerobic co-digestion bioprocess of RCW and DCM at different RCW concentrations. Additionally, the trend of decreasing TS levels with increasing RCW concentration suggests an improvement in the efficiency of this bioprocess. This phenomenon can be attributed to the synergistic effect of co-digestion, which enhances microbial activity and solid degradation. Thus, the increase in RCW concentration appears to contribute to better solid removal, resulting in higher quality effluent and reflecting a potential optimization of the bioprocess (Xie et al. 2018). These results highlight the potential of anaerobic co-digestion for practical applications and may guide future research to further improve system efficiency.

The combination of residues with complementary characteristics, such as RCW and DCM, in anaerobic co-digestion bioprocesses demonstrated a significant positive effect on solid removal and organic matter degradation. The increase in RCW concentration in this bioprocess resulted in a higher organic matter content, which, in turn, led to greater VS removals and biogas production. This behavior can be attributed to the higher organic load provided by RCW, which offers additional substrates for microbial activity, promoting more complete degradation of organic compounds (Cozmuta et al. 2020).

These results corroborate the effectiveness of anaerobic co-digestion of dairy effluents with complementary residues, such as DCM, in optimizing anaerobic co-digestion bioprocesses efficiency, providing not only greater organic matter degradation but also increased biogas production, which is crucial for the economic and environmental viability of this type of treatment (Bella and Rao 2022). Moreover, they indicate that anaerobic co-digestion is a promising strategy for optimized organic waste treatment, especially in systems where effective solid removal and complete organic matter degradation are critical goals (Karki et al. 2021).

These results align with previous studies that investigated anaerobic co-digestion of dairy effluents, such as whey and DCM, in continuously stirred tank reactors. For instance, Rico et al. (2015) observed that a CSTR operating at 35 °C with an HRT of 80 days, fed with cheese whey and DCM, achieved VS removals of 56.2 and 69.9% for 15 and 85% whey concentrations, respectively. These results indicate that higher proportions of whey are associated with more efficient VS removal, corroborating with biogas production.

Similarly, another study by Jihen et al. (2015) demonstrated that a CSTR fed with a mixture of 80% cheese whey and 20% DCM, operating at 35 °C with an HRT of 20 days, achieved a maximum VS removal of 88.6%.

These results underscore the importance of optimizing substrate proportions in anaerobic co-digestion processes to maximize volatile solid removal and biogas production. Adjusting operational conditions, such as RCW concentration, can play a crucial role in improving the efficiency of anaerobic systems, especially in industries dealing with large volumes of organic waste, such as dairy industries (Bella and Rao 2022).

Removal of Biochemical and Chemical Oxygen Demand

Although no significant differences were detected in the removal of BOD520 and COD in the reactors operated with 20 and 40% RCW, these results suggest a moderate efficiency in the removal of organic load. Similar to solid removal, COD removal showed a significant increase with the rise in RCW concentrations in the anaerobic co-digestion bioprocess. This behavior indicates that the introduction of only 20% DCM, an inoculum not only adapted but highly effective, played a crucial role in assisting the biodegradation of 80% of the RCW.

This efficiency can be attributed to the combination of RCW with DCM, which created a synergistic microbiological environment, where the microorganisms present in the DCM played a crucial role in degrading the complex organic compounds in the RCW (Singh et al. 2023). Ricotta cheese whey, rich in organic matter and nutrients, served as an excellent substrate for microbial activity, while DCM acted as a process stabilizer, providing microbiological diversity and enhancing the system's resilience to potential variations in operational conditions (Cozmuta et al. 2020; Bella and Rao 2022).

In contrast, in the control reactor operated with 100% DCM, a lower COD removal was observed. This suggests that while DCM contributes to process stabilization, its standalone efficiency in organic matter removal is limited (Bella and Rao 2022). This limitation was corroborated by Khalid et al. (2019), who reported a COD removal of only 15% in a control reactor containing 100% DCM operated at 35 °C with an HRT of 24 days.

These results reinforce the importance of co-digestion as an efficient strategy for treating complex organic waste, where the combination of different wastes not only improves organic matter removal but also optimizes biogas production, enhancing the potential for energy recovery (Karki et al. 2021).

Biogas production

Ricotta cheese whey was an important by-product for maximizing CH_4 production in the operated reactors. The significant CH_4 yield is associated with the high organic loads and biodegradability of RCW, which can thus be considered a potential by-product for biogas generation with a relevant volumetric production of CH_4 (Rico et al. 2015). The CH_4 yield per kg of COD removed showed promising results, considering that the theoretical maximum CH_4 yield is 0.35 m^3CH_4 per kg of COD removed, under conditions of 1 atm and 0 °C (Park et al. 2016).

From a practical perspective, electricity generation using an Otto cycle engine generator (with low efficiency), with a CH_4 concentration of 65% in the biogas, can result in up to 1.8 kWh of energy for each m^3 of biogas produced (EMPRESAS FOCKING; BRANCO MOTORES 2009). Thus, in this study, plug flow reactors, when fed with a 40% RCW concentration, demonstrated greater biogas productivity.

These results are promising and indicate significant practical potential for biogas generation, offering a new alternative for renewable energy production. Specifically, agricultural producers with biodigesters on their farms could receive RCW to increase electricity generation, thereby reducing operational costs. Additionally, this approach helps solve an environmental problem faced by the dairy industry by providing an appropriate destination for RCW.

Ammonia nitrogen

In anaerobic reactors operated with DCM, $N\text{-}NH_3$ tends to accumulate due to the high concentrations of this chemical compound in DCM. Concentrations above 150 mg L^{-1} of $N\text{-}NH_3$ are recognized for causing significant detrimental effects on anaerobic systems, as reported by Bella and Rao (2022). Despite these challenges, in this study, the biogas productivity and CH_4 yields demonstrated that the anaerobic co-digestion process was efficient even in the presence of high concentrations of N-NH_3. The observed N-NH_3 concentrations in reactors operated with 20, 40, and 80% RCW were 2.68, 2.50, and 2.14 times higher, respectively, than those reported in the literature for systems with detrimental effects related to N-NH_3. The results demonstrate that it is essential to recognize the limitations imposed by ammonia toxicity in anaerobic co-digestion processes.

5. Conclusions

The reactor operated with 40% RCW was the most effective, yielding significantly higher biogas volumes while maintaining optimal pH and alkalinity. This concentration also achieved CH_4 yields close to the theoretical maximum, demonstrating it to be the most efficient for both maximizing biogas production and stability of the bioprocess.

This ratio is a highly effective strategy for valorizing organic waste and enhancing anaerobic co-digestion processes, significantly contributing to sustainability and cost reduction through biogas production. By transforming RCW into a valuable resource, it offers significant benefits for renewable energy production and efficient waste management.

Anaerobic co-digestion of RCW and DCM not only maximizes energy yields and efficiently utilizes biomass, benefiting farmers and dairy industries by promoting sustainability and cost savings, but also fosters innovation in waste-to-energy conversion processes, providing a sustainable solution for waste management in both rural and industrial production chains.

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