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Article

Biogas Production from Anaerobic Co-Digestion of Cow Manure, Residual Edible Oil with Two Qualities of Waste Activated Sludge

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Abstract: The quality of waste activated sludge produced in urban wastewater treatment systems varies according to the efficiency of the operation of treatment units along the content of microflora and organic carbon. However, these represent a biogas-energy alternative through anaerobic co-digestion, contributing to reducing the environmental impacts caused by their inadequate disposal. Biogas production by the Two-stage production method in batch digesters was evaluated by two qualities of waste activated sludge (SLB₅₀ y SLB₉₀) and with a mixture of two co-substrates: cattle manure (CEV₅₀ y CEV₉₀) and residual edible oil with mixing, pH and temperature control. Bacteria in good quality sludge (SLB₉₀) showed a faster adaptation of 2 days than those in low quality (SLB₅₀) with 25 days lag phase. The highest CH₄ production was for SLB₉₀ (303.99 cm³ d⁻¹) compared to SLB₅₀ (4.33 cm³ d⁻¹); while cow manure sludge mixture (CEV₉₀) contributed to increasing production of CH₄ (42 422.8 cm³ d⁻¹) compared to CEV₅₀ (12 881.45 cm³ CH₄ d⁻¹), while in residual edible oil mixture they were 767.32 cm³ d⁻¹ and 211.42 cm³ d⁻¹ for CAV₉₀ y CAV₅₀, respectively. The addition of sludge co-substrates improves the nutrient balance, C/N rate and micro flora diversity; consequently methane production improves too; this methodology could be integrated into concepts of Circular economy.

Keywords: biogas; method two-stage; waste activated sludge; sludge calidad; co-substrata

1. Introduction

Rapid economic growth in numerous cities has led to a high dependence on fossil fuels and gas for energy production, causing a significant increase in greenhouse gas emissions [1,2]. On the other hand, liquid bio-fuels (biodiesel y bio-ethanol) have been part of the strategies for reducing emissions of pollutants into the atmosphere. Likewise, biogas is another alternative considered as a source of renewable energy that can be used for the production of energy and heat, as well as, fuel for vehicles or for synthesis of chemicals or materials [3] and thus contributes to reducing emission of greenhouse gases.

Biogas is commonly produced using one-stage fermentation techniques, consisting in fermentation process and biogas production occurring in a single reactor. In this process, bacteria degrade complex organic compounds under an acidic environment, subsequently collaborating with methane-producing bacteria requiring a neutral pH environment. This process causes complex organic compounds degradation into simple organic compounds during the acetogenic stage, with these simple organic compounds converting, in parallel, into biogas at the methanogenic stage. Negative effects can occur with (one-stage) biodigestores due to the increase of organic load causing acetogenic bacteria to work more actively than methanogenic bacteria; When this occurs, inhibitors such as volatile fatty acids and hydrogen appear, decreasing biogas production [4]. To solve this problem, studies have proposed the use of anaerobic biodigesters using a two-stage production system, meaning, the separation of the acidification phase (acetogenesis stage) and the biogas formation stage (methanogenesis stage). This method has proven versatile since a better biogas

production performance can be achieved even with a mixture of substrates, with pH being the variable to control in each reactor in which acetogenic (pH 5) and methanogenic (pH 7) fermentation occurs.

It is a fact that large cities present great challenges such as environmental sustainability and the creation of strategies for the treatment of waste produced by anthropogenic activities [5]. Although, the combustion of fossil fuel as an energy source is a contributor to greenhouse gas emissions, untreated waste biomass also contributes to greenhouse gas emissions, in addition to soil and water pollution [6]. The Agenda for Sustainable Development proposes energy as an important component and support of economic growth [7]; Therefore, the use of organic waste (food waste, domestic sewage and biological sludge) can contribute to energy production and consequently to environmental sustainability.

In this context, the topic of study is that biogas production can vary in quantity and quality, depending on the raw material source (organic substrate); Since the diverse substrates (cow manure, food waste, agro-industrial waste, waste activated sludge, among others) used for biogas production have different chemical compositions [8–10]. Some of the control parameters considered to improve biogas production are: Water content, pH, nitrogen content, organic carbon content and C/N ratio. Studies suggest a minimum humidity requirement in the range of 81.9 % - 97.25% to carry out fermentation and a C/N rate of the raw material or mixture with substrates within the range of 20-30; meaning a lower C/N rate would cause the accumulation of ammonium, becoming toxic to bacteria [11]. Likewise, the mixture content of total solids will determine the efficiency of the process; the optimal value for continuous digesters is estimated to be 8-12% of total solids, while in discontinuous digesters it can be between 40-60%.

A relevant raw material for the production of biogas that is available and abundant is waste activated sludge obtained from wastewater treatment systems. According to Lee et al. [12] in the U.S., Many wastewater treatment systems spend between 40-50% of their budget for the treatment and disposal of waste activated sludge (WAS) and approximately 62 % of these WAS are treated in landfills or incinerated [13]. WAS management costs have increased due to rapid population growth and strict environmental regulations. In México, the generation of municipal wastewater for the years 2014 and 2016, indicated that each Mexican generated an average of 60 m³/year (~164 l/d) [14]. Therefore, the national volume of wastewater increased to 228.9 m³/s, concluding that water treatment at the national level is insufficient. The National Water Commission of Mexico (CONAGUA) estimates that worldwide between 80-95% of wastewater is discharged directly into rivers, lakes and oceans without receiving prior treatment. As a consequence of this, residual sludge remaining from the municipal wastewater is produced with considerable concentrations of organic matter, microorganisms, and other non-biodegradable toxic compounds.

However, these sludges with high concentrations of biodegradable organic matter could be used as a substrate in anaerobic digestion [15]. However, studies have considered that with these biowastes that have been previously processed by a biological system, the production of biogas is significantly lower than that of other substrates [16]. For this reason, the nutrient balance can be improved through the co-digestion of sludge with other organic substrates. The objective of this work is to evaluate the production of biogas using biological sludge from wastewater treatment systems with low and high removal efficiency, with a mixture of two co-substrates (cow manure and edible residual oil) in complete mixing reactors.

2. Materials and Methods

2.1. Substrate and Co-Substrate Preparation

The substrate used was residual biological sludge (SLB) obtained from two aerobic biological treatment plants from urban wastewater (PTAR) from Ciudad del Carmen and Campeche. This geographical location in the southeast of Mexico places the city within the Flora and Fauna Laguna de Terminos Protection area, an ecosystem that is a refuge for many species of flora and fauna and is threatened by oil companies, fisheries and urban development [17]. The treatment plants were selected according to the removal efficiency (50 and 90%) of the biochemical oxygen demand (DBO). Approximately 20 L of sludge (SLB) were collected from biological reactors and kept refrigerated at 4°C for at least 2 to 4 days until use and subsequent analysis.

To evaluate the effect of the characteristics of the two biological sludges on the biogas production, they were mixed with two Co-substrates: 1) cattle manure (CEV), collected (20 Kg) from the location's livestock region (18° 37' 47.30'' N, 91° 56' 36.12'' W) preserved at room temperature (20-25 °C) until later use and the co-substrate 2) residual edible oil (CAV) was collected (700 mL) from restaurants, previously filtered to eliminate the presence of solid particles in the oil (food waste) stored 4°C. The analysis of the composition of SLB and CEV consisted of previously centrifuging approximately 200 mL of biological sludge (SLB) and 20 g of bovine manure (CEV) at 2500 rpm for 15 minutes at 17° C. These samples were dried in an oven at 40±5 °C for 48 hours [18], later on they were ground and stored in airtight glass containers to avoid humidity in the samples. Both SLB and CEV samples were analyzed by determining pH using a Thermo Orion Star A211 instrument; while organic carbon (CO), organic matter (MO), total nitrogen (NT) and total phosphorus (PT) were analyzed according to the techniques described by the official Mexican standard [19]. Total solids (ST) and volatile solids (SV) were determined by standard gravimetric methods according to Mexican standard [20].

2.2. Experimental Design

The experimental design was proposed for a two-stage anaerobic fermentation by separating the acidification phase (hydrolysis/acetogenesis) and the biogas formation stage (methanogenesis). The biogas production process was carried out in batch mode in triplicate in reactors with 1.5 L operating volume consisting in adding the raw material (biological-sludge) to each reactor with mixture volume ratio (v/v) sludge: water (1:1). The treatments (Table 1) were in triplicate and consisted of the installation of a control reactor (SLB) containing only sludge-biological substrate, another two series of reactors in triplicate were installed with SLB plus the addition of used vegetable oil (CAV) and beef manure (CEV), respectively, with a proportion of 25% of total volume of the bioreactor, in such a way as to allow correct mixing within the biodigester (Table 1).

Table 1. Experimental design for biogas production on a laboratory scale.

Reactor	Clue	Treatment
Substrate (control)	SLB ₅₀	Biological sludge (Efficiency 50%)
Substrate (control)	SLB ₉₀	Biological sludge (Efficiency 90%)
Co-substrate 1	CEV ₅₀	SLB ₅₀ + cattle manure (CEV)
Co-substrate 1	CEV ₉₀	SLB ₉₀ +cattle manure (CEV)
Co-substrate 2	CAV ₅₀	SLB ₅₀ +recovered vegetable oil (CAV)
Co-substrate 2	CAV ₉₀	SLB ₉₀ + recovered vegetable oil (CAV)

Prior to the acetogenesis and methanogenesis processes, the raw material was fermented for 15 days at pH 7.0 regulated by the addition of Na₂CO₃ (10%) buffer. Subsequently, the first stage (acetogenic process) consisted of adjusting the pH of approximately 5.3-5.7 by adding solution of H₂SO₄ at 20% to fermented effluent at room temperature (26°C), which promotes the conversion of organic matter into short-chain organic acids (acetic acid, propionic acid and butyric acid), which are intermediate products of the anaerobic degradation of organic matter and are essential for the next stage of the process.

After a period of 14 days, the fermented effluent was neutralized to pH 7.0-7.5 with sodium carbonate solution (Na₂CO₃ al 10%), stimulating the methanogenic stage (second stage), where organic acids produced in the first stage (hydrolysis-acidogenesis) are transformed into biogas, mainly methane and carbon dioxide, by methanogenic bacteria. During the two-stage co-digestion process, different microorganisms are involved and pH is an important variable because acidogenic and methanogenic bacteria are highly sensitive to changes in acidity or alkalinity of the medium [21]. Thus, the system was monitored with pH control and agitation (approximately120 rpm) to maintain the optimal conditions of each stage. For the bio-digestion process of the matter, some factors were considered (Table 2), which allowed maintaining the ideal conditions for the production of biogas.

Table 2. Factors considered for methane production (cm³ CH₄ min⁻¹).

Control factors	Units
pH	6-8
Mixture (sludge: water)	1:1
Co-substrate	25%
Agitation	120 rpm
Hydraulic holding time	30 days
Initial C/N	~20
Temperature	20-30 °C

The reactors used were type *Batch Corning*® brand polycarbonate with a total volume of 3000 ml (1500 ml operating volume) with magnetic stirring. For each reactor, a pH electrode was incorporated that allowed daily monitoring of the acidity or alkalinity of the fermented medium. Likewise, the reactor maintained a direct connection to the methane flow sensor using *Gas Flow Meter* (Cole-Parmer, serie: 32908-51) (Figure 1).

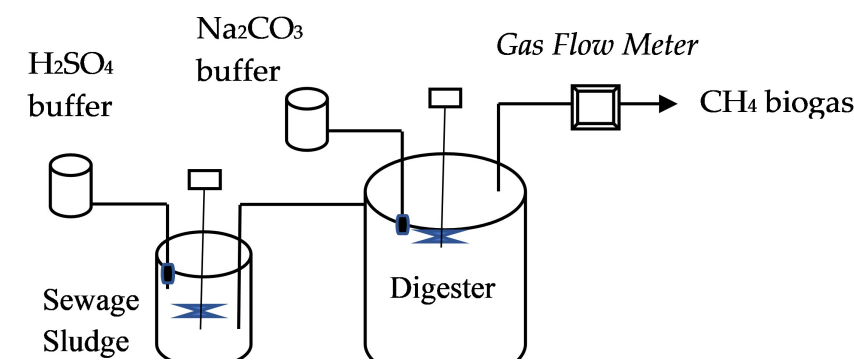


Figure 1. Schematic diagram for the treatment of sewage sludge and biogas production in batch reactor.

2.3. Statistical Analyses

For statistical comparisons, the methane production rate for the different mixtures of raw materials was analyzed by analysis of variance (ANOVA) using Statistic software (StatSoft Inc., Tulsa, OK, USA). The Tukey test ($P \leq 0.05$) was applied when results showed significant differences.

3. Results

Effect of Sewage Sludge and Co-Substrate

Anaerobic digestion is a widely used technology for the degradation and stabilization of organic matter. Under these conditions, the organic matter is oxidized and transformed into biogas. The metabolic process involves various reaction mechanisms where anaerobic bacteria intervene in the transformation through sequenced biological reactions. However, during the transformation stages, various inhibitors can intervene and affect the production of biogas and although they have been studied in bioreactors for better control, the manipulation of the variables aims to improve the transformation of organic matter and consequently the production of biogas, but this depends in most

cases on the type of substrate, inoculum size, period of adaptability of the bacteria and the balance of nutrients.

The quantity and quality of biogas that can be produced will depend on the characteristics of the raw materials. One of the raw materials of greatest interest is the use of biological sludge produced from biological wastewater treatment plants. This waste is a promising material to be used for the production of biogas. It is of outmost importance within the circular economy objectives; however, it is not possible to generalize its use because one of the factors to consider is the origin and quality of the biological waste sludge. In the present study, two qualities of biological sludge (SLB₅₀ y SLB₉₀) were considered based on the operating efficiency of biological wastewater treatment plants. The result show that a low quality sludge (SLB₅₀) can present a higher ST ($44.91 \pm 0.056 \text{ g L}^{-1}$) compared to a high quality sludge (SLB₉₀) ($35.58 \pm 0.055 \text{ g L}^{-1}$); while the SV content tends to be inversely proportional to the ST content, which was observed with a higher SV content in SLB₉₀ ($24.24 \pm 0.339 \text{ g L}^{-1}$) compared to SLB₅₀ ($11.56 \pm 0.346 \text{ g L}^{-1}$) (Table 1), suggesting that treatment plants that function efficiently carry out efficient digestion of organic matter, providing a remaining substrate and bacteria with better adaptability (biological sludge) that could contribute to anaerobic digestion. The above was possible to confirm by the high content (%) of M.O and C.O in biological sludge (SLB₉₀), which proposes greater bioavailability for the diversity of biogas-producing bacteria. Therefore, good quality biological sludge could be used as raw material and bacterial inoculum to achieve efficient biogas production in biodigesters (Table 1).

Table 1. Composition ($\pm$ standard deviation) of the two types of sewage sludge (SLB₉₀ and SLB₅₀) from wastewater treatment plant and cattle manure (CEV).

Parameter	Units	SLB ₉₀	SLB ₅₀	CEV
pH	-	7.04 $\pm$ 0.084	7.30 $\pm$ 0.014	7.4 $\pm$ 0.091
ST	g L ⁻¹	35.58 $\pm$ 0.056	44.91 $\pm$ 0.056	156 $\pm$ 0.02
SV	g L ⁻¹	24.24 $\pm$ 0.339	11.56 $\pm$ 0.346	32.5 $\pm$ 0.02
C.O	%	18.82 $\pm$ 0.542	8.22 $\pm$ 0.433	18.99 $\pm$ 0.051
M.O	%	32.46 $\pm$ 0.362	14.17 $\pm$ 0.544	32.74 $\pm$ 0.089
N.T	%	20.11 $\pm$ 0.829	11.46 $\pm$ 1.965	1.11 $\pm$ 0.0003
P.T	g kg ⁻¹	1.76 $\pm$ 0.016	1.74 $\pm$ 0.001	10.97 $\pm$ 0.001
C/N	-	3.080	0.558	17.01

To evaluate the effect of co-substrates for improving the balance of organic matter and nutrients in biodigesters and methane production; the present study used two type of waste: Cow manure and Edible residual oil. In fact, unlike residual oil, cattle manure contributes a high content of ST and SV of $+156 \pm 0.02 \text{ g L}^{-1}$ and $32.5 \pm 0.02 \text{ g L}^{-1}$, respectively (Table 1); therefore, the mixture with the co-substrate bovine manure (CEV) increases the content of M.O and C.O in CEV₉₀, CEV₅₀ reactors (Table 2).

Table 2. Composition ($\pm$ standard deviation) of the two types of sewage sludge from wastewater treatment plant with a mixture of co-substrates (cow manure and residual edible oil).

Parameter	Units	CEV ₉₀	CEV ₅₀	CAV ₉₀	CAV ₅₀
pH	-	7.04 $\pm$ 0.084	7.30 $\pm$ 0.014	7.4 $\pm$ 0.091	7.4 $\pm$ 0.091
ST	g L ⁻¹	27.85 $\pm$ 0.026	26.46 $\pm$ 0.056	22.87 $\pm$ 0.02	56.21 $\pm$ 0.02
SV	g L ⁻¹	11.56 $\pm$ 0.029	23.15 $\pm$ 0.034	21.81 $\pm$ 0.02	45.16 $\pm$ 0.02

C.O	%	23.39± 0.054	17.56± 0.423	18.55± 0.051	12.46± 0.051
M.O	%	40.34± 0.32	30.28± 0.544	31.99± 0.089	21.48± 0.089
N.T	%	2.52± 0.082	18.12± 0.465	4.74± 0.06	21.48± 0.06
C/N	-	23.16	6.26	6.16	0.58

This significant improvement in organic matter content, organic carbon and nitrogen resulted in the C/N ratio increasing from 3.080 obtained in SLB₉₀ sludge to 23.16 in CEV₉₀ reactors and for SLB₅₀ sludge from 0.558 to 6.26 in CEV₅₀ reactors (Table 2), while, the mixture of biological sludge with residual oil did not observe a significant increase due to the particular chemical properties of the oil. According to what is reported in the literature the C/N rate of the CEV₉₀ mixture suggests a desirable range for anaerobic digestion process, with a recommended C/N rate of 20 -30 [22,23]. In the present study, the control of this variable limited the accumulation of ammonium in the reactors, which can be a toxic compound for bacteria.

According to Ellacuriaga et al. [21] excessive nitrogen levels within digesters can cause inhibition of methanogenesis; Therefore, it is recommended a range of 2.7 -3.1 g L⁻¹ of nitrogen, becoming tolerant for previously adapted bacteria, while concentrations greater 4.0 g L⁻¹ cause an inhibitory effect on methane production. This was confirmed in the present study for the CEV₉₀ reactor showing low nitrogen content and indicating a potential use for methane production. Various studies have evaluated the effect of ammonium-nitrogen in anaerobic digestion systems and determined the high complexity between ammonium concentration, pH, temperature and acclimatization of the microflora [24–26]; For this reason, in the present study each of the variables in the two-stage anaerobic digestion system were controlled and the biogas production could be evaluated.

• *Biogas production*

In the present study, the biosludge: co-substrate mixture was considered to evaluate the effect of biosludge quality on methane production. The average methane production (cm³ d⁻¹) for only good quality sludge (SLB₉₀) was higher (303.99cm³ CH₄ d⁻¹) compared to a low quality sludge (SLB₅₀) of 4.33 cm³ CH₄ d⁻¹ (Table 3). An advantage of using SLB₉₀ is being able to reduce the lag phase or adaptation by approximately 2 days, compared to low quality SLB₅₀ sludge which showed a prolonged lag phase of 25 days (Table 3), suggesting that for anaerobic digestion purposes and methane production, it is proposed that sludge from treatment plants operate with high treatment efficiency since this could guarantee bioavailable M.O, C.O contents and bacteria with greater adaptability in the biodigesters.

Table 3. Daily methane production (cm³ d⁻¹) using biological waste sludge (SLB) with a mixture of cattle waste (CEV) and residual edible oil (CAV).

Day	SLB ₉₀	CAV ₉₀	CEV ₉₀	SLB ₅₀	CAV ₅₀	CEV ₅₀
1	9.10		66.96			26.94
2	13.94		223.72			26.87
3	65.52		239.90			175.42
4	186.01		375.6			534.33
5	177.22		701.76			589.48
6	307	141.02	971.66			566.08
7	455.58	574.89	925.34			571.01
8	466.47	865.53	838.56			547.66
9	409.05	945.45	820.22			481.64
10	360.55	1266.76	870.57			420.98
11	350.26	969.79	1097.08		88.01	390.24
12	365.31	1305.41	1497.52		155.81	396.11
13	344.65	1410.62	1670.73		174.07	399.07

14	390.62	621.79	1909.77		175.55	343.15
15	413.67	619.52	2232.19		172.69	317.50
16	435.88	599.02	2325.74		165.21	321.53
17	428.84	523.44	2425.05		173.94	326.26
18	388.91	636.09	2255.23		214.02	364.84
19	342	498.14	2066.54		250.32	348.62
20	379.19	569.66	2097.6		219.91	333.18
21	384.21	540.48	1944.67		196.62	362.03
22	315.9	726.28	1873.15		187.69	403.05
23	296.19	929.47	1609.2		198.19	430.84
24	234.04	784.51	1742.88		223.74	463.14
25	88.07	782.4	1836.52	1.062	222.53	498.18
26	0.74	865.63	1853.66	2.843	235.04	569.71
27	-	742.56	1747.72	4.549	269.82	626.36
28	-	893.47	1427.95	5.628	283.32	654.67
29	-	881.76	1416.91	6.229	291.48	699.17
30	-	489.50	1358.4	5.68	330.54	693.39
ΣCH ₄	7599.82 ^a	19183.19 ^b	42422.8 ^c	25.99 ^a	4228.5 ^a	12881.45 ^{ab}
Media	303.99	767.32	1414.09	4.33	211.42	429.38
SD	149.39	344.37	676.62	2.00	55.53	167.04

*different letters mean significant differences (Tukey P≤0.05); standard deviation (SD).

In the present study, the highest accumulated methane production was obtained for the anaerobic digester (CEV₉₀) with a mixture of biological sludge and cattle manure of 42 422.8 cm³ d⁻¹; Compared to CEV₅₀ digesters with a lower production of 12 881.45 cm³ d⁻¹. On the other hand, the co-substrate residual edible oil (CAV), which has been present in significant quantities in the treatment plants, was evaluated in the anaerobic digesters, showing that the mixture (CAV₉₀) showed a greater production of methane (767.32 cm³ d⁻¹) with 5 days of adaptation, compared to the CAV₅₀ digesters of 211.42 cm³ d⁻¹ of methane produced and 11 days of adaptation. The results suggest that the residual edible oil present in treatment plants and biological sludge could provide a source of energy for bacteria, which can be observed in the increase in methane production unlike cultures only with sludge (SLB₅₀ and SLB₉₀) (Table 3).

The CEV₉₀ treatment was the mixture with the highest methane production and was proposed to improve biogas production given that both sludge and bovine manure provide nutrients and carbon sources, which establishes a favorable (C/N) balance for methane production, coupled with that both substrates provide diversity of bacteria with high adaptability; Therefore, this treatment can be considered as part of the Circular economy to convert environmental damage into social and economic benefits for the use of waste biomass as a renewable energy source [21].

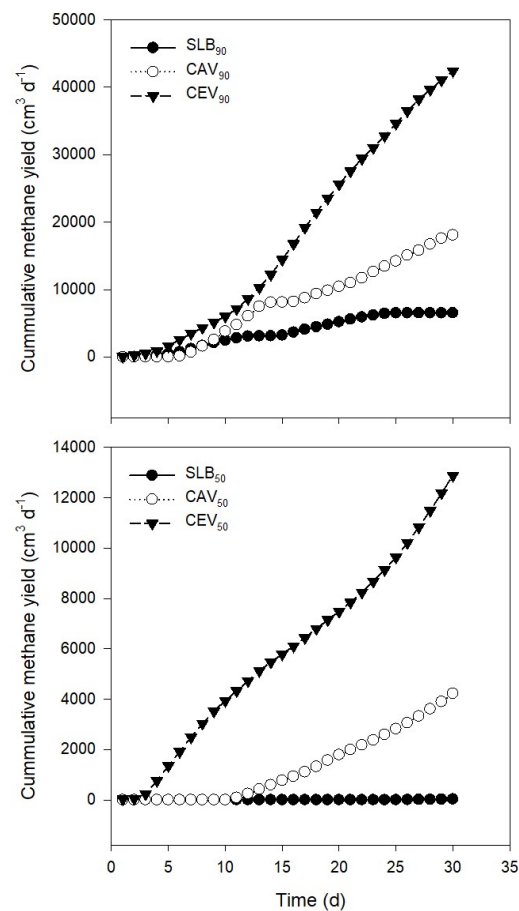


Figure 1. Cumulative methane yields for batch reactors with only waste activated sludge (SLB) and different mixing substrate (CAV and CEV).

The production of methane obtained in the present analysis in the different mixtures of substrate with waste activated sludge showed an improvement in the production of methane, being higher in the digesters with a mixture of cow manure. The methane contents were higher than those reported by other studies (Table 4) in which municipal solid waste, vegetable waste, and food waste were used and with a mixture of cow manure and cattle slurry. This proposes sludge for two reasons; be a source of organic carbon and, on the other hand, provides a micro-flora with a high acclimatization capacity. Studies such as those reported by Díaz et al. [4] suggest that the use of activated sludge is possible to obtain an improvement in methane production of $63.6 \pm 1.1\%$ v/v. Similarly, Lee et al. [12] reported a methane production of $191.0 \pm 8 \text{ ml CH}_4 \text{ g}^{-1} \text{ SV}$, similar to that reported by Cabbai et al. [27] de $248.7 \pm 4.13 \text{ ml CH}_4 \text{ g}^{-1} \text{ VS}$. However, in the present study, unlike those reported by other authors, it can also be concluded that biological sludge from treatment plants with low operating efficiency does not significantly favor methane production in anaerobic biodigesters ((Figure 1).

Table 4. Methane production reported in the literature under different predominant substrate and inoculum.

Digestion	SLB ₉₀	CAV ₉₀	CEV ₉₀	[28]	[29]	[30]	[5]
Predominant substrate	Waste activated sludge	waste edible oil	Waste activated sludge	Municipal solid waste	Vegetable waste	Food waste	Food waste

Inoculum	-	Waste activated sludge	Cow manure	Cow manure	Cow manure	Cattle slurry	Bovine manure
Biogas yield (m ³ d ⁻¹)	7.59x10 ⁻³	19.18x10 ⁻³	42.42 x10 ⁻³	9.3x10 ⁻³ 56.95x10 ⁻³	0.005 x 10 ⁻³	3.16	11.83 x 10 ⁻³

4. Conclusions

The anaerobic co-digestion of a wide variety of waste has taken on great relevance in energy production issues and various control variables have been studied, in order to avoid the inhibition of methanogenic processes and optimize the obtaining of methane under solid conditions of production. The quantity and quality of methane that can be produced will depend on the characteristics of the raw material. One of the most interesting and abundant raw materials is the biological sludge produced by wastewater treatment processes, which have been proposed for the biogas production and included in the Circular economy objective; However, its use depends on the origin and quality level of the waste sludge. The contribution of carbon sources and micro-flora with a high capacity for adaptability are some of the advantages of sludge, coupled with the fact that they can be mixed with co-substrates, improving the C/N ratio by around 20-30, desirable for the anaerobic process digestion.

Good quality sludge (SLB₉₀) can carry out methanogenesis with a reduced adaptation phase of 2 days, compared to low quality sludge (SLB₅₀) with methane production after 25 days. Therefore, SLB₉₀ sludge shows a higher production (303.99cm³ CH₄ d⁻¹) compared to SLB₅₀ sludge (4.33 cm³ CH₄ d⁻¹). Methane production increases with the sludge cow manure mixture to levels of 42 422.8 cm³ CH₄ d⁻¹ and 12 881.45 cm³ CH₄ d⁻¹ for CEV₉₀ and CEV₅₀, treatments respectively; As with co-substrate residual edible oil of 767.32 cm³ d⁻¹ and 211.42 cm³ d⁻¹ for CAV₉₀ and CAV₅₀, respectively. The best mixture in the present study was waste sludge cow manure contributing both nutrients and carbon sources, with a balance of nutrients favorable for methane production, coupled with the contribution of microflora diversity with high adaptability. However, it can be concluded that biological sludge from treatment plants with low operating efficiency does not significantly favor methane production in anaerobic biodigesters.

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Data Availability Statement: The concentration of PAHs and granulometry data used to support the findings of this study are included within the article and could be made available from the corresponding author upon request.

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