Optimization of Biogas Production from Sewage Sludge: Impact of Combination with Bovine Dung and Leachate from Municipal Organic Waste

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Abstract: Biogas is a bioenergy produced from organic or all types of biological degradable wastes and could make it possible to limit energy dependence. Sludge is the best alternative substrate for biogas production at a community-level biogas plant. The literature shows that co-digestion can increase the efficiency of sludge anaerobic digestion. This research, thus, focused on (i) determining the conditions of optimal biogas production in the co-digestion of primary sludge (PS) and bovine dung (BD), (ii) evaluating the impact of leachate from organic waste and cellulose on biogas production. Primary sludge was collected in Bacau town wastewater treatment plant in Romania. The sampling of municipal solid waste was carried out in Ouagadougou pre-collect centers (Burkina Faso). Batch tests were conducted in glass bottles through anaerobic digestion (1 L). The following parameters were monitored during the digestion process: pH, volatile fatty acid (VFA), volatile solids (VS) and biogas production. Primary sludge, bovine dung and leachate showed 50.51%, 72.41% and 70.48% of volatile solids content, respectively. Sludge showed good stability, unlike the other two substrates, such as bovine dung and leachate, with VFA to alkalinity ratio 0.54. Leachate from organic waste had high values of VFA to alkalinity ratio > 3600. Co-digestion could make it possible to raise the levels of organic matter and improve microbial growth and the stability of anaerobic biomass. The best biogas production yield of 152.43 mL/g VS was obtained with a combination of 30% bovine dung and 70% primary sludge at 45 °C, with a 21.57% reduction in organic matter. An improvement in biogas productivity was effective with the addition of leachate, which could be used as an additive element during anaerobic digestion.

Keywords: anaerobic digestion; co-digestion; sludge; bovine dung; municipal organic waste; bioenergy

1. Introduction

According to the World Water Assessment Programme (WWAP) [1] and United Nations Water [2], more than 80% of wastewater worldwide (over 95% in some developing countries) is released into the environment without any treatment. Therefore, the issue of wastewater management is highlighted in the 2030 Agenda for Sustainable Development, through Sustainable Development Goal (SDG) 6, on water and sanitation, and more
specifically, Target 6.3, to halve the proportion of untreated wastewater and significantly increase safe recycling and worldwide water reuse [3]. This increasing interest in wastewater treatment will certainly generate additional quantities of sludge that must be managed. According to Canler and Perret (2013) [4], three types of sewage sludge could be produced: primary sludge (PS) obtained by gravitational sedimentation, secondary sludge (SS) from biological treatment, which is possible thanks to purifying microorganisms in the environment, and tertiary sludge, most often the result of physico-chemical treatment after biological treatment; mixed sludge corresponds to the mixture of primary and secondary sludge, see tertiary. The annual sewage sludge production has been estimated to be 10 million tons (dry matter), 20 million tons and 49 trillion liters in Europe, China and the United States, respectively [5,6] and is expected to increase to 13 million by 2010 [7].

In Burkina Faso, specifically in Ouagadougou, sludge production was estimated at 91,126 m³ per year, corresponding to 250 m³ per day [8]. Several techniques are used for sludge management: landfill, agricultural use, incineration, biological treatment, such as composting, and anaerobic digestion, etc. [9,10]. Landfill and agricultural use are increasingly constrained; they are a weak technology and legally prohibited in many countries [11]. Sludge incineration is prohibitively expensive and poses a risk related to the impact of toxic gases, such as dioxin, on the environment [12]. Composting is a green technology that transforms sludge into an organic amendment, thus, contributing to the reintegration of mineral and organic elements in soils [9,13]. From these techniques, anaerobic digestion allows energy recovery through biogas production that can be used as source of heat and electricity [9,14]. Among the problems related to energy, there is an imbalance between areas, the use of energy as a political weapon, environmental risks, the political and military consequences of nuclear programs, the risk of energy dependencies with their implications [15]. The war in Ukraine has exacerbated the crisis and allowed countries around the world to rethink the need for energy independence, hence the need to find local means within this framework. Biogas, recognized by the European directive 2001/77/EC, known as the “Energy Directive”, as a source of new renewable energy could achieve the objective of increasing the share of renewable energies in the mix, which could help solve the energy crisis for a while [16]. Many works were realized to optimize this system. Hao et al. [17] concluded, upon research of alternative sludge disposal methods, that anaerobic digestion was not the solution for excess sludge and that direct incineration without anaerobic digestion had the lowest energy deficit and investment and operation costs. These authors recommended more efficient dewatering instead of anaerobic digestion. For the better recovery of sludge, it is necessary to exploit the possibilities of biogas production and digestate use to boost agricultural productivity. Hao et al. [17] reported anaerobic digestion from thermo-treated sludge, ultrasonic-treated sludge and the combined-treated sludge obtained promotion of 30.62%, 32.80% and 36.98% in methane yield compared with raw sludge. Work investigated by Tian et al. [18] on the effects of physico-chemical parameters, such as ultrasonic, ultrasonic–ozone and ultrasonic + alkaline post-treatments, on the anaerobic digestion of sewage found that sludge improved the biogas yield (277 mL CH₄/g VS). Odirile et al. [19] worked on the anaerobic digestion of fine mesh, sieved primary sludge and sedimented primary sludge, with, respectively, 83% and 78.77% of volatile solids (VS). The cumulative biogas production over the 30 days of anaerobic digestion was in the same vein (442.29 mlbiogas/gvs for sedimented primary sludge versus 434.73 mL biogas/gvs for fine-mesh-sieved primary sludge). For sludge with low organic matter content, the co-digestion system could make it possible to avoid costly pre-treatments and boost biomethane productivity. Lacour [20] showed co-digestion has synergistic effects with biomethanogenic potentials of mono-digestion co-substrates. In addition, several studies showed co-digestion of bagasse and animal dung, such as pig slurry, bovine dung and chicken droppings, improved biomethane production [9–11]. For example, Zhai et al. [21] demonstrated that the co-digestion of manure and food waste obtained high methane production. Yoon et al. [22] and Li et al. [23] reported that the co-digestion of organic fraction of municipal solid waste (OFMSW) and fraction vegetable waste (FVW) with
animal manure represents an option for controlling the anaerobic digestion stability process and maximizing biogas production. Therefore, the aim of this study is to optimize biogas production through the co-digestion of primary sludge, bovine dung and leachate from municipal solid waste. Specifically, this research, thus, focused on (i) determining the conditions of optimal biogas production in the co-digestion of primary sludge (PS) and bovine dung (BD), (ii) evaluating the impact of leachate from organic waste and cellulose on biogas production.

2. Materials and Methods

2.1. Sludge and Bovine Dung Sampling

The primary sludge was collected in Bacau town wastewater treatment plant in Romania (46.536824° N, 26.937782° W) and placed in a five L bottle. The bovine dung slurry was collected in five bags from a farm in Bacau town (46.550209° N, 26.965780° W) in Romania (Figure 1). The samples collected were both kept at 4 °C until usage.

![Figure 1. Localization of wastewater and bovine dung sampling site.](image)

2.2. Organic Waste Sampling

The municipal organic solid waste was collected from three pre-collect centers in Ouagadougou (Burkina Faso). The geographic coordinates of the sampling sites are 12°22′ N, 1°31′ W; 12°19′ N, 1°31′ W; and 12°23′, 1°32′ W respectively. Pre-collected waste was mixed, sorted and dried (Figure 2), then the composition of the organic fraction was determined. The organic fraction waste samples were crushed and sieved in order to obtain particles with a size of ≤1 mm.

![Figure 2. Types of municipal waste sampled in pre-collection centers: (a) waste bin; (b) unsorted waste; (c) sorted and dried waste.](image)

2.3. Feedstocks Characteristics

pH, Salinity, Total Dissolved Solids (TDS) (conductivity X, resistivity and Total Solids (TS)) were measured using a WTW digital multiparameter system (inoLab Multi 9420 IDS,
Darmstadt, Germany). The total solid (TS), volatile solid (VS) and ash were estimated according to the standard procedures described by APHA [24]. Biochemical oxygen demand for five days (BOD5) was performed using an OxiTop® Box incubator WTW. Total Organic Carbon (TOC) was determined from the theoretical VS (solid volatile)/TOC ratio (generally estimated at 1.74) [25] using the following Equation (1):

\[
\text{TOC(\%)} = \left( \frac{\text{VS(\%)} \cdot 1.74}{100} \right)
\]

(1)

2.4. Experimental Setup and Design

The experiment was carried out in Department of Environmental Engineering and Mechanical Engineering at Vasile Alecsandri University of Bacau. The experimental design (Figure 3) was made of 4 series of glass bottles (1 L) used as batch anaerobic digesters. Each bottle was filled with 500 mL of a mixture of primary sludge and bovine slurry (at different proportions) supplemented with 20 mL of mineral solution and incubated at 30 or 45 °C (Table 1). Incubation time was 30 days. Figure 3 presents five points: (1) bottle containing 2% NaOH solution, (2) water outflow, (3) biogas input, (4) becher for water recovery, (5) digester.

![Figure 3.](image)

**Figure 3.** (a) Classic liquid displacement system configuration. (b) Biogas quantification during experimentation. Adapted from: Esposito et al. [26].

**Table 1.** Experimental design matrix.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Combination</th>
<th>Temperature</th>
<th>MS (mL)</th>
<th>Bovine Dung (mL)</th>
<th>Primary Sludge (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>10% BD + 90% PS</td>
<td>30 °C</td>
<td>20</td>
<td>50</td>
<td>450</td>
</tr>
<tr>
<td>R2</td>
<td>10% BD + 90% PS</td>
<td>45 °C</td>
<td>20</td>
<td>50</td>
<td>450</td>
</tr>
<tr>
<td>R3</td>
<td>30% BD+ 70% PS</td>
<td>45 °C</td>
<td>20</td>
<td>150</td>
<td>350</td>
</tr>
<tr>
<td>R4</td>
<td>30% BD+ 70% PS</td>
<td>45 °C</td>
<td>20</td>
<td>150</td>
<td>350</td>
</tr>
</tbody>
</table>

MS: Mineral solution, PS: Primary Sludge, BD: bovine dung.

Table 1 showed experimental design matrix. These designs present 04 digesters R1, R2, R3 and R4. R1 and R2 have the same combination of Bouse Bovine 10% and Primary Sludge 90% with different incubation temperature (30 and 45 °C). R3 and R4 combination was Bouse Bovine Dung 30% and Primary Sludge 70%.

2.4.1. Monitoring of Physico-Chemical Parameters during Anaerobic Digestion

pH, volatile fatty acid (VFA), organic matter (OM) and biogas production were monitored during the digestion process. pH, VFA and OM were estimated according to the procedures in the standard methods for examination of water and wastewater [27]. VFA was measured titrimetrically for mainly acetate VFA: The sample was collected from the reactor and the pH was determined. To a 20.0 mL sample (E), 0.1 M H2SO4 (C2) was added to adjust the pH to 4.0 (V1). The sample was heated on a hot plate (100 °C) for 3 min, cooled,
and 0.1 M NaOH was added until the pH was 7.0 \((V_2)\). The volumes of \(H_2SO_4\) \((V_1)\) and \(NaOH\) \((V_2)\) used in the process were recorded. The total VFA (volatile fatty acid) content was calculated as mg acetic acid \(L^{-1}\) according to the following Equation (2):

\[
VFA = 6.10^4 \cdot \left( \frac{C_2\cdot V_2}{E} \right) \text{ (mg acetic acid} \ L^{-1})
\]  

(2)

Biogas production was measured at a fixed time each 5 days by water displacement method (Figure 3) according to procedures described by APHA [27].

2.4.2. Effect of Organic Waste Leachate and Cellulose on Biogas Production by a Mixture of Wastewater and Bovine Dung

Batch reactors were prepared in 1 L borosilicate glass bottle. The experiment was carried out in two reactors R1-C and R1-L for microcrystalline cellulose and organic waste leachate respectively, with the aim of using leachate as an additive during anaerobic digestion for ameliorate biogas production. A mixture of 30% BD and 70% PS were used in each reactor and the biogas production was monitored until the substrate was depleted. To compare the susceptibility to biomethane production, it was necessary to let substrate run out during anaerobic digestion in two reactors, before adding a new substrate (cellulose or organic waste leachate). The quantity of cellulose and organic waste leachate added to R1-C and R1-L reactors were 100 mL. Reactors were finally incubated at 45 \(^\circ\)C. Microcrystalline cellulose was a good control, with interest yield 371 mL \(CH_4/g \) VS [28].

2.4.3. Statistical Analysis

XLSAT software was used for statistical analysis. The amounts of biogas generated were compared using analysis of variance (ANOVA) at 5% threshold.

3. Results and Discussion

3.1. Physico-Chemical Characteristics of the Feedstocks

The characteristics of the substrates used for biogas production are presented in Table 2. Primary sludge, as well as bovine dung, exhibited neutral pH, suitable for optimum biogas production. The slightly acidic pH (5.17) of the leachate, comparatively to primary sludge (7.25) and Boving dung (7.21), could be related to microbial fermentation during its extraction. Total dissolved solid (TDS), Conductivity (X) and Resistivity (e) can indicate the presence of dissolved organic matter, major mineral compounds and traces of heavy metals. These micronutrients are indispensable for anaerobic digestion [29]. Bovine dung had TDS 6.82 g \(L^{-1}\), conductivity 6.38 mS\(cm^{-1}\) and resistivity 146.70 \(\Omega\)\(cm\). Leachate from organic waste had a TDS of 7.29 g \(L^{-1}\), conductivity 7.29 mS\(cm^{-1}\) and resistivity 137.1 \(\Omega\)\(cm\). Primary sludge presented TDS 9.23 g \(L^{-1}\), conductivity 9.22 mS\(cm^{-1}\) and resistivity 108.30 \(\Omega\)\(cm\). During the anaerobic digestion of animal dung, Zeng et al. [30] found 6.80 g \(L^{-1}\) and 13.61 mS\(cm^{-1}\) for TDS and conductivity, respectively. Graterol [31] found 2.58 to 4.28 mS\(cm^{-1}\) and 16.13 to 26.75 g \(L^{-1}\), respectively for conductivity and TDS during industrial wastewater anaerobic digestion. TDS, conductivity and resistivity values showed the presence of micronutrients in these substrates. According to Suarez et al. [32], the presence of micronutrients is essential for biogas production.
Table 2. Physical-chemical characteristics.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary Sludge</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>7.25</td>
</tr>
<tr>
<td>Sal</td>
<td>g/L</td>
<td>5.2</td>
</tr>
<tr>
<td>TDS</td>
<td>g/L</td>
<td>9.23</td>
</tr>
<tr>
<td>e</td>
<td>mS/cm</td>
<td>9.22</td>
</tr>
<tr>
<td>VFA</td>
<td>mg(acetic acid)/L</td>
<td>1120</td>
</tr>
<tr>
<td>VFA/TAC</td>
<td>mg(CaCO₃)/L</td>
<td>2058.33</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>23.32</td>
</tr>
<tr>
<td>SV</td>
<td>%</td>
<td>50.51</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
<td>49.49</td>
</tr>
<tr>
<td>TOC</td>
<td>%</td>
<td>29.03</td>
</tr>
<tr>
<td>BOD₅</td>
<td>mg/L</td>
<td>-</td>
</tr>
</tbody>
</table>

Sal = Salinity; TSD = Total dissolved solid; X = Conductivity; e = Resistivity; VFA = Volatile Fatty Acids; TAC = Title Alkalinity; VS = Volatile Solid; TOC = Total Organic Carbon; BOD₅ = Biochemical Oxygen Demand in five days.

Primary sludge presented a high value of salinity (5.2 g·L⁻¹) compared to bovine dung (3.8 g·L⁻¹) and leachate from organic waste (4 g·L⁻¹). Moletta [33] conducted tests on the anaerobic digestion of marine microalgae, obtained the highest yield of CH₄ with 15 g·L⁻¹ of salinity. The salinity is estimated at 4.90 g·L⁻¹, which is suitable for anaerobic digestion; increased salinity is involved in decreasing CH₄ yield. Chen et al. [34] showed that a salinity of 15 g·L⁻¹ corresponds to a concentration of 4.6 g·L⁻¹ NaCl. According to Soto et al. [35], sodium is more toxic than propionic acid and acetic acid, resulting from the use of volatile fatty acids during bacteria metabolism.

The substrates used had high levels of TSV 50.51%, 72.41% and 70.48% for primary sludge, bovine dung and leachate, respectively. This shows the bioavailability of organic matter for microorganisms involved in anaerobic digestion. Hack et al. [36] found a TSV of 74.2% from language lodge. The production of primary sludge comes from particular functioning of the water sector, so characteristics are functions of city or country. High levels of volatile fatty acids (VFAs), suitable for biogas production, were monitored from the primary sludge (1120 mg·L⁻¹ acetic acid) and leachate (3600 mg acetic acid·L⁻¹). According to Tampio et al. [37], VFAs are intermediates in the methane formation pathway of anaerobic digestion and they can be produced in similar reactors as biogas to increase the productivity of a digestion plant, as VFAs have more varying end uses compared to biogas and methane. The VFA to alkalinity ratio was 0.54, 2.96 and >3600 for primary sludge, bovine dung and leachate from organic waste, respectively. The VFA to alkalinity ratio in the digesters was compared as an indicator of digester stability. Ward et al. [38] reported that a digester VFA to alkalinity ratio of above 0.5 indicates that a digester is unstable. Sludge showed good stability, unlike the other two substrates (bovine dung and leachate). Leachate from organic waste has high values of VFA to alkalinity ratio; this could be explained by the presence of organic acids in large quantities due to the fermentation process. Therefore, the leachate could be a booster for anaerobic microbial performance. Co-digestion could make it possible to raise the levels of organic matter and improve the microbial growth and stability of the anaerobic biomass.

The BOD₅ of the leachate was 2025 mg·L⁻¹, indicating a high content of dissolved organic matter. Indeed, during the first phases of degradation, volatile fatty acids are predominant and can be up to 95% of the organic load of leachates [39], with BOD₅ ranging from 5000 to 68,000 mg·L⁻¹ [30]. In the methanogenesis phase, acids were assimilated over time, resulting in a drop in BOD₅, ranging from 0.5 to 1770 mg·L⁻¹, according to Kjeldsen et al. [40]. The BOD₅ value for leachate could be explained by a long pre-fermentation time (10 days in pretreatment process). Leachate has characteristics compara-
ble to those used in anaerobic digestion, but a decrease in fermentation time was required to increase organic load and maintain a high number of micronutrients.

3.2. Evolution of Anaerobic Digestion Parameters

3.2.1. pH and Volatile Fatty Acid

Figure 4a shows the pH evolution of different reactors as a function of time. In reactor R1, the pH rose from 7 to about 7.4, and remained stable around that value. The temperature increase in reactor R2 at 45 °C led to a rise in pH, up to 8. This could be explained by the higher degradation of alkaline compounds (urea, proteins) with temperature [41,42]. An increase in the bovine dung proportion resulted in acidification of the medium in the R3 reactor, which led to pH reduction of 7.8 to around 7.2. Under these conditions, the temperature increase in reactor R4 caused a sharp drop at pH 7.8 to about 7.4.

![Figure 4a](image1.png)  
![Figure 4b](image2.png)

**Figure 4.** Evolution of pH (a) and VFA (b) during anaerobic digestion: R1: 10% BD + 90% PS at 30 °C; R2: 10% BD + 90% PS at 45 °C; R3: 30% BD + 70% PS at 30 °C; R4: 30% BD + 70% PS at 45 °C.

Volatile fatty acids (VFA) evolution, presented in Figure 4b, shows that VFA production stabilized in reactor R1 at about 2000 mg acetic acid·L$^{-1}$. Increasing the temperature to 45 °C leads to an increase in the production of volatile fatty acids above 6000 mg acetic acid·L$^{-1}$ and a sharp drop after the 7th day, to 1000 mg acetic acid·L$^{-1}$. R3 and R4 kept volatile fatty acids production, which increased slightly, up to 2500 mg acetic acid·L$^{-1}$ and dropped after the 20th day, around 1000 mg acetic acid·L$^{-1}$. The results showed that the combination type and temperature have an effect on volatile fatty acids production.

3.2.2. Organic Matter and Biogas Evolution

The organic matter decreases during the process of anaerobic digestion, in all of the reactors (Figure 5a). Organic matter reductions of 8.51%, 16.85%, 4.32% and 21.57% were obtained, respectively, in reactors R1, R2, R3 and R4. During anaerobic digestion, organic matter was transformed into volatile fatty acids, used by methanogens bacteria for biogas production [43–45]. Laskri et al. [46] showed that the degradation of organic matter is more readable with a COD decrease during biogas production from sludge. In their experience, biogas volume formed was correlated with the organic load degradation of sludge. Studies showed this reduction is more accentuated in reactors R2 and R4, operated at a temperature of 45 °C. The effect of temperature on hydrolysis has been studied by several authors, between 20 and 70 °C [47,48]. According to Amodeo et al. [47], thermophilic (55 °C) was the best condition for the hydrolytic stage. The effects of operating conditions (temperature and substrate mixture) on biogas production are presented in Table 3. For both substrate mixtures, the increase in operating temperature, from 30 to 45 °C, significantly enhanced the biogas production. Indeed, for the same substrate mixture, the biogas generated at 45 °C (R3 and R4) was significantly higher than that generated at 30 °C (R1 and R2) ($p < 0.0001$). These results are in concordance with Ross et al. [49], who reported increased activity in methane-producing bacteria with temperature increases. However, it has been shown that
the biogas yield was not influenced by temperature in the range of 40–55 °C when the ammonia concentration was moderate [50].

When operated at the same temperature, the results show that the increase in Bovine Dung from 10 to 30% also significantly increased the biogas production (p < 0.0001). Indeed, the biogas generated was significantly higher in R4 (152.43 mL/g VS) compared to R2 (95.38 mL/g VS). The same results were found with reactor R3 (124.17 mL/g VS) compared to R1 (80.74 mL/g VS) (Table 3). In a similar study, Nikièma et al. [51] reported a significant influence of 30% Bovine Dung + 70% Primary Sludge mixture on biogas production, compared to the mixture of 10% Bovine Dung + 90% Primary Sludge. Co-digestion with dung could increase organic load and improve biogas production [36]. According to [4], primary sludge has a gaseous production between 0.85 to 1.2 Nm³/kg SV. The quantities of biogas generated in our study are comparable to those previously reported. Thus, Stan et al. [52] found values of 172 mL/g SV from the co-digestion of organic fraction of municipal solid waste (OFMSW) with cow manure, and Hack et al. [36] reported an average yield of 407 NmL CH₄/g SV for sludge from the Bern effluent treatment plant in Germany. Monson et al. [53], Rapport et al. [54] and Pavi et al. [55] reported that at an industrial level, specific biogas production is between 80–120 m³/tons of waste. It should be noted that the methanogenic potential of sludge is high compared to the values obtained in our study. This could be explained by the high organic matter content in sludge from the effluent treatment plant studied (about 74.2%).

3.2.3. Effect of Leachate from Organic Waste and Cellulose on Biogas Production

The biogas production with activated sludge reactors, supplemented with leachate (R1-L) and cellulose (R2-C), respectively, increased after day 25 days (Figure 6). This shows the depletion of the initial substrate used, marking the end of the methanization process. Biogas production resumes with the addition of cellulose and leachate, respectively, in R1-L

![Graph](image-url)

**Figure 5.** Evolution of organic matter (a) and cumulative biogas during anaerobic digestion (b): R1: 10% BD + 90% PS at 30 °C; R2: 10% BD + 90% PS at 45 °C; R3: 30% BD + 70% PS at 30 °C; R4: 30% BD + 70% PS at 45 °C.

**Table 3.** Effect of operating conditions on biogas production.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Combination</th>
<th>Temperature</th>
<th>Biogas mL/g SV</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>10% BD + 90% PS</td>
<td>30 °C</td>
<td>80.74</td>
</tr>
<tr>
<td>R2</td>
<td>10% BD + 90% PS</td>
<td>45 °C</td>
<td>95.38</td>
</tr>
<tr>
<td>R3</td>
<td>30% BD + 70% PS</td>
<td>30 °C</td>
<td>124.17</td>
</tr>
<tr>
<td>R4</td>
<td>30% BD + 70% PS</td>
<td>45 °C</td>
<td>152.43</td>
</tr>
</tbody>
</table>

a, b, c and d significantly different according to Fischer LSD test at probability p = 0.05.
and R2-C. Quantities of 450 and 400 mL were obtained, at 16 days digestion of cellulose 5% (m/v) and leachate, respectively. Studies have shown that cellulose is a good substrate for anaerobic digestion, with methane yields ranging from 340 to 366 L (CH\textsubscript{4})·Kg\textsuperscript{−1} SV [56]. Raposo et al. [57] used cellulose as a reference substrate with theoretical methane production of 373 mL (CH\textsubscript{4})·g\textsuperscript{−1} SV, or 90% of maximum production if 100% was converted into biogas. There is no significant difference between biogas production with cellulose and leachate from the organic fraction of municipal waste fermentation (p = 0.155). Cellulose is a substrate with a simple structure that can maintain the microbial flora of anaerobic digestion. Matejka [29] showed leachate from municipal solid waste was composed of dissolved organic matter, major mineral compounds (Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, K\textsuperscript{+}, Na\textsuperscript{+}, NH\textsubscript{4}\textsuperscript{+}, Fe\textsuperscript{2+}, etc.) and traces of heavy metals (Cd, Zn, Ni, Pb, etc.). Leachate from the organic fraction of municipal waste fermentation could be used for the maintenance of microbial flora to boost anaerobic digestion.

Figure 6. Biogas production by comparing leachate and cellulose uses: R1-C = R1-Cellulose; R1-L = R1-Leachate.

4. Conclusions

This experimental study demonstrated that the co-digestion of primary sludge and bovine dung, using leachate from municipal organic waste, increased the cumulative biogas yield. The various co-substrates had volatile solid contents of 50.51%, 72.41% and 70.48% for primary sludge, bovine dung and leachate, respectively. Sludge showed good stability, with a VFA to alkalinity ratio 0.54. The VFA to alkalinity ratio > 3600 for leachate from organic waste could boost the anaerobic microbial performance. The latency of biogas production is reduced to less than 2 days with the co-digestion system. An increase in bovine dung proportion and a rise in temperature, to around thermophilic temperatures of 45 °C, increase biogas production to 152.43 mL/g SV. Bovine dung lead to an increase in the organic matter proportion of sludge, and waste leachate, composed of VFAs and major mineral compounds, could be used for the maintenance of microbial flora to boost anaerobic digestion. These processes could be enhanced in further investigations by evaluation of the cost, production, and stability. Furthermore, agronomic tests could be carried out on the digestates for a greater value of sewage sludge valorization.

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