Production of Biogas and Biofertilizer by the Co-digestion of Effluent From Cellulose Industry With Addition of Glycerin

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Received: October 21, 2018      Accepted: November 30, 2018      Online Published: February 15, 2019
doi:10.5539/jas.v11n3p242          URL: https://doi.org/10.5539/jas.v11n3p242

Abstract

The present study aimed to assess the anaerobic digestion of sewage from cellulose industry mixed with doses of glycerin, in order to produce biofertilizer and biogas, for agriculture and energy purposes. Five anaerobic reactors were developed, which were fed with effluent and doses of glycerin (0, 5, 10, 15 and 20% v v⁻¹) and evaluated during the hydraulic retention time of 42 days. The physical and chemical parameters were evaluated every seven days: temperature; pH; turbidity; series of solids; total phosphorus (P total); Kjeldahl nitrogen (N total) and COD; as well as the levels of K, Ca, Mg, Cu, Zn, Fe, Mn and Cd, Pb and Cr; biogas production was evaluated daily. The anaerobic treatment produced biofertilizer with excellent nutritional conditions, with adequate levels of P, K, Ca, Mg, Mn, Fe and Zn. The addition of glycerin increased biogas production, with 15% and 5% of glycerin, respectively producing 0.54 and 0.40 mL L⁻¹ d⁻¹. The treatments of 15% and 5% of v v⁻¹ glycerin also exhibited greater removal of COD. However, the low levels of N have caused problems in the anaerobic process. Anaerobic digestion of the cellulose industry effluent with 5 and 15% of glycerin (v v⁻¹) provided increase in biogas production of 0.54 and 0.40 mL L⁻¹ d⁻¹, respectively. K, Ca, Mg, Zn, Fe and Mn at the final biodigestion remained with excellent levels for use in crops as biofertilizer.

Keywords: renewable energy, bioenergy, glycerin, biodigestion, biofuels

1. Introduction

Brazil is a major world producer of paper, besides supplying the domestic market, it exports products mainly to countries of Latin America, the European Union and North America (Bracelpa, 2013). This industrial segment is one of the most competitive in the country, the quality standard is one of the best in the world, and the market is globalized and strongly active. The majority of paper production comes from the pulp of short eucalyptus fibers, which the country is the largest producer in the world. These fibers are mainly used in the manufacture of papers with high benefit (Montebello & Bacha, 2013).

In contrast to the considerable economic benefits, the pulp and paper industry is responsible for the emission of pollutants in the solid, liquid and gaseous states (CETESB, 2008). The sector generates a large amount of effluent, reaching a value of 1 m³ per ton of produced paper (Priadi et al., 2014), and therefore has been seeking development in environmental issues, especially liquid effluents generated in production processes, which, due to highly polluting characteristics and high volume, when released without adequate treatment, aggressively impair the quality of the receiving water body (CETESB, 2008).

Among the new technologies that have emerged in recent years, which can be applied in the treatment of effluents from the pulp and paper industries, effluent co-digestion has gained prominence because, besides biostabilizing the effluent, it enables the production of biogas during the process. The addition of glycerin to the effluent may potentiate the biogas production, making it an activity of greater attractiveness and profitability (Priadi et al., 2014).

Some studies have demonstrated that anaerobic digestion of crude glycerin, mixed with effluents from different
Crude glycerin is a residue from the biodiesel production process. In general the production of biodiesel is carried out by the catalyzed transesterification process. In addition to the desired methyl esters, such reaction also provides some other products, including glycerin and pressed oil pie. All these by-products provide attractive raw materials for use in the production of biogas (Kolesárová et al., 2011).

However, the introduction of biodiesel into the Brazilian energy matrix by the National Program for the Production and Use of Biodiesel has increased the supply of glycerin, which is produced at the rate of 10 kg for every 100 L of biodiesel (Larsen et al., 2013).

In this context, the process of anaerobic biodigestion of residues from different origins and compositions, in this case, effluents from cellulose and the biodiesel industries, may be characterized as a solution to improve the treatment and biostabilization of these, possibly increasing production of biogas, and, in addition, enabling the correct destination of this waste, for its eventual use as a biofertilizer in agriculture.

The objective of the present study was to evaluate the process of co-digestion of the effluents from the cellulose industry with added doses of glycerin, aiming the production of biogas, as well as the possibility of biofertilizer production.

2. Methods

2.1 Development of Alternative Anaerobic Reactors

The biodigestors were constructed with recycled plastic material, for that, 5 plastic bottles with capacity of 50 liters were used; 5 taps; 4.5 meters of hose Rodrigo soft blue PU 8 mm; 5 metal and silicone fittings used for sealing, keeping the internal environment completely anaerobic.

The taps were installed to allow sampling of the liquid effluent along the hydraulic retention time (HRT), the reactors were arranged horizontally, with the end containing the tap down and a spigot, facing upwards (Figure 1A).

Figure 1. Biodigesters and gasometer linked to the reactor used in the experiment

2.2 Experiment Setup and Doses of Glycerin

The experiment was carried out at the Pontifical Catholic University of Paraná (PUCPR), located in the municipality of Toledo, Paraná (24°43’08.72” S and 53°46’08.72” W) with an average elevation of 575 meters.

Before the supply of the biodigestors, physical and chemical parameters of the cellulose industry effluent were analyzed, in order to evaluate the efficiency of the process.

The volume of 25 liters of effluent were added in each biodigester, as well as increasing doses of glycerin (5, 10, 15 and 20% v v⁻¹). The biodigesters were operated without maintenance of temperature, with hydraulic retention time (HRT) of 42 days, with daily monitoring of gas volume and weekly monitoring of physical and chemical parameters of the effluent.

In Table 1 are exhibited the data regarding the amount of cellulose effluent and glycerin (Vetec®, 99.5%) added to the biodigesters.
2.3 Gasometer

Five gasometers were made in plastic bottles, which were filled with water and sealed with a lid to which two hoses were connected, one for biogas inlet and another for the water outlet. As the gas was produced, it was directed through the hoses for the bottles, the volume of biogas produced ended up generating pressure and displacing the water to a second bottle, thus providing quantification of the volume of the produced gas (Figure 1B).

For the construction of the gasometers, 10 plastic bottles of 2 liters were used; 4.5 meters of hose Rodoar soft blue PU 8 mm; 5 metal fittings; 5 plastic valves and silicone for sealing.

2.4 Sampling of Waste and Evaluated Parameters

The cellulose effluent sampling was carried out in a pulp paper production unit located in the state of Paraná, southern Brazil.

Physical and chemical parameters evaluated were: pH; turbidity; total (TS), fixed (FS) and volatile (VS) solids; total phosphorus (P_{total}); total nitrogen (N_{Kjeldahl}) (N_{total}), and chemical oxygen demand (COD) (APHA, 2012).

Table 1. Doses of glycerin and total volume of biodigesters

| Doses of glycerin % (v v⁻¹) | Cellulose industry effluent (L) | Glycerin (L) | Total volume of the mixture (L) |
|----------------------------|--------------------------------|--------------|-------------------------------|
| 0                          | 25.00                          | 0.00         | 25.00                         |
| 5                          | 23.75                          | 1.25         | 25.00                         |
| 10                         | 22.50                          | 2.50         | 25.00                         |
| 15                         | 21.25                          | 3.75         | 25.00                         |
| 20                         | 20.00                          | 5.00         | 25.00                         |

Note. Amount of cellulose effluent and glycerin (Vetec®, 99.5%) exposed to biodigesters.

Total concentration of potassium (K), calcium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd), lead (Pb) and chromium (Cr) were determined by atomic absorption spectrometry, flame mode (FAAS) model GBC 932 AA (Victoria, Australia) with deuterium lamp for background correction (Welz and Sperling, 1999).

In addition, the volume of the biogas produced was determined daily, by the gasometers linked to the biodigesters, the measurement was carried out with graduated cylinder.

3. Results

The reactor containing 0% of glycerin exhibited low variation of pH, with maximum and minimum values of 6.16 to 6.81. The presence of glycerin acidified the medium, reaching the lowest pH (4.77) in the dose of 15%.

The higher the dose of glycerin added to the system, the higher is the concentration of TS, because with such increase, the concentration of VS is increased, since the glycerin [C₃H₅(OH)₃] is basically composed of carbon, hydrogen and oxygen. In addition to increasing TS levels, the addition of glycerin favors the production of biogas components, mainly CH₄, CO₂, NH₃ and H₂S.

The turbidity is higher in the glycerin treatments than in the 0% dose treatment, due to the multiplication of microorganisms.

The treatments with glycerin addition exhibited higher values for COD, this is an expected fact, since a liquid with higher turbidity and total and volatile solids tends to have higher COD values.

In this research, the treatments with the highest COD removal at the end of HRT are the treatments with the highest biogas production (Dose 0%, 5% and 15%).

Both, the industrial effluent and the glycerin, did not have N contents in their initial composition, a condition that was maintained for almost all treatments until the end of the process.

It was observed a reduction of P_{otal} for the treatments with glycerin addition. The stability of P becomes attractive for biofertilization purposes, since the final effluent contains considerable P contents, being this one of the most important macronutrients for the development of plants.
The doses of 15% and 5% of glycerin, respectively, resulted in higher rate of biogas production (0.543 and 0.400 mL L⁻¹ d⁻¹). Doses higher than 15% affected the development of microorganisms and, consequently, the production of biogas.

That most of the studied nutrients had their levels elevated throughout HRT. In general, we observed a significant increase of the final K, Ca, Mg contents of the bio stabilized effluent, as well as the maintenance and availability of Fe, Mn in the glycerin treatments, as a function of the maintenance of the liquid medium at more acidic pH, facilitating more soluble forms in the aqueous medium.

4. Discussion

The acidification of the medium occurs due to the presence of the glycerin, which directly affects the microbial metabolism inside the digesters, causing a substantial increase of the substrate (organic material) and raising the population of bacteria quickly, thus provoking the presence of organic acids in the biodigester origin of the acidogenic phase (Sim et al., 2017).

According to Figure 2, the reactor containing 0% of glycerin exhibited low variation of pH similar to Priadi et al. (2014), when evaluating the biogas production from the anaerobic digestion of effluent from the cellulose industry found a variation of 6.30 to 6.90, considered by the authors as low fluctuation range and with adequate pH values for the development of methanogens.

According to Ruiz et al. (2017), the pH drop of the effluent is due to the accumulation of these organic acids in the biodigester, which occurs when the system is unable to use all the material coming from the steps of hydrolysis and acidogenesis in the next phase, that is, in the methanogenic phase.

![Figure 2](https://example.com/figure2.jpg)

Figure 2. pH and air temperature (mean, maximum and minimum, in °C) during the anaerobic co-digestion of industrial effluent (cellulose) with glycerin doses (0, 5, 10, 15 and 20% v v⁻¹), during the hydraulic retention time (HRT) of 42 days

Acid or alkaline pH values negatively influence the metabolism of methanogenic microorganisms, the ones responsible for the production of methane (CH₄), which are quite sensitive to variations in the pH of the medium. Calli et al. (2005) state that inhibition of biogas production may occur at pH values above 7.4. In general, low pH systems are not able to produce buffer solution to maintain pH stability, and it is necessary to alkalinize the medium to raise biogas and methane production (Awe et al., 2017).

At first, the anaerobic microorganisms perform anabolic reactions, aiming their cellular growth. In this way, by means of enzymatic processes, there is a breakdown of the substrate (effluent and glycerin components) and assimilation by the bacterial cells (anaerobic digestion phases, such as hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Shah et al., 2014).

In this initial stage, due to the high availability of nutrients in the effluent, intense multiplication of microorganisms occurs (exponential and stationary phase of microorganisms development) (Shah et al., 2014), resulting in turbidity increase, since the microorganisms are also biomass particulate matter or suspension
(Jerônimo et al., 2012). This also explains the fact that turbidity is higher in glycerin treatments than in the treatment with 0% Dose.

In this initial stage, the intense multiplication of microorganisms result in a marked increase in turbidity, since the microorganisms are also particulate biomass, or biomass suspension (Jerônimo et al., 2012). This also explains the fact that turbidity is higher in glycerin treatments than in Dose 0%.

![Figure 3. Turbidity (NTU) and COD (mg L⁻¹) (left), VS and TS (right) during anaerobic co-digestion of industrial effluent (cellulose) with glycerin doses (0, 10, 15 and 20% v v⁻¹) during the hydraulic retention time (HRT) of 42 days](image)

The treatments with glycerin addition exhibited higher values for COD, this is an expected fact, since a liquid with higher turbidity and total and volatile solids tends to have higher COD values.

This occurs because COD is the parameter that indirectly represents the amount of organic compounds in the sample, measuring the necessary amount of oxygen for chemical degradation of organic matter in the liquid medium (Kolesárová et al., 2011). As the organic matter content in the glycerin treatments is higher, a proportional increase of COD values occurs in these reactors (Verussa et al., 2017).

Because of this close relationship, several authors studied the influence of COD on the production of biogas in anaerobic systems (Kolesárová et al., 2011; Fleck et al., 2017; Sumardiono et al., 2013; Ebrahimi et al., 2018).

Sunada et al. (2012), when evaluating the biogas production in the anaerobic digestion of refrigerator effluent with and without the removal of COD (solid fraction > 1 mm), observed, in the treatments with initial COD removal, a decrease in the biogas production rate, while in the reactors with organic load maintenance the rate remained practically constant.

In this research, the treatments with the highest COD removal at the end of HRT are also the treatments with the highest biogas production (Dose 0%, 5% and 15%). Panpong et al. (2017), in their studies, also observed that the treatments with higher COD removal produced higher amounts of biogas.

The only treatments with N contents were Dose 0% and 5%, with peaks observed at 7 and 14 days (Figure 4). The low values for the N₁₀₀ concentration are justified by its easy degradation in the anaerobic biodigestion process, as well by the low N₁₀₀ initial values in the substrates (Sunada et al., 2012).
Figure 4. $N_{\text{total}}$ and $P_{\text{total}}$ (mg L$^{-1}$) in anaerobic digestion of industrial effluent (cellulose) with glycerin doses (0, 5, 10, 15 and 20% v v$^{-1}$) during the hydraulic retention time (HRT) of 42 days

The limitation of N is an obstacle to the efficient digestion of glycerin (Aguilar et al., 2017), which molecule (propane-1,2,3-triol) is composed of carbon atoms (C), hydrogen (H) and oxygen (O), without N in its composition.

Likewise, N deficiency may restrict the conversion efficiency of organic matter to biogas. Therefore, the ideal for anaerobic digestion and biogas production would be to use effluents with medium-high content of N, such as the swine effluent, and glycerin, which has a high carbon content, because this way the effluent corrects the N restriction of glycerin. Thus, it is possible to obtain a good C/N ratio in the final substrate, with values in the range of 20/35, causing an increase in the production of biogas (Aguilar et al., 2017).

As glycerin contains a high concentration of C of easy assimilation, its quality, as a co-product of anaerobic digestion, enriches the process, since C in more complex structures, such as long chain fatty acids, can become toxic for microorganisms. Thus, glycerin doses with animal wastes with N contents can balance the medium (Schwingel et al., 2017).

The concentrations of $P_{\text{total}}$ did not exhibit variations during HRT (Figure 4). For Sunada et al. (2012), an increase of the N, P and K concentrations in the biofertilizer is common, because during the anaerobic digestion the majority of the C is lost in the form of CH$_4$ and CO$_2$, causing the concentration of other non-volatile elements. However, as in the present study, these authors observed a significant reduction of $P_{\text{total}}$ concentrations, which, according to them, is related to the physical and chemical removal by the precipitation of these compounds (Sunada et al., 2012).

The doses of 15% and 5% of glycerin, respectively, resulted in higher rate of biogas production (0.543 and 0.400 mL L$^{-1}$ d$^{-1}$). Doses higher than 15% affected the development of microorganisms and, consequently, the production of biogas (Figure 5).

Fountoulakis, Petousi, and Manios (2010) mention that when applied in adequate doses, glycerin may have represent advantage in the biodigestion process, increasing biogas production, but glycerin adding must be cautious, in order not to cause organic overload in the biodigester. It occurs that in this case the overload hinders the action of anaerobic microorganisms, making it desirable to dilute the residue, which would facilitate the process of anaerobic biodigestion (Sgorlon et al., 2011).
This subject is discussed in the studies of Astals et al. (2011), where the presence of acetic, propionic and butyric acids in the co-digestion of swine effluent associated with high levels of glycerin (80%), being the presence of these acids the result of the lack of conditions (due to the acid medium) for the digestion of the samples. In addition, for these authors, the treatment with 80% of glycerin produced less biogas when compared to the treatment with 0%.

A fundamental aspect of anaerobic biodigestion associated with glycerin is the importance of N-rich substances, due to the scarcity or absence of this nutrient in the crude glycerin, thus, for an ideal balance of C/N ratio, in some cases, the use of N supplementation in the biodigester is necessary (Pazuch et al., 2017).

In order to achieve higher biogas production rates, the substrate used should exhibit carbon-nitrogen (C/N) ratio of about 30 carbon molecules by 20 nitrogen molecules (Aguilar et al., 2017).

Aguilar et al. (2017) reports the biogas production as a function of the anaerobic digestion of crude glycerin dosed in swine effluent, and for these authors, better results are observed in the mixing range between 0.4 and 0.5%, resulting in an amount of 521.5 mL of biogas per g of total COD. For them the relationship between inoculum volume and the total amount of VS was adequate, and this was an important factor for biodigestion. However, in this same research, the highest COD removal occurred with the higher doses of glycerin (0.10 and 0.11%).

In the present study, the treatments with the highest biogas production were also the ones with the highest removal of COD, evidencing the transformation of the organic matter in liquid medium into biogas (C$_{org}$ in CH$_4$ and CO$_2$). Panpong et al. (2017) also observed such correlation between these parameters, when they studied the biogas production through the digestion of sardine processing effluent, with production up to 8.30% more biogas in relation to the treatment without glycerin, with the highest biogas production following the highest removal of COD.

In Table 2 and Figure 6 it is possible to observe that most of the studied nutrients had their levels elevated throughout HRT, Sunada et al. (2012), explains that the elevation of these levels is expected, because during digestion, C tends to be lost in the formation of CH$_4$ and CO$_2$, and in this way occurs the concentration of other nutrients.

In the treatment without addition of glycerin, where the pH values remained in the range of 6.16 to 6.8, it is observed a lower availability of metals, such as Fe, Mn, Cu and Zn, due to the formation of insoluble forms, i.e. occurrence of precipitated forms of these compounds.

The presence of certain metals in the medium may be of fundamental importance during biogas production, since methanogenesis is one of the most metal-enriched enzyme pathways in biology, however, the amount of metal required at this stage of biodigestion depends on the biochemical pathway of the biogas. However, there are trends that generally prevail. Fe, for example, is the most required metal, followed by nickel (Ni) and cobalt (Co) and remnants of molybdenum (Mo) and Zn (Goswami et al., 2016).
Table 2. Total contents of K, Ca, Mg, Cu, Zn, Fe and Mn

| Organic waste | N    | P    | K    | Ca   | Mg   | Cu   | Zn   | Fe   | Mn   | Authors                  |
|---------------|------|------|------|------|------|------|------|------|------|--------------------------|
|               | mg L⁻¹ |      |      |      |      |      |      |      |      |                          |
| Cellulose effluent + Glycerin (HRT = 7 days, Dose 0%) | 0.00 | 0.17 | 78.71 | 171.97 | 58.36 | 0.00 | 0.26 | 0.21 | 0.44 | This research             |
| Cellulose effluent + Glycerin (HRT = 14 days, Dose 5%) | 0.14 | 0.01 | 91.86 | 202.01 | 65.88 | 0.00 | 0.04 | 0.18 | 0.51 |                         |
| Cellulose effluent + Glycerin (HRT = 21 days, Dose 10%) | 0.04 | 0.01 | 109.59 | 212.36 | 67.42 | 0.00 | 0.01 | 0.18 | 1.52 |                         |
| Cellulose effluent + Glycerin (HRT = 28 days, Dose 15%) | 0.07 | 0.005 | 131.12 | 216.80 | 63.98 | 0.00 | 0.00 | 0.20 | 0.50 |                         |
| Cellulose effluent + Glycerin (HRT = 35 days, Dose 20%) | 0.03 | 0.02 | 136.32 | 207.92 | 64.47 | 0.00 | 0.04 | 0.18 | 0.23 |                         |
| Mean values obtained in this study after 42 days of HRT | 0.04 | 0.04 | 109.52 | 202.21 | 64.02 | 0.00 | 0.07 | 0.19 | 0.64 |                         |
| Swine waste | 19,600.00 | 23,600.00 | 159.10 | 27.70 | 12.20 | 30.00 | 117.00 | 303.00 | 110.00 | Bócoli et al. (2016) |
| Bovine biofertilizer | 140.00 | 50.00 | 0.05 | 0.07 | 0.03 | 0.10 | 1.40 | 1.00 | 0.20 | Chicondo et al. (2013) |
| STS sludge, whey industry | 6,530.00 | 980.00 | 0.78 | 1.89 | 1.29 | 5.34 | 15.00 | 440.00 | 13.85 | Schwantes et al. (2017) |
| Vinasse | 710.00 | 170.00 |      |      |      |      |      |      |      | Silva et al. (2016)     |
| Bovine biofertilizer | 60.00 | 4.33 | 6.77 | 8.50 |      |      |      |      |      | Ferreira et al. (2015) |
| Cattle manure | 780.00 | 730.00 | 1.19 | 0.59 | 0.28 | 2.04 | 5.90 | 73.04 | 9.32 | Alencar et al. (2015) |
| Spraying, urine and dietary remains of dairy cows | 4,250.00 | 20.00 | 0.13 | 0.30 | 0.37 |      |      |      |      | Soares Filho et al. (2015) |

*Note. Total contents of K, Ca, Mg, Cu, Zn, Fe and Mn of cellulose industry effluent and glycerin, and comparison with other organic wastess.*

In addition to being used in electron transport, Fe is a constituent of the metalloenzymes involved in methanogenesis, in the form of Fe₃S₂, Fe₂S₂ or Fe₄S₂ (Rao et al., 2011). Another important element is Ca, this macronutrient when in the substrate contributes to pH equilibrium by neutralizing H⁺, thus avoiding intensive acidification of the medium (Matheri et al., 2016). On the other hand, Suárez et al. (2014) states that excess of Ca can inhibit biogas production by favoring the precipitation of carbonate and calcium phosphate, which can result, for example, in the loss of buffer capacity and essential nutrients for anaerobic digestion.

It is also necessary to be cautious about Zn, because in high concentrations it can cause toxicity to the microorganisms, inhibiting important biochemical reactions. However, adequate levels of Zn have been shown to potentiate the formation of CH₄ (Matheri et al., 2016).

The Mn is another element that can positively interfere in the production of biogas, since besides accelerating the methanogenesis, can increase the final produced volume. In addition, the element can also serve as an oxidizing agent to donate electrons to form methane from carbon dioxide (Qiao et al., 2015).
Mg acts as a necessary enzymatic cofactor in the stabilization of nucleotides and nucleic acids. It is related to enzymatic reactions associated with energy transfer and phosphorylation/dephosphorylation. It occurs that much of the energy of the cell is stored in the form of ADP and ATP, for this energy to be released the presence of Mg is fundamental, as it assists in the formation of a “bridge” between the oxygen atoms of two adjacent phosphate groups and an N atom at the catalytic site of the protein (Suárez et al., 2014).
Similarly, K+ ions also are not directly involved in methanogenesis, however, a large part of the methanogenic enzymes exert their function effectively when K+ is present in the medium at high concentrations (Glasemacher et al., 1996).

Since several soils have micronutrient deficiency (Silva et al., 2014), the final concentrations of these in the biofertilizer, although not expressive, are a good alternative for supplying Zn, Fe, Mn, K, Ca and Mg to the soil, without causing toxicity to the plants.

Anaerobic digestion of the cellulose industry effluent with 5 and 15% of glycerin (v/v) provided a satisfactory increase in biogas production (0.54 and 0.40 mL L⁻¹ d⁻¹, respectively).

Anaerobic digestion provided considerable reductions for TS and VS, confirming the feasibility of using biodigesters as treatment of these residues.

The concentrations of P were reduced during HRT, and the nutrients (K, Ca, Mg, Zn, Fe and Mn), although with high levels during the process (which is considered normal by several authors) are in adequate concentrations for use in crops as biofertilizer.

Acknowledgements

To the Fundação Araucária for the funding of this research.

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