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EVALUATION OF METHANE PRODUCTION IN THE ANAEROBIC DIGESTION OF VINASSE PRETREATED WITH OZONE

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ABSTRACT

This paper presents the evaluation of removing phenolic compounds present in vinasse using an advanced oxidation process (AOP) based on ozone coupled with an anaerobic biological process. The evaluations of the treatments were performed by determining the changes in the concentrations of the phenolic compounds as a function of ozone application time (7 – 15 – 30 minutes) and pH of the substrate (4.2 – 7.47) in order to determine the influence of these factors on methane production in anaerobic digestion. The results indicate that neutral pH in the vinasse favors by an additional 27% the removal of phenolic compounds when compared to an acid pH. It was also observed that after the ozonation of vinasse at a neutral pH, the total concentration of phenol decreased by 62%, influencing methane production by 70% with respect to the methane production obtained from raw vinasse.

KEYWORDS: AOP, ozonation, anaerobic digestion, methane production.

EVALUACIÓN DE LA PRODUCCIÓN DE METANO EN LA DIGESTIÓN ANAEROBIA DE VINAZAS PRETRATADAS CON OZONO

RESUMEN

Este artículo presenta la evaluación de la remoción de compuestos fenólicos presentes en la vinaza, utilizando un proceso avanzado de oxidación (PAO) basado en ozono acoplado a un proceso biológico anaerobio. Las evaluaciones de los tratamientos se realizaron determinando los cambios en la concentración de compuestos fenólicos en función del tiempo de aplicación de ozono (7 – 15 – 30 minutos) y pH del sustrato (4,42 – 7,47) con el fin de determinar la influencia de estos factores sobre la producción de metano en el proceso de digestión anaerobia. Los resultados indican que el pH neutro en la vinaza favorece un 27 % más la remoción de compuestos fenólicos en comparación al pH ácido. Se observó que después

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del proceso de ozonización de vinazas a pH neutro la concentración de fenoles totales disminuye un 62 % influenciando la producción de metano en un 70 % con respecto a la producción de metano obtenida a partir de vinazas crudas.

PALABRAS CLAVE: PAO, ozonización, digestión anaerobia, producción de metano.

AVALIAÇÃO DA PRODUÇÃO DE METANO NA DIGESTÃO ANAERÓBIA DA VINHAÇA PRÉ-TRATADA COM OZÔNIO

RESUMO

Este trabalho apresenta a avaliação da remoção dos compostos fenólicos presentes no bagaço, usando um processo de oxidação avançada (PAO), com base no ozono acoplado a um processo biológico anaeróbio. As avaliações dos tratamentos foram realizados por determinação das alterações na concentração de compostos fenólicos em função do tempo de aplicação de ozono (7 - 15 - 30 minutos) e pH do substrato (4,42 a 7,47), a fim de determinar a influência desses fatores sobre a produção de metano no processo de digestão anaeróbia. Os resultados indicam que o pH neutro favorece um 27% de remoção de compostos fenólicos em comparação com pH ácido. Observou-se que após do processo de ozonização de vinhaças ao pH neutro a concentração total de fenol em um 62% influenciando a produção de metano em 70% em relação a produção de metano em bruto obtido a partir de vinhaça.

PALAVRAS-CHAVE: PAO, ozonização, digestão anaeróbia, produção de metano.

1. INTRODUCTION

The goal of replacing fossil fuels with biofuels has generated unbridled growth in the production of fuel alcohol (ethanol), biodiesel, and, as a consequence, of waste produced by each process (Gil, 2011). In the particular case of ethanol production, it is estimated that in 2014, 90.38 billion liters of this alcohol were produced worldwide (GRFA, 2014), of which Colombia, the tenth largest worldwide producer of ethanol from sugarcane, produced nearly 450 million liters (Federación Nacional de Biocombustibles de Colombia, 2015). Vinasse is a liquid waste resulting from ethanol production either from distillation of the fermented molasses or direct fermentation of sugarcane (García et al, 2008), and for each liter of ethanol produced, between 0.8 and 3.0 liters of vinasse are obtained (Asocaña, 2011).

In Colombia, sugar mills in the geographical valley of the Cauca River, which produce ethanol, have undertaken rigorous management of their waste, with fertilizers being the widest use given to vinasse. However, other alternative methods for final disposal of this waste have arisen in response to the increase in salinity in land fertilized with vinasse, including incineration, anaerobic digestion, dehydration, and composting (Cenicaña, 2010). However, it is important to consider additional measures for its use given that none of these alternatives on its own is viable for managing the entire volume of vinasse produced. Dávila et al. (2009) evaluated the electroflotation/oxidation process for vinasse, obtaining reductions in the chemical demand for oxygen by 58%. Similarly, Yusuf (2007) achieved a 90% reduction in total organic carbon in vinasse through electrocoagulation with the use of a supporting electrolyte and the gradual addition of hydrogen peroxide. In 2012, España-Gamboa et al. treated vinasse from ethanol distillation with anaerobic digestion, achieving a 69% reduction in the chemical oxygen demand and 0.263 m^3 kg COD_{added} of methane production, which is considered a promising source of renewable energy.

In this sense, in a biological anaerobic digestion system, the organic content of vinasse, 156g $COD.L^{-1}$ (Caicedo, 2010), can be utilized and transformed into methane. However, the toxic compounds present in the vinasse, mainly phenols and polyphenols (Robles-González et al., 2012), affect the biological system's development and necessitate a treatment process prior to the biological process, which allows for the degradation of inhibitor compounds for comprehensive use of the vinasse.

Advanced oxidation processes (AOPs) have been used as an alternative for water decontamination since they are capable of completely mineralizing organic contaminants (Malato et al. 2009) and as pretreatments for enzymatic hydrolysis processes in fermentation systems in order to increase the digestibility of lignocellulosic materials (Travaini et al., 2013). However, these technologies require further development in order to be economically viable. As such, couplings with biological systems are one of the best options to reduce the implementation costs and environmental impacts they produce (García-Montaño et al., 2008).

The purpose of this study was to evaluate ozone as an advanced oxidation process to degrade phenolic compounds in vinasse generated by the production of ethanol from sugarcane, coupled with an anaerobic digestion process to obtain methane as a second-generation biofuel.

2. MATERIALS AND METHODS

2.1. Location

All trials were completed in the Sanitary and Environmental Engineering Program's Environmental Microbiology Laboratory on the Melendez campus of Universidad del Valle in Santiago de Cali – Colombia.

2.2. Origin of vinasse and inoculum

The vinasse was donated by the Mayaguez sugar mill located in the municipality of Candelaria – Valle del Cauca and came from the process of sugarcane distillation for fuel alcohol production. The inoculum used in the anaerobic digestion process was taken from the UASB reactor at the Cavasa animal slaughtering center in the municipality of Candelaria – Valle del Cauca.

2.3. Vinasse characterization

Before and after ozone treatment, the vinasse was characterized using parameters of chemical oxygen demand (COD), pH, total suspended solids (TSS), biochemical oxygen demand (BOD_5), ammoniacal nitrogen ($N\text{-}NH_3$), sulfur (S^{-2}), and total phenols according to *Standard Methods (APHA, 2005)*.

2.4. Removal of phenolic compounds using an AOP

The vinasse was pretreated using an advanced oxidation process (AOP): ozonolysis using a 40cm tall and 5cm diameter glass column, which operates with a flow of 0.5 l/min of an oxygen/ozone mixture under room temperature conditions (26°C). The ozone concentration used was 50mg O^3 L^{-1}. The vinasse was placed raw (55% TSS) in the glass column. In order to evaluate the bubbling system and guarantee the flow of ozone through the sample, the foam generated during the process was re-circulated, thereby achieving constant contact between the vinasse and the ozone flow (**Figure 1**).

The diffuser used is made of porous fine-bubble glass in which the transfer efficiency for ozone is maximized by increasing the interfacial area (Gogate & Pandit, 2004).

An ozone concentration of 50mg L^{-1} was used in accordance with the recommendations of Caicedo (2010) since this concentration is considered to be the most efficient for eliminating phenolic compounds from vinasse. The oxygen/ozone flow used was 0.5 l/min. Considering that this project's objective was to remove phenolic compounds from vinasse causing the least change in its organic composition so that it could be utilized in anaerobic digestion, the substrate was exposed to the ozone for short application periods, evaluating the pretreatment at a pH of 7.47 and 4.42 (**Table 1**).

Figure 1. Vinasse ozonation system.

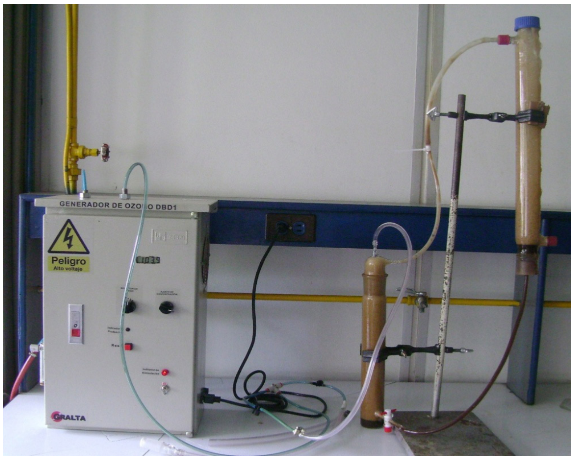


TABLE 1. OPERATING CONDITIONS FOR THE OZONOLYSIS PROCESS *

Pretreatment	*Application time (min)*	*pH*
1	7	7.47
2	7	4.42
3	15	7.47
4	15	4.42
5	30	7.47
6	30	4.42

*The flow of oxygen/ozone used was 0.5 l/min in standard conditions

2.5. Setup of trial and anaerobic digestion

The anaerobic digestion trial was performed in 120ml reactors with screw-on lids and fitted rubber septa with 20cm diameter metal seals. Seeding was performed in an anaerobic chamber. The liquid phase (usable volume) was composed of 33.5ml of distilled water, 1.52ml of inoculum (1.5g SSV. L^{-1}), 0.4ml of vitamin solution, and between 0.56 and 0.84ml of substrate due to the fact that the vinasse show different COD values after each ozone treatment. However, the same load (4g COD. L^{-1}) was guaranteed in all the reactors through mass per volume balances. The balance of nutrients performed on the vinasse (see section 3.1 results) shows that it fulfills the carbon/nitrogen ratio required by anaerobic microorganisms corresponding to C/N = 20-30 (Lorenzo & Obaya, 2005, 2005) without needing the addition of nutrients in the anaerobic reactors.

Six anaerobic digestion trials were performed with three replications of each and three replications for each control (C_1= inoculum, C_2= volatile fatty acids (VFA) (73:23:4) and C_3= vinasse not treated with ozone or raw vinasse), as is shown in **Table 2**. The repetitions were maintained for the 25 days of the experiment. The reactors were inoculated in anaerobiosis and incubated during the trial at 35°C in an inverted position such that a hydraulic seal was created at the rubber stopper (**Figure 2**).

TABLE 2. TREATMENTS OF THE ANAEROBIC DIGESTION TRIAL

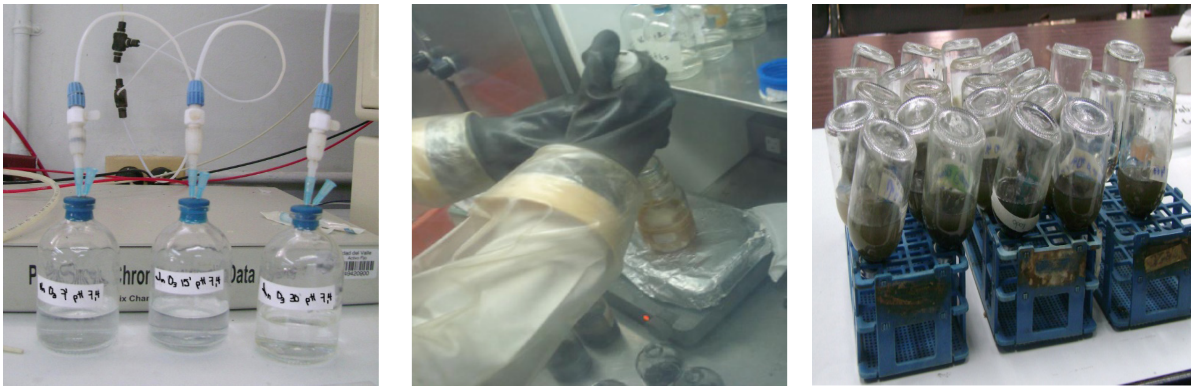
Treatment	*Substrate*
1	Vin Oz 7' (pH 7.4)
2	Vin Oz 7' (pH 4.4)
3	Vin Oz 15' (pH 7.4)
4	Vin Oz 15' (pH 4.4)
5	Vin Oz 30' (pH 7.4)
6	Vin Oz 30' (pH 4.4)
Control 1	inoculum
Control 2	VFA
Control 3	Raw vinasse

Vin Oz 7': vinasse ozonated for 7 minutes; Vin Oz 15': vinasse ozonated for 15 minutes; Vin Oz 30': vinasse ozonated for 30 minutes. VFA volatile fatty acids.

2.6. Methane production monitoring

Every three days, methane production was measured using gas chromatography. The gas chromatograph used (Shimadzu GC 14, FID) has a Carbowax 20M column (3%), H_3PO_4 (1%), and Supelco 80/100. The methane peaks were detected around 0.5 seconds using Peak Simple 3.0 software.The chromatograph's operating conditions were: H_2 =

Figure 2. Anaerobic digestion trial. (a). Displacement of O_2 with nitrogen. (b). Seeding of reactors for anaerobiosos. (c). Reactors incubated in an inverted position.



2a 2b 2c

40psi, air = 30psi, carrier 1 = 300psi, and carrier 2 = 140psi. The gas drag of both carriers was molecular nitrogen (N_2). The methane reading was made in duplicate, the injection volume was 1ml, and a needle with a gas valve was used.

2.7. Statistical analysis

Two-way ANOVA tests were performed for six treatments with defined factors of pH and ozonation time, and the response variable was the total phenol concentration in mg L^{-1} of gallic acid. A confidence level of 95% was used. Two-way ANOVA tests were also used for six treatments with factors of pH and ozonation time and a response variable of methane production in ml, using a level of confidence of 95%. Normality, homoscedasticity, and observation independence tests were performed for each of the tests, and the determination of homogenous groups was made using the HSD Tukey test. The statistics software Minitab and StatGraphics Plus 5.1 for Windows (Manugistics, Inc., Rockville MD, USA) were used.

3. RESULTS AND DISCUSSION

3.1. Substrate characterization

Table 3 presents the physiochemical characteristics of the vinasse used in all of the trials in this study, which came from the Mayaguez sugar mill, Candelaria - Colombia.

The anaerobic digestion process requires a moderate amount of nutrients when compared to aerobic systems due to the low growth speed of the microorganisms (Lorenzo & Obaya, 2005). Therefore, the values obtained for carbon (288787mg L^{1}), nitrogen (888mg L^{-1}), and phosphorus (194 mg L^{-1}) in the vinasse evaluated ensure growth of the microorganisms in the anaerobic treatment biological reactors since the substrate fulfills the minimum functioning ratio for the carbon/nitrogen system = 20-30.

TABLE 3. PHYSIOCHEMICAL CHARACTERIZATION OF THE VINASSE USED

Parameter	Value*
COD	288787
BOD_5	96030
TSS	52000
pH	4.42
Nitrogen	888
Phosphorus	194
S^{-2}	182
$N\text{-}NH_3$	180
Total phenols	306.120

*All values except pH are expressed in mg L^{-1}.

According to this characterization, the anaerobic biodegradability of the vinasse is BOD_5/COD: 0.33. The result of this ratio means that the difference between the COD and BOD_5 content is large, indicating that there is a higher proportion of non-biodegradable components. España-Gamboa and collaborators (2011) report a high concentration of phenols in the vinasse from the wine industry of 474mg L^{-1}, which results in an inhibited growth of the microorganisms for anaerobic digestion conditions. The sugarcane vinasse evaluated in this study shows a concentration of 306.120mg L^{-1}, considered to inhibit the anaerobic process. In addition, ammoniacal nitrogen and sulfur contents between 1700 – 14000mg L^{-1} and 30 – 250mg L^{-1}, respectively, have been reported to have inhibiting effects on the anaerobic digestion process and, therefore, on methane production (Cirne et al., 2008; Parawira et al., 2008). However, for the case of ammoniacal nitrogen, concentrations of 200mg L^{-1} have been reported to be beneficial in the anaerobic digestion process (Cirne et al., 2008). In this study, the concentration of ammoniacal nitrogen in the vinasse is 180mg L^{-1}, and the concentration of sulfur is 182mg L^{-1}. The ammoniacal nitrogen concentration is beneficial to the anaerobic biological process, as opposed to the sulfur concentration.

3.2. Reduction of organic compounds using AOP

Using the crude vinasse (see **Table 3**), the pretreatment was performed with ozone and short application times (7, 15, and 30 minutes). The values of initial and final concentration as well as percentage removal of COD and phenolic compounds are included below in **Tables 4** and **5**, respectively.

TABLE 4. REDUCTION OF ORGANIC MATTER IN PRETREATMENTS IN TERMS OF COD

Pretreatment	*Initial COD (mg L^{-1})*	*Final COD (mg L^{-1})*	*% COD removal*
Vin Oz 7' (pH 7.4)	288787	277058	4.1
Vin Oz 15' (pH 7.4)	288787	265015	8.23
Vin Oz 30' (pH 7.4)	288787	201805	30.12
Vin Oz 7' (pH 4.4)	288787	261910	9.31
Vin Oz 15' (pH 4.4)	288787	215175	25.49
Vin Oz 30' (pH 4.4)	288787	191945	33.53

The percentage of removal of organic matter using ozone as a pretreatment is more representative in vinasse with an acid pH, reaching the highest values of removal, 25.49 – 33.53, for the pretreatment applied at 15 and 30 minutes at a pH of 4.4, respectively. The efficiencies of COD removal at an acid pH are in agreement with those reported by Caicedo (2010), who concludes that vinasse's natural pH(4.42) is adequate for applying ozonation in order to decrease the organic load in this substrate. However, despite this decrease, the efficiency obtained by applying this pretreatment is low, in agreement with the information reported by Martin Santos et al. (2003). The ozonation of vinasse does not present high levels of organic matter removal, but rather the transformation of compounds present in the waste. It is therefore convenient to opt for this technology to transform compounds that are difficult to degrade into simpler compounds without greatly decreasing the waste's organic concentration.

Martín Santos et al. (2003) state that a pretreatment with ozone of vinasse with a natural pH (4.42) is indicated for removal of phenolic compounds. On that occasion, vinasse with an acid pH (4.5) and an alkaline pH (8.8) was evaluated, showing that the direct oxidation reaction (oxidation through the molecular ozone) is more efficient for decreasing phenols compared to indirect reactions (oxidation through the hydroxil radicals). However, the results obtained in this study (see **Table 5**) indicate that at a nearly neutral pH, the decrease of phenolic compounds occurs in a greater proportion than at an acid pH. This could be explained by the fact that a neutral pH condition favors both oxidation reactions (direct and indirect) in agreement with Singer & Gurol (1983), who show that the oxidation reaction of phenol by ozone is influenced by pH, with the reaction increasing as the pH increases, and with Jeworski & Heinzle (2000), who indicate that the efficiency of the ozonation process is greater at neutral pH levels with the amount of ozone required to oxidize organic molecules. In addition, Martín et al. (2005) state that ozone is a selective oxidant to eliminate phenolic compounds in vinasse (ozone-phenol reaction between 20 and 4 times faster than with the other organic compounds present in the waste) without greatly decreasing the organic load that can be transformed into methane. Siles et al. (2011) mention that ozonation has a high affinity and selection for phenolic compounds, obtaining sub-products from this reaction which are easily assimilable by microbial communities present in the anaerobic processes, such as aldehydes, ketones, alcohols, and carboxylic acids.

TABLE 5. REMOVAL OF TOTAL PHENOL CONTENT IN PRETREATMENTS..

Pretreatment	Initial phenol content *	Final phenol content *	% phenol removal
Vin Oz 7' (pH 7.4)	306.120	158.070	48.36
Vin Oz 15' (pH 7.4)	306.120	134.200	56.16
Vin Oz 30' (pH 7.4)	306.120	115.090	62.40
Vin Oz 7' (pH 4.4)	306.120	243.180	20.56
Vin Oz 15' (pH 4.4)	306.120	213.320	30.31
Vin Oz 30' (pH 4.4)	306.120	196.770	35.72

* Values expressed in mg equivalent to gallic acid L^{-1}

According to the variance analysis shown in **Table 6**, the decrease in the concentration of phenolic compounds in vinasse does not depend on the interaction between ozone application time and pH; rather, it depends on the intensity of the factors, that is, the ozone time of application factor and the pH factor individually influence the decrease in phenol in the substrate.

TABLE 6. TWO-WAY ANOVA: PHENOL CONCENTRATION IN TERMS OF OZONATION TIME AND PH

Factor	*Sum of squares*	*F*	*P*
Ozonation time	2877.2	39.89	0.000
pH	22904.0	635.14	0.000
Time - pH	183.1	91.6	0.159

Table 7 shows the variation in phenol concentration for the different ozone application times (7 – 15 – 30 minutes). It can be observed in the table that the phenolic compounds decrease as the time becomes longer. The percentage removal of the concentration of phenolic compounds in the vinasse is 34% at minute 7 and 43% and 46% at minutes 15 and 30, respectively.

TABLE 7. VARIATION IN PHENOL CONCENTRATION IN TERMS OF OZONATION TIME

Ozonation time (min)	7	15	30
Phenol concentration (mg L^{-1} gallic acid)	200.57 ± 44.43^{a}	173.53 ± 46.11^{b}	164.0 ± 56.55^{b}

Mean ± standard deviation (n=4)

Data with different letters in the same row indicate a significant difference (p<0.05)

Likewise, **Table 8** shows the variation in phenol concentration at different pH levels (7.4 – 4.4). It can be observed in the table that the phenolic compounds show a greater decrease at pH 7.4, which indicates that the oxidation reaction between the ozone and the phenol increases when the vinasse has a nearly neutral pH with a percentage removal of 62% and 35% for pH levels of 7.4 and 4.4, respectively.

TABLE 8. VARIATION IN PHENOL CONCENTRATION IN TERMS OF SUBSTRATE PH

pH	7.4	4.4
Phenol concentration ($mg\ L^{-1}$ gallic acid)	135.68±19,87[a]	223.06±16,15[b]

Media ± desviación estándar (n=4)

Datos con diferentes letras en la misma fila indican diferencia significativa (p<0,05)

3.3. Methane production

Table 9 shows a summary of the accumulated methane production for all of the anaerobic digestion tests performed during the 25 days of the trail. The table is organized by arithmetic averages and standard deviations based on the final ready (25 days).

TABLE 9. SUMMARY OF METHANE PRODUCTION IN TREATMENTS.

Substrate	*Mean (ml de CH_4 g COD L^{-1})*
Vin Oz 7' (pH 7.4)	35.5 ± 16.6
Vin Oz 7' (pH 4.4)	23.3 ± 3.37
Vin Oz 15' (pH 74)	42.7 ± 3.88
Vin Oz 15' (pH 4.4)	24.0 ± 2.82
Vin Oz 30' (pH 7.4)	104.3 ± 27.5
Vin Oz 30' (pH 4.4)	44.5 ± 9.44
Inoculum	0.12 ± 0.02
Raw vinasse	28.7 ± 19.7
AGV	114.3 ± 8.4

Mean ± standard deviation (n=3)

As can be observed in **Table 9**, the treatment Vin Oz 30' (pH 7.4) showed the highest accumulated methane production of 104.3mL of CH_4 g COD L^{-1}, similar to that produced by control 3 (AGV, 114.3ml g COD L^{-1}). Control 3 does not contain inhibitor compounds, and methane production is the result of the digestion of acetic acid, propionic acid, and butyric acid with a proportion of 73:23:4, which served as a substrate for the anaerobic digestion process.

The other treatments had a methane production of between 23.3 and 45ml g COD L^{-1}. It can be observed that the methane production values are higher with pretreated vinasse with a nearly neutral pH compared to those obtained with pretreated vinasse with an acid pH. Likewise, the greatest production of methane was obtained at 30 minutes of ozone application compared to times of 7 and 15 minutes.

According to the variance analysis of the methane production results for all of the treatments presented in **Table 10**, the increase in methane production for pretreated vinasse does not depend on the interaction between ozone application time and pH in the treatments; ozone application time and pH individually affect methane production.

TABLE 10. TWO-WAY ANOVA FOR METHANE PRODUCTION IN TERMS OF TIME AND PH

Factor	*Sum of squares*	*F*	*P*
Ozonation time	11677	30.70	0.000
pH	8991.67	47.28	0.000
Time - pH	535.48	1.41	0.2824

Table 11 shows the variation in methane production for the different ozone application times (7 – 15 – 30 minutes). As can be observed, methane production increases as the ozonation time becomes longer, showing the importance of applying ozone as an oxidant for compounds that are difficult to degrade, favoring the growth of microorganisms in the anaerobic system and thereby increasing the efficiency of methane production.

TABLE 11. VARIATION IN METHANE PRODUCTION IN TERMS OF OZONATION TIME

Ozonation time (min)	7	15	30
Methane production (ml de CH_4)	18.33 ± 21.54[a]	22.67 ± 22.07[a]	74.4 ± 37.5[b]

Mean ± standard deviation (n=6)

Data with different letters in the same row indicate a significant difference (p<0.05)

Table 12 shows the variation in methane production in terms of pH. It can be observed that the greatest methane production occurs at a pH of 7.4, which indicates that the decrease of toxic compounds in the vinasse occurs in a greater proportion at a nearly neutral pH. This could be attributed to the fact that the oxidation reaction between ozone and phenol is influenced by pH. This is a directly proportional relationship; that is, as pH increases, so does the oxidation reaction (Singer & Gurol, 1983).

TABLE 12. VARIATION IN METHANE PRODUCTION IN TERMS OF SUBSTRATE PH

pH	4.4	7.4
Methane production (ml de CH_4)	16.11 ± 21.8[a]	60.82 ± 36.53[b]

Mean ± standard deviation (n=6)

Data with different letters in the same row indicate a significant difference ($p<0.05$)

4. CONCLUSIONS

Ozone pretreatment (50mg L^{-1} O3 – 30 min) showed removal of phenolic compounds from sugarcane vinasse of 62% at pH 7.4 and of 35% at pH 4.4.

The ozone oxidation reaction in a pure phenol solution generates an 89% removal of phenolic compounds. In the vinasse evaluated, the removals of 27% and 54%, with the lower percentage removal at pH 7.4 and pH 4.4, respectively. This difference is mainly due to the nature of the phenolic compound mixes in the substrate and to the elevated content of organic matter that can act as inhibitors in the phenol compound oxidation process (Forero et al., 2005).

The greatest methane production (104.3ml g COD L^{-1}) was obtained from vinasse treated with ozone at 50mg L^{-1} at 30 minutes of application at pH 7.4 for 25 days.

AOP treatment (O_3) is a good option for improving the production of biogas from agro-industrial waste since a 70% increase was demonstrated in methane production using pretreated vinasse compared to gas production using raw vinasse.

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