Diocléa Almeida Seabra Silva
(Organizadora)

Agronomia: Elo da Cadeia Produtiva 6

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APRESENTAÇÃO

A cadeia produtiva é um termo amplo que define com clareza onde cada segmento tem seu grau de importância seja na produtividade de frutos, venda de semente de capineira, na pesca, na aquicultura, na formação de resíduos para a indústria, no controle determinado de vírus, bactérias, nematóides para a agricultura e até mesmo na comercialização de espécies florestais com potencial madeireiro. Na verdade, o termo cadeia produtiva é um conjunto de ações ou processos que fazem presente em estudos científicos que irá dar imagem para o avanço de um produto final.

A imagem de um produto final se torna possível quando trabalhamos todos os elos da cadeia, como por exemplo: para um produtor chegar a comercializar o feijão, ele precisará antes preparar seu solo, ter maquinários pra isso, além de corririr o solo com corretivo, definindo a saturação de base ideal, plantar a semente de boa qualidade, adubar, acompanhar a produção fazendo os tratos culturais adequados, controlando pragas, doenças e ervas daninhas, além de encontrar mercados para que o mesmo possa vender sua produção. Esses elos são essenciais em todas as áreas, ao passo que na produção de madeira será necessário técnicas sofisticadas de manejo que começa na germinação de sementes, quebra de dormência para a formação de mudas, e além disso padronizar espaçamento, tratos silviculturais para a formação de madeira em tora para exportação.

Na pesca a cadeia produtiva segue a vertente do ganho de peso e da qualidade da carne do pescado, que está vinculada a temperatura, pH da água, oxigenação, alimentação e o ambiente para que haja produção. Também a cadeia se verticaliza na agregação de preço ao subproduto do pescado como o filetamento para as indústrias, mercado de peixe vivo e etc.

Na cadeia cujo foco são os resíduos da indústria açucareira, há mercados para a queima de combustível no maquinário da indústria, através da qualidade deste resíduo, além de mercados promissores para a fabricação de combustíveis, rações e até mesmo resíduo vegetal para incorporação nos solos, com a finalidade de manter ou melhorar as características químicas, físicas e biológicas, além de controlar erosão e elevar os níveis de produtividade nas áreas agrícolas, através da adição de nutrientes.

Contudo, sabemos que todos os elos que compõem a cadeia produtiva são responsáveis por agregar valor e gerar de maneira direta e indireta renda aos produtores e pescadores, possibilitando-os na melhoria da qualidade de vida, além da obtenção de produtos de alta qualidade. No entanto, aqui se faz presente a importância das pesquisas mostradas neste E-Book, v. 6 – Agronomia: Elo da Cadeia Produtiva para que o leitor possa perceber novidades que são contextualizadas, através dos trabalhos aqui publicados.

Diocléa Almeida Seabra Silva

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BIOGAS PRODUCTION FROM SECOND GENERATION ETHANOL VINASSE

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ABSTRACT: Anaerobic digestion has raised as an efficient solution for both biowastes management and renewable energy supply. In Brazil, the sucroenergetic industry generates great amounts of vinasse, the biowaste from ethanol process, which has high contents of soluble organic matter. Such feature makes vinasse a very suitable wastewater for biogas production. Very recently, second generation (2G) ethanol process has been implemented in the Brazilian industry and 2G vinasse is expected to a different chemical composition than first generation (1G) vinasse, since the 2G ethanol process has different operations. In this study we aimed to investigate the 2G vinasse potential for biogas production. Affluent 2G vinasse was characterized and high concentrations of acetic acid, total phenolic compounds (TPC) and sulphate were determined. No detectable concentration of furans was found. The experiment was carried out in a 5 L up flow reactor and the process was monitored by volumetric biogas production and concentration of Chemical Oxygen Demand, acetic acid, propionic acid, butyric acid, TPC and sulphate. Effluent recirculation was employed in the process for a ten days period. The experiment reached a mean value for biogas production of 0.41 (± 0.19) L_{biogas} DQO_{remov}^{-1}, which is a satisfactory result in comparison to previous studies with 1G vinasse. Effluent recirculation

had a positive impact on biogas production process, which was observed by organic acids concentrations decrease. Results indicate that 2G vinasse has a very interesting potential for biogas production especially due to its high acetic acid content.
KEYWORDS: biogas; anaerobic digestion; second generation vinasse; lignocellulosic ethanol; effluent recirculation

PRODUÇÃO DE BIOGÁS A PARTIR DE VINHAÇA DE ETANOL DE SEGUNDA GERAÇÃO

1 I INTRODUCTION

Brazil is among the countries with the largest shares in renewable energy contribution. In 2018, 83.3% of electricity in Brazil came from sustainable energy. In the same year, renewable sources contributed with 45.3% in total energy generation. Among renewable sources, sugarcane biomass had the largest participation in the Brazilian energy supply, with 17.4% (EMPRESA DE PESQUISA ENERGÉTICA, 2019).

In Brazil, biogas represents only 0.07% of the total energy supply. Meanwhile, in other countries, especially in Europe, biogas production by anaerobic digestion is seen as a crucial technology for achieving sustainable energy and an efficient alternative for waste management (EMPRESA DE PESQUISA ENERGÉTICA, 2019; ANDRITZ GROUP, 2013).

More recently, the second generation (2G) ethanol process has expanded, which is the ethanol produced from lignocellulosic hydrolysate. Such process is not available all over the country yet, although it is expected to expand soon, since its implementation might increase ethanol production by about 160% (DIAS et al., 2012).

Meanwhile, the first generation (1G) ethanol process generates about 10-15 L of vinasse per liter of ethanol. Considering that Brazil produced 33.1 billion liters of ethanol in 2018/2019, vinasse production was estimated at about 330 billion liters for the same period (UNICADATA, 2019; CORTEZ, 2014; PANT; ADHOLEYA, 2007).

Although 2G ethanol process has become feasible in the Brazilian industry very recently, it is already known that it does not reduce vinasse generation. In general, 2G process allows higher sugar concentrations in fermentation broths and, besides, it also carries byproducts from sugarcane bagasse physical chemical treatments along the process. Because of these factors, it is very likely for fermentation broths in 2G process to be even more diluted than those from 1G process, what might lead to higher vinasse volumes (JARDINE; DISPATO; PERES, 2009; MONLAU et al., 2014).

Vinasse has a polluting potential about a hundred times higher than domestic sewage and its composition is very variable, depending on sugarcane variety, yeast

strain, ethanol production process, etc (CORTEZ, 2014).

For 2G ethanol process, vinasse composition is expected to be different from 1G ethanol vinasses. Physical, chemical and enzymatic treatments are applied on sugarcane bagasse and, during the process, compounds such as organic acids and phenolic compounds are released into the fermentation broth. Since they are not significantly consumed during alcoholic fermentation, those compounds might be found in vinasse. Some of these compounds are organic acids, phenolic compunds and furans, such as furfural and 5-hydroxymethylfurfural (CORTEZ, 2014; PANT; ADHOLEYA, 2007; JARDINE; DISPATO; PERES, 2009; PALMQVIST; HAHN-HAGERDAL, 2000; KLINKE; THOMSEN; AHRING, 2004).

In this study, our purpose was to investigate the 2G vinasse potential for biogas production. Special attention was given to 2G vinasse compounds such as total phenolic compounds (TPC) and organic acids, which are potential microbial inhibitors and byproducts from sugarcane bagasse pretreatment. Anaerobic digestion was carried out in laboratory scale and monitored by biogas volumetric production, COD removal, organic acids production, phenolic and sulphate consumption.

2 I MATERIAL AND METHODS

Both methanogenic inoculum and 2G vinasse were obtained from industrial plants in São Paulo state, Brazil.

The experiment was performed in a 5 L up flow reactor, operated with 4 L working volume, at 38 ± 2 ºC. The reactor was set up with 3 L of granular sludge and 1 L of 2G vinasse. Experiment was carried out as a fed-batch process. During operations, the feeding volume was 0.5 L. Hydraulic retention time was 16.61 (± 2.3) days and data collection took 64 days of experiment.

In operating the reactor, effluent recirculation was employed between 47th and 57th days of experiment in order to increase biogas production by returning hydrolysate organic matter (PEREIRA-RAMIREZ, 2004). During effluent recirculation period, the same feeding volume was used and affluent consisted of a mixture of 2G vinasse and reactor effluent (1:1).

For our experiments, *in natura* 2G vinasse was diluted and the affluent Chemical Oxygen Demand (COD) concentration was 13,751.12 (± 2,598.21) $mgO_2\ L^{-1}$. Vinasse 's pH was adjusted to 7.3 (± 0.5) using $NaHCO_3$.

2.1 Analytical Methods

Vinasse organic matter content was analyzed by COD concentration, which was determined using the colorimetric method described in (APHAa, 2012). Samples were diluted fifty times. Inoculum was characterized by its volatile total solids (VTS) content before starting the experiment and in the end of data collection. Analyses

were performed in triplicate (APHAb, 2012).

Biogas volumetric production was measured daily, using a Wet Tip Gas Meter®. Total phenolic compounds (TPC) analyses were performed following the procedure described elsewhere (JULKUNEN-TIITTO, 1985). Samples were previously diluted fifty times.

Organic acids (acetic acid, butyric acid and propionic acid) were analyzed in a UFLC Prominence high performance liquid chromatography system (Shimadzu®) with Aminex HPX-87H (300 mm x 7.8 mm; Bio-Rad®) column. Analyses were carried out as previously described by other authors (PENTEADO et al., 2013). Samples were diluted a hundred times and filtered with hydrophilic membrane syringe filters, pore size 0.45 µm.

Furfural and HMF analyses consisted of a system with a Shim-pack VP-ODS (5 µm) de 250 x 4.6 mm column, Shimadzu®, at 25 ºC, eluted with acetonitrile and acetone (1:8 v v^{-1}) in acetic acid (1% v v^{-1}), at a flow rate of 0.8 mL min^{-1}, and DAD detector (SPD-M20A) (275 nm). Samples were diluted fifty times, filtered with hydrophilic membrane syringe filters, pore size 0.45 µm and analyzed in a volume of 20 µL. Sulphate was determined using 930 Compact IC Flex system (Metrohm®), with Metrosep A Supp 5 250/4.0 column, at 25ºC. Analyses were carried out as described elsewhere (NARAYARAN, 2016).

Dispersion statistics analyses and histograms were performed using Microsoft Excel® 2010.

3 I RESULTS AND DISCUSSION

3.1 Inoculum and affluent 2G vinasse characterization

Inoculum VTS contents in the beginning and in the end of experiment were 17,900 (± 2,969) mg L^{-1} and 80,920 (± 5,830) mg L^{-1}, respectively, indicating that methanogenic inoculum had important cellular growth during 2G vinasse treatment process.

Data shown in Table 1 present affluent 2G vinasse composition in other important compounds for anaerobic digestion.

$COD_{affluent}$ (mg L^{-1})	13,751.12 (± 2,598.21)
Acetic $Acid_{affluent}$ (mg L^{-1})	9,999.71 (± 1,889.40)
Butyric $Acid_{affluent}$ (mg L^{-1})	737.59 (± 139.36)
Propionic $Acid_{affluent}$ (mg L^{-1})	ND
Total Phenolic $Compounds_{affluent}$ (mg L^{-1})	812.14 (± 225.69)
Furfural (mg L^{-1})	ND
HMF (mg L^{-1})	ND
Sulphate (mg L^{-1})	949.69 (± 214.70)

Table 1. Chemical composition of affluent vinasse

ND: not detectable.

No detectable concentrations of furfural and HMF were found. However, it is noteworthy how relevant acetic acid concentration was, as high as 9,999.71 (± 1,889.40) mg L^{-1}. Some authors report that acetic acid concentration in 1G vinasses might be up to 2,200 mg L^{-1} (DOWD et al., 1994; ESPAÑA-GAMBOA et al., 2012).

Besides acetic acid, affluent TPC and affluent sulphate were present in relatively high concentrations. In 2G vinasse, the high TPC concentration (812.14 ± 225.69 mg L^{-1}) is a consequence of lignocellulosic material hydrolysis (MONLAU et al., 2014). In 1G vinasses, TPC are not supposed to be found in detectable concentrations since fermentation broth is not derived from lignocellulosic feedstock (MORAES; ZAIAT; BONOMI, 2015).

In regards to sulphate concentration, it is very variable in anaerobic systems. Some authors have reported working with affluent sulphate concentrations from 140 to 1,100 mg L^{-1} (LÓPEZ-LÓPEZ et al., 2015; NACHEVA et al., 2009). In our analyses, affluent 2G vinasse had 949.69 (± 214.70) mg L^{-1} of sulphate, which is a high concentration according to literature.

3.2 Biogas Production

Biogas daily production throughout 64 days of experiment is given in Figure 1. The mean value for daily volume was 0.79 (± 0.33) L day^{-1}. The process had an increasing biogas production until 16th day, when it reached maximum volumetric production (1.65 L day^{-1-}).

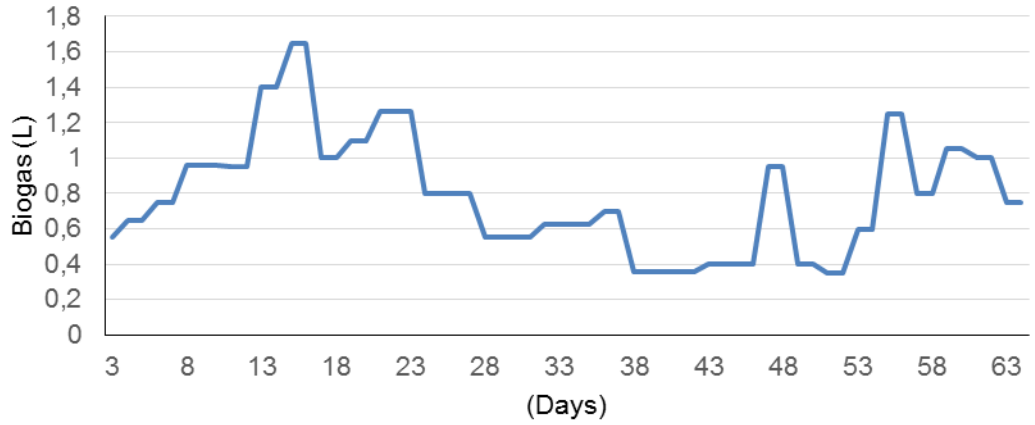

Figure 1. Daily biogas production

In our studies, the average COD removal was 39.47% (± 17.19) and biogas production per COD removal was 0.41 (± 0.19) L_{biogas} $gCOD_{removed}^{-1}$. Anaerobic systems have potential for removing up to 90% of affluent COD (MOLINA et al., 2007; GAO;

SHE; JIN, 2007). However, lower COD removal efficiencies, such as 30-45%, have also been reported for other anaerobic systems (FRANCO et al., 2007; HARADA et al., 1996). Concerning biogas production, other authors (HARADA et al., 1996; ESPAÑA-GAMBOA et al., 2012) have reported results such as 0.21 and 0.35 L_{biogas} $gCOD_{removed}^{-1}$ for 1G vinasse as substrate in processes with 20-69% COD removal efficiency.

Some authors have reported that systems under operation for longer periods of time (about 200 days) are likely to achieve higher COD removal efficiencies (≥ 80%) (LÓPEZ-LÓPEZ et al., 2015; HARADA et al., 1996; OMIL et al., 1998).

Firstly, biogas production and COD removal are strongly linked to many factors in an anaerobic system, such as organic matter content and its composition, inoculum characteristics, reactor design and many others (McCARTY, 1964). Secondly, it is important to state that COD concentration is a non-specific determination of organic matter. Comparing our study with the results obtained by the authors cited above (ESPAÑA-GAMBOA et al., 2012; HARADA et al., 1996), it is possible to notice that although higher COD removal efficiencies were achieved by the authors, lower volumes of biogas (in terms of L_{biogas} $gCOD_{removed}^{-1}$) were produced as well.

It is important to point out that the vinasse used in one of those studies (ESPAÑA-GAMBOA et al., 2012) had a very different composition in acetic acid (2,237 mg L^{-1}) when compared to the 2G vinasse we employed in our experiment (Table 1). Acetic acid is a substrate directly assimilated by acetoclastic methanogenic archea. Since 2G vinasse had a high concentration of acetic acid, it is suggested that such supply made acetoclastic methanogenic archea nearly independent from bacterial organic matter degradation for acetic acid availability. Biogas production due to high acetic acid concentration is strongly supported by previously elucidated biochemical pathways in anaerobic systems (MOSEY, 1982; McCARTY, 1964). However, further research would be required to definitely determine if that was the only 2G vinasse compound involved in such performance.

Acetic acid concentration as high as what was determined in 2G vinasse is a consequence of sugarcane bagasse pretreatment. Hemicellulose, one of bagasse components, is majorly consisted of sugars and acetyl groups. Once enzymatic treatment releases fermentable sugars into hydrolysate, acetyl groups are released as well and might be converted into acetic acid (JARDINE; DISPATO; PERES, 2009). Since acetic acid is not significantly consumed during alcoholic fermentation, it might be carried along the process until vinasse generation. Acetic acid may be detected in 1G vinasses as well, but in those cases the main source is contamination by acetic bacteria during alcoholic fermentation (DOWD et al., 1994).

Thus, our results indicate that despite lower COD removal efficiency, the system was capable of giving satisfactory biogas production when compared to other authors' results (ESPAÑA-GAMBOA et al., 2012; HARADA et al., 1996), most probably due to 2G vinasse content in acetic acid.

By the 40th day biogas production efficiency decreased, it did not recover satisfactorily and organic acids concentration increased. For this reason, effluent recirculation was employed (47th - 57th days) and biogas production was recovered after 47th day of experiment.

It was later observed that preferential flow channels were formed inside the reactor, what might have disturbed organic matter diffusion and metabolites transfer among microrganisms .The reactor we used in the experiment had a single channel for affluent entrance. The implementation of structures to facilitate efficient affluent diffusion once they reach the reactor interior would probably have minimized this kind of problem.

Once complex organic matter is hydrolyzed by bacteria, carbohydrates, proteins and others organic polymers are converted majorly into butyric acid, propionic acid and acetic acid. Effluent recirculation means recirculating hydrolyzed organic matter, decreasing organic acids concentrations because it minimizes complex organic matter input in the system (PEREIRA-RAMIREZ, 2004; LÓPEZ-LÓPEZ et al., 2015).

Between 24th and 46th days, when decreasing production was observed, average biogas production was 0.55 L day^{-1}. Between 47th and 57th days, average biogas production reached 0.72 L day^{-1}.

Although acetic acid concentrations were very high until 14th day (Table 2), the system was able to handle the acid conditions and biogas production did not drop. From 21st day on, the reactor effluent presented unstable acetic acid concentrations. By the 40th day, biogas production dropped and in the 44th day the reactor had very elevated concentrations of organic acids. By the 60th day, after effluent recirculation, acetic acid, propionic acid and butyric acid concentrations dropped by, respectively, 95.21%, 100% and 75.86%.

Day	Acetic acid (mg L^{-1})	Propionic acid (mg L^{-1})	Butyric acid (mg L^{-1})	TPC removal efficiency (%)
3	829.98	ND	ND	66.44
7	1,927.66	ND	605.24	-
14	6,132.42	2495.52	712.20	59.91
21	9,326.25	2,686.2	2,796.51	-
24	-	-	-	31.18
28	10,693.62	3178.34	1768.57	-
35	-	-	-	67.12
36	,7,081.2	3,155.46	1,753.99	-
40	2,715.57	2,133.3	1,311.96	-
42	2,920.23	2,094.9	2,560.62	70.57
44	3,982.77	4,817.64	2,423.59	-
49	2,428.65	3,334.26	2,240.94	40.87
52	1,663.20	3,290.34	1,468.965	-
56	1,557.27	ND	818.35	-
58	764.69	ND	ND	-

60	190.88	ND	585.14	46.14
64	-	-	-	60.10

Table 2. Analyses of acetic acid, propionic acid and butyric acid quantification and analysis of TPC removal efficiency in the effluent of anaerobic digestion of 2G vinasse.

ND: not detectable. (-) Analyses were not performed in this date.

Although it is desirable to keep organic acids at the lowest possible concentrations, previous studies have also reported high organic acids concentrations. Some determined acetic acid concentrations as high as 1,370 and 6,000 mg L^{-1}; butyric acid between 886 and 1,193 mg L^{-1}; and propionic acid concentrations such as 2,800 and 3,009 mg L^{-1} (LEITE et al., 2015; GOODWIN; STUART, 1994; ESPAÑA-GAMBOA et al., 2012; LÓPEZ-LÓPEZ et al., 2015).

Total phenolic compounds concentration was monitored during the experiment (Table 2). Due to their hydrophobicity, phenolic compounds might have negative effects on different microbial groups. Cellular membrane permeability might be destabilized and intracellular components might leak out. Besides, once phenolic compounds reach the cytoplasm, they might inactivate enzymes or lead to the formation of reactive oxygen species (H_2O_2, O_2^-, OH^-). Apoptose cell process might be initiated or cell metabolism is decreased (MONLAU et al., 2014).

It was observed that the system was able to steadily consume affluent TPC, with a mean value of 55.29% (± 14.22) of removal efficiency. It was noticed that between 24th and 42nd days, when lower biogas production was achieved and higher organic acids concentration were detected, TPC removal efficiencies did not drop.

In anaerobic systems, some authors reported that TPC concentrations up to 500 mg L^{-1} are well tolerated by microorganisms involved in biogas production (CHAPLEUR et al., 2016) and in the presence of high acetic acid concentrations (above 2,000 mg L^{-1}), microorganisms' TPC tolerance is increased to up to 1,200 mg L^{-1} of phenol (FEDORAK; HRUDEY, 1984). Therefore, our results indicate that not only the system was not inhibited by 2G vinasse composition in terms of TPC, but it was also able to steadly consume them.

Regarding sulphate removal, the system had an average efficiency of 85.76% (±16.14). Anaerobic systems are usually very efficient in removing sulphate, since SRB are commonly present in anaerobic inocula. Other authors (GODOI; DAMIANOVIC; FORESTI, 2015) reached from 85.2% (± 4.6) to 97.3% (±1.4) of sulphate removal efficiency.

Anaerobic sludges are very complex inocula. Besides archea and bacterial groups important for methanogenesis, there are also sulphate reducing bacteria (SRB). Whenever there is sulphate in an anaerobic system, SRB compete with hydrogenotrophic archea for H_2 and H_2S is produced. Aiming at biogas production, such metabolic pathway is not desirable for biogas production, because H_2S is a toxic, corrosive gas and it decreases biogas quality (GODOI; DAMIANOVIC; FORESTI,

2015; STAMS; PLUGGE, 2009).

4 I CONCLUSION

Biogas production was not inhibited by 2G vinasse composition and it reached satisfactory performance, achieving mean value of 0.41 (± 0.19) L_{biogas} $gDQO_{remov}^{-1}$. Acetic acid is a crucial compound for biogas production and it was determined at very high concentration in affluent 2G vinasse, 9,999.71 (± 1,889.40) mg L^{-1}. Then, the 2G vinasse composition in terms of acetic acid might have strongly contributed for the satisfactory biogas production results. Furans, which could have led to biogas production inhibition, were not detected in the 2G vinasse used in this study. Other important compounds in 2G vinasse were total phenolic compounds. Total phenolic compounds were detected at 812.14 (± 225.69) mg L^{-1} and removal efficiency was 55.29% (± 14.22).

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