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ARTIGO ORIGINAL

VINASSE FROM THE BRAZILIAN LIGNOCELLULOSIC ETHANOL PROCESS: CHEMICAL COMPOSITION AND POTENTIAL FOR BIOPROCESSES

VINHAÇA DO PROCESSO DE ETANOL LIGNOCELULÓSICO BRASILEIRO: COMPOSIÇÃO QUÍMICA E POTENCIAL PARA BIOPROCESSOS

VINAZA DEL PROCESO DE ETANOL LIGNOCELULÓSICO BRASILEÑO: COMPOSICIÓN QUÍMICA Y POTENCIAL PARA BIOPROCESOS

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ABSTRACT

Brazil is the second-largest producer of ethanol and the alcoholic fermentation wastes have become a concern for both environmental and economic reasons. Recently, the Brazilian industry has implemented the second generation (2G) process to attend the growing for biofuel. In this study, we aimed to investigate whether the 2G vinasse faces the same environmental challenges that first generation (1G) vinasses do, meaning vinasses from ethanol processes using sugarcane juice and/or molasses. Thus, vinasse was obtained from one of the recently-started 2G ethanol facilities in São Paulo State and then chemically characterized. Considering glycerol, mannitol, residual sugars, and organic acids concentrations altogether, it was determined that 2G vinasse had a total carbon source of 23,050 mg L-1 (compared to 4,800 mg L-1 in 1G vinasse). Magnesium, calcium, potassium, and others salts were determined as well. Based on its chemical composition, vinasses could be considered as nutrient sources for other bioprocesses. Finally, we brought some perspectives into bioprocesses with nutritional requirements that might be fully or partially provided by vinasses, leading to the production of bioenergy or bioproducts.

Keywords: Sucroenergetic sector. Chromatographic analyses. Waste valorization. Biorefinery. 2G vinasse.

1 INTRODUCTION

The sucroenergetic sector plays a very important role in the Brazilian economy, accounting for about 2% of the Brazilian Gross Domestic Product (UNICA, 2019a). Internationally, Brazil is an important player in ethanol production as the second-largest producer in the world. During the 2018/19 crop, Brazil produced 33 billion L of ethanol, and the largest ethanol producer, the United States, produced about 60 billion L in 2017 (UNICA, 2019b; U.S. ENERGY INFORMATION ADMINISTRATION, 2019). In this scenario, the Brazilian industry aims to more efficient processes so the growing demand for biofuels may be met.

According to recent studies on technical-economic evaluation, second generation (2G) ethanol processes have great potential in systems integrated with first-generation (1G) processes. Because 2G ethanol production requires obtaining fermentable sugars from lignocellulosic feedstocks, different methods and operations are required, such as physical-chemical pretreatments and enzymatic hydrolysis, so production costs are higher when compared to 1G ethanol process (STEPHEN *et al*., 2012). Thus, a stand-alone 2G ethanol process might be more expensive than 1G processes. However, once they are integrated, 2G process provides a higher ratio of volumetric production per sugarcane ton. In addition to the optimization of facilities use, the integrated process becomes more attractive than specialized 1G or 2G production facilities (MACRELLI *et al*., 2014; DIAS *et al*., 2012).

Co-fermentation of hexoses and pentoses is still challenging for industrial scales. Therefore, integrated 1G and 2G processes might be configured as separate operations for 1G and 2G fermentations and a single distillation process, with a mixed (1G + 2G) fermented broth as input (MACRELLI *et al*., 2014).

Feedstocks, operations and process conditions have significant effects on industrial wastes composition. As for the 2G ethanol process, it requires different sugar sources and different operations by employing severe physical-chemical treatments and enzymatic hydrolysis on lignocellulosic feedstock to obtain fermentable sugars. Regarding ethanol from sugarcane, 2G ethanol process means using bagasse to obtain a sugar hydrolysate, rich in hexoses and pentoses. Though some undesired byproducts are commonly generated as well, such as organic acids, phenolic compounds, and furfuraldehydes (furfural and 5-hydroxymethylfurfural). During alcoholic fermentation, these byproducts are not significantly consumed, therefore they might be found in vinasse (JARDINE *et al*., 2009).

The 2G ethanol process is an emerging technology in the Brazilian industry and extensive research is needed so that effective treatment and management may be established for 2G organic wastes, based on their specific characteristics and composition.

Vinasse is the most important waste from alcoholic fermentation since it is generated at very large amounts: in the sugarcane 1G process, every 1 L of ethanol generates an average ratio of 10-15 L of vinasse (CORTEZ, 2010; ESPAÑA-GAMBOA *et al*., 2012; MORAES *et al*., 2015). For the 2G ethanol process, there is no information for such a production ratio yet, although the undesired byproducts mentioned above might have inhibitory effects on fermentative microorganisms and the process might require diluted fermentation broths.

Vinasse fertirrigation became a common practice in Brazil during the 80s, when ethanol production had a fast increase because of government incentives. In the 1980/81 crop, Brazil produced 3.7 billion L of ethanol and about 37 billion L of vinasse. By the 1989/90 crop, Brazil produced 11.9 billion L of ethanol and about 119 billion L of vinasse, meaning an increase of over three times in less than ten years (UNICA, 2020).

Before the 80s, vinasses with no previous treatment were disposed of in rivers or other water bodies (RIBEIRO *et al*., 1983; SANTOS *et al*., 1981). Given the significant increase in vinasse generation during the 80s, fertirrigation came up as an immediate and satisfactory alternative for an increasing volume of vinasse in such a short time.

Over the last forty years, the Brazilian industry has continuously increased the annual ethanol production, which also led to an increase of almost ten times in vinasse volume from the 1980/81 crop until 2018/19 crop. In the meantime, the Brazilian industry has kept the same practices regarding vinasse management from the 80s until nowadays.

So, considering a process that employs severe physical-chemical treatments on lignocellulosic material, such as 2G ethanol process, would vinasse from such process bring even more risks of soil contamination once applied in fertirrigation? Would legislation based on potassium content be enough to regulate the safe amounts of 2G vinasse for fertirrigation? Would there be any other applications for vinasses that could be safer for the environment, human health, and bring economic benefits as well?

In this study, we characterized the vinasse generated in one of the recently-started 2G ethanol facilities in Brazil, which employs the integrated 1G and 2G process. By analyzing vinasse composition, we aimed to propose biotechnological applications that might lead to the production of important bioproducts and bioenergy.

2 MATERIALS AND METHODS

2.1 MATERIALS AND SAMPLES PREPARATION

In this study, two types of vinasse were analyzed. A sample of vinasse from an ethanol process using sugarcane molasse (1G) was used as control for analyses. The 2G vinasse was obtained from an integrated production unit, 1G + 2G ethanol process. Thus, in this study, we name 2G vinasse the one composed of a mixture of 1G and 2G vinasses. Further details on the integrated process, such as 1G and 2G ratios, which operations are common to 1G and 2G processes or characteristics of fermentative microorganisms were not provided by the industry due to corporate and legal reasons related to patent deposit.

Both vinasses were obtained in a concentrated form from distilleries in São Paulo State, Brazil. Before analyses, they were both diluted to in natura concentrations: 1G vinasse, 3,2 ºBx; 2G vinasse, 3,8 ºBx. Diluted vinasses were not submitted to any other pre-treatments, except those required by the analytical methods described below. Samples were stored in 4ºC and kept in room temperature before analyses.

2.2 ANALYTICAL PROCEDURES

2.2.1 Chemical Oxygen Demand (COD)

Vinasses were characterized using the colorimetric method (APHA, 2012). The following solutions were prepared: (i) 2.04% K2Cr2O7 (m v-1), 3.33% HgSO4 (m v-1) and 0.0167% H2SO4 (v v-1) in distilled water; (ii) 1.012% Ag2SO4 (m v-1) in concentrated H2SO4. Reactions were prepared using 2.0 mL of diluted sample (1:50), 1.2 mL solution (i) and 2.8 mL solution (ii).

2.2.2 Total Phenolic Compounds (TPC)

We employed the procedure described by JULKUNEN-TIITO (1985). Samples were diluted 50 times to fit into the calibration curve.

2.2.3 Organic acids and furfuraldehydes

Organic acids, furfural, and 5-hydroxymethylfufural (HMF) were analyzed in a UFLC Prominence high-performance liquid chromatography system (SHIMADZU).

For organic acids characterization, acetic acid, propionic acid, butyric acid, iso-butyric acid, and lactic acid were analyzed. The system consisted of Aminex HPX-87H (300 mm x 7.8 mm; Bio-Rad) column, at 64 ºC, eluted with 0.005 M H2SO4 at a flow rate of 0.4 mL min-1, and a UV-Vis/DAD detector (208 nm). Samples were diluted 100 times, acidified with concentrated H2SO4, until pH ≤ 2.0, as described elsewhere (PENTEADO *et al*., 2013), and filtered in 0.45 µm cellulosic membranes. The volume sample was 100 µL.

The furfural and HMF analyses consisted of a system with a Shim-pack VP-ODS (5 µm) of 250 x 4.6 mm column, 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 (SHIMADZU SPD-M20A) (275 nm). Samples were diluted 50 times, filtered in 0.45 µm cellulosic membranes and analyzed in a volume of 20 µL.

2.2.4 Carbohydrates, anions, and cations

Glycerol, mannitol, sugars, cations, and anions were analyzed using ionic chromatographic systems (930 IC Compact, Metrohm). All samples were diluted 100 times, filtered in 0.45 µm cellulosic membranes and analyzed with a sample volume of 20 µL.

Glycerol mannitol, sucrose, glucose, fructose, xylose, and arabinose were determined in ionic chromatographic system, using Metrosep Carb 1 150/4.0 column, at 30 ºC, eluted with 100 mM NaOH and 10 mM Sodium acetate at a flow rate of 1.0 mL min-1 and amperometric detector (METROHM, 2016).

Sodium, potassium, ammonium, iron, magnesium, and calcium were determined using the cation system in ionic chromatograph: Metrosep C4 250/4.0 column, at 30 ºC, eluted with 7.5 mM tartaric acid, 0.135 mM dipicolinic acid and 3.0 mM ascorbic acid, at a flow rate of 0.9 mL min-1 and a conductivity detector (METROHM, 2015a).

Chloride, nitrate, nitrite, phosphate, and sulfate were determined in ionic chromatograph using the anion system: Metrosep A Supp 5 250/4.0, at 25 ºC, eluted with 3.2 mM Sodium carbonate and 1.0 mM Sodium bicarbonate, at a flow rate of 0.7 mL min-1 and a conductivity detector (METROHM, 2015b).

2.2.5 Statistical analysis

All analyses were carried out in triplicates. Calibration curves, descriptive statistical analyses of means and standard deviation were performed using Microsoft Excel 2010® software.

3 RESULTS AND DISCUSSION

3.1 VINASSES COMPOSITION: FEEDSTOCKS AND OPERATIONS INFLUENCE

Table 1 details the chemical characterization for 1G and 2G vinasses. It also provides reference values, which were obtained from previous studies with 1G vinasse characterization.

Table 1 – Physical-chemical characterization of 1G vinasse and 2G vinasses.

(mg L^{-1})	1G Vinasse	2G Vinasse	Reference	
COD (mgO_2 L^{-1})	26,715.19 ± 161.49	30,969.25 ± 28.18	21,000 - 33,600	CORTEZ, 2010; MORAES *et al*., 2014
Sucrose	285.50 ± 16.02	67.95 ± 7.14		
Glucose	88.78 ± 8.91	66.51 ± 1.98		
Fructose	130.71 ± 13.05	324.70 ± 11.29	Total residual sugars: 962	FERREIRA *et al*., 2011
Xylose	ND	105.00 ± 3.29		
Arabinose	ND	12.16 ± 0.78		
Furfural	ND	ND		
HMF	ND	ND		
Glycerol	1,970.12 ± 3.43	701.28 ± 2.10	1,400	ORTIZ-MUNIZ *et al*., 2010
Lactic Acid	571.27 ± 14.21	6,869.19 ± 739.71	7,740	DOWD *et al*., 1994
Propionic Acid	ND	ND		
Iso-butyric Acid	ND	ND		
Butyric Acid	ND	1,021.18 ± 1,444.17	325.61	PRADO *et al*., 2016
Acetic Acid	1,641.90 ± 700.45	13,844.38 ± 4,189.51	2,200	ESPAÑA-GAMBOA *et al*., 2012; DOWD *et al*., 1994
Mannitol	130.41 ± 1.50	112.98 ± 1.03	89,00	DOWD *et al*., 1994
TPC	510.28 ± 8.26	2,407.21 ± 21.17	1,100	FERREIRA *et al*., 2011

Sulfate	3,440.85 ± 237.40	2,467.17 ± 29.54	23.93 - 5,336	ESPAÑA-GAMBOA *et al*., 2012; PRADO *et al*., 2016
Potassium	8,746.78 ± 526.10	38,966.43 ± 764.26	600 - 13,000	ESPAÑA-GAMBOA *et al*., 2012; MORAES *et al*., 2015; PATHAK *et al*., 1999; BISWAS *et al*., 2009
Sodium	4,959.23 ± 236.05	22,264.89 ± 259.31	50 - 31,300	FERREIRA *et al*., 2011; PEDRO-ESCHER *et al*., 2014; COELHO *et al*., 2018
Calcium	960.59 ± 87.77	6,850.61 ± 347.12	450 - 5,180	ESPAÑA-GAMBOA *et al*., 2011; SOUZA *et al*., 2015
Magnesium	1,739.63 ± 420.63	8,015.92 ± 231.21	420 - 520	ESPAÑA-GAMBOA *et al*., 2011; COELHO *et al*., 2018
Phosphate	109.52 ± 0.61	ND	1.3 - 3,796	PEDRO-ESCHER *et al*., 2014; REIS *et al*., 2015
Nitrate	513.74 ± 0.54	631.69 ± 0.55	1.3 - 17.6	PEDRO-ESCHER *et al*., 2014; CASSMAN *et al*., 2018; SYDNEY, 2013
Chloride	1,546.98 ± 5.08	978.21 ± 10.22	3,500	NASPOLINI *et al*., 2017
Ammonium	ND	ND		

ND: Not Detectable

Source: Original results.

COD in vinasses may vary between 21,000 and 33,600 mgO2 L-1 and it is influenced by many factors, such as type of feedstocks and their quality (concerning microbial contamination), unit operations, process conditions and fermentative microorganisms' metabolism (CORTEZ, 2010; MORAES *et al*., 2015). COD content in both 1G and 2G

vinasses were in accordance with previous studies, however, detailed chemical analysis revealed very different compositions.

Because lignin is the main source of phenolic compounds, detectable concentrations are not expected to be found in 1G vinasses, which are obtained from ethanol processes using sugarcane juice and/or molasses. In our study, TPC concentration in 2G vinasse was over four times higher than the concentration determined for 1G vinasse. Such a difference is clear evidence of how feedstock and operations from the ethanol processes might have an influence on vinasse composition.

The higher concentration we found in 2G vinasse was expected because of the physical-chemical treatment used in sugarcane bagasse for the 2G ethanol process. Lignin is a very complex heterogeneous vegetable polymer, formed by several aromatic compounds. Once they are submitted to severe conditions, such as high temperature and pressure in physical-chemical treatments, several phenolic compounds may be released and eventually found in the hydrolysate. Since they are not significantly consumed during alcoholic fermentation, they are eventually found in vinasse (JARDINE *et al*., 2009).

As a less usual case for 1G vinasses, FERREIRA *et al*. (2011) reported 1,100 mg L- 1 of total phenolic compounds in 1G vinasse, almost twice higher than the concentration we determined for 1G vinasse in this study. That might be explained by the fact that some lignocellulosic parts, such as leaves and straw, are possibly processed during sugarcane milling for syrup production. During sugar production, syrup is submitted to very high temperatures and then molasse is obtained as a byproduct, which is further used as carbon source in alcoholic fermentation (SAHU, 2018). Such residual compounds are not supposed to be consumed during the fermentation process, so, as a result, they will be found in 1G vinasse.

Lactic acid concentration in 2G vinasse was considerably higher than that determined in 1G vinasse. However, our results were consistent with the literature (DOWD *et al*., 1994), which reports that elevated lactic acid concentrations are common in industrial alcoholic fermentation since they are a bacterial contamination product.

Other organic acids may be indicators of bacterial contamination as well, such as propionic, iso-butyric, butyric, and acetic acids. Neither propionic acid nor iso-butyric acid was found in detectable concentration in any of vinasses. Butyric acid was determined only in 2G vinasse, in higher concentrations than those previously reported in the literature for 1G vinasses (PRADO *et al*., 2016).

In comparison to the 1G vinasse we analyzed, and also previous reports in the literature, acetic acid concentration in 2G vinasse was notably higher (ESPAÑA-GAMBOA *et al*., 2012; DOWD *et al*., 1994).

In the 1G ethanol process, the acetic acid in fermented broths and vinasse results mostly from yeast metabolism and, possibly, from acetic bacteria metabolism as well, whenever there is contamination (LOPES *et al*., 2016).

In the 2G ethanol process, however, there might be acetic acid production from both yeasts and bacteria metabolisms, but the enzymatic treatment on sugarcane bagasse may be the main source. Enzymes are used to extract fermentable sugars from hemicellulose, which is a heterogeneous polymer composed by units of pentoses, hexoses, and acetyl groups. After acetyl groups are released into the fermentation broth, they are converted into acetic acid and they are not significantly consumed during alcoholic fermentation. As a result, high acetic acid concentrations might be found in 2G vinasses (JARDINE *et al*., 2009). For these reasons, acetic acid concentration was indeed expected to be found in high concentration in the 2G vinasse.

Furfural and HMF were expected to be found in 2G vinasse, since the 2G ethanol process may lead to sugars degradation due to high temperature and elevated pressure conditions in physical-chemical treatments. Furfural is formed from pentoses degradation and HMF results from hexoses degradation (JARDINE *et al*., 2009). However, our analyses found no detectable concentration of such compounds.

Residual sugars were detected in both vinasses. In 2G vinasse, xylose and arabinose were detected, as well as other residual sugars from alcoholic fermentation, such as sucrose, glucose, and fructose. Residual pentoses were expected to be found in 2G vinasse since 2G ethanol exploits both hexoses and pentoses from sugarcane bagasse.

Similarly, 1G vinasse was also characterized in residual hexoses. Total residual sugars concentrations were similar between vinasses in our study: 504.99 mg L-1 in 1G vinasse and 576.32 mg L-1 in 2G vinasse. Because sugars are exhausted in fermentation, very low concentrations of residual sugars are commonly detected in vinasses (FERREIRA *et al*., 2011).

Glycerol was determined in both vinasses and concentrations were consistent with previous studies (ORTIZ-MUNIZ *et al*., 2010). Glycerol is one of the main yeast metabolites in alcoholic fermentation since its metabolic pathway is related to redox balance maintenance and osmotic stress response (NEVOIGT & STAHL, 1997).

Mannitol, along with organic acids, is an indicator of bacterial contamination and the concentrations we determined for both 1G and 2G vinasses in our study were higher than those reported elsewhere (LOPES *et al*., 2016; EGGLESTON *et al*., 2007).

Vinasses usually have very high concentrations of sulfate due to many operations along the global process. During the sugar production process, sulphitation is applied for crystal sugar production. Thus, some residual forms of sulphur are generated in molasses and converted into sulphate, which will remain in the broth during alcoholic fermentation, and finally in vinasse (SAHU, 2018).

As for the ethanol production process, after alcoholic fermentation, it is common to apply H_2SO_4 on cream yeast, which is known as the acid treatment. The operation is useful to decrease bacterial contamination during inoculum recycling for the next fermentation batch (OLIVA-NETO & YOKOYA, 2001). Acid treatment may also generate residual forms of sulphur, such as sulphate, which are eventually found in vinasse. Still, some authors suggest H_2SO_4 to be used in physical-chemical pre-treatments on sugarcane bagasse for 2G ethanol process (JARDINE *et al*., 2009), which could be yet another possible source of sulfates in 2G vinasses. However, this type of information was not confirmed by the industry that provided the 2G vinasse for this study. Sulfate concentrations from our analyses were consistent with those in literature for 1G vinasses (ESPAÑA-GAMBOA *et al*., 2012; PRADO *et al*., 2016).

In our study, nitrate was determined in higher concentrations than those found in literature, for both 1G and 2G vinasses (PRADO *et al*., 2016; PEDRO-ESCHER *et al*., 2014; CASSMAN *et al*., 2018; SYDNEY, 2013). Ammonium was investigated and no detectable concentration was found for neither vinasses.

Other components, such as salts of sodium, calcium, magnesium, chloride, and potassium were determined in important concentrations. These salts have been previously reported by other authors with highly variable concentrations in 1G vinasses. In our analyses, 2G vinasse had higher concentrations for calcium and magnesium salts than those reported elsewhere, for 1G vinasses. Sodium and chloride concentrations in 2G vinasse were following other authors' results with 1G vinasse samples. As for our analysis with 1G vinasse, concentrations of sodium, calcium, chloride, and phosphate salts were consistent with those reported elsewhere. No detectable concentration for phosphate was determined in 2G vinasse (COELHO *et al*., 2018; PEDRO-ESCHER *et al*., 2014; FERREIRA

et al., 2011; ESPAÑA-GAMBOA *et al*., 2012; SOUZA *et al*., 2015; REIS *et al*., 2015; NASPOLINI *et al*., 2017).

Potassium content in vinasses is commonly very high, ranging from 600 to 13,000 mg L-1 (ESPAÑA-GAMBOA *et al*., 2012; MORAES *et al*., 2015; PATHAK *et al*., 1999; BISWAS *et al*., 2009). In our analyses, potassium content in 1G vinasse was consistent with those found by other authors, whereas potassium content in 2G vinasse was highly above that range.

São Paulo State accounts for the most important share of ethanol production in Brazil and is the only region in the country where vinasse fertirrigation must follow a governmental technical regulation. Since potassium salts are usually the most abundant in vinasses, its concentration should be considered for vinasse application in soil in order to avoid nutrients imbalance and salinization (CETESB, 2013).

In this regard, 2G vinasse management could be an even greater challenge if fertirrigation is to be considered. According to the São Paulo regulation, lower volumes of 2G vinasse would be allowed per area, which means transporting vinasse to further fields. Costs with fuel for trucks, labor and specialized material for vinasse storage and transportation (by trucks, channels or lagoons) are implicated.

Considering fertirrigation, 2G vinasse might be economically very challenging. Moreover, calcium, sodium, magnesium salts, and organic acids were found in very high concentrations as well. So, not only from the economic point of view but also for environmental concerns, 2G vinasse might arise the urgent need for alternative and more innovative technologies in its management strategies.

3.2 VINASSE MANAGEMENT CHALLENGES: ADVANTAGES, DISADVANTAGES OF FERTIRRIGATION AND OTHER TECHNOLOGICAL OPPORTUNITIES

Vinasses might have a variable composition, but in general, they are interesting sources of salts, carbon (organic acids and glycerol), other nutrients, and even water. In Brazil vinasse fertirrigation is an important supply of these nutrients for sugarcane fields. Moreover, previous studies have showed that vinasse fertirrigation might indeed promote higher sugar production by increasing the sugarcane growth and rooting (MEDINA *et al*., 2002; PAULINO *et al*., 2002).

An important factor about vinasse fertirrigation is that it means a low-cost investment, with low-cost maintenance, as well. The use of vinasse in sugarcane fields demands facilities such as piping, pumps, channels, trucks, and storage lagoons. The nutrients recycling by fertirrigation also means purchasing less fertilizers for sugarcane crops (CHRISTOFOLETTI *et al*., 2013).

In the last years many studies have been focusing on the impacts that fertirrigation might have on the quality of soil and groundwaters.

According to literature, the continuous vinasse fertirrigation in a certain area means the continuous addition of specific nutrients, such as salts and organic acids, which might lead to an unbalanced composition of nutrients in the soil (SILVA *et al*., 2007; OLIVEIRA *et al*., 2015; PEDRO-ESCHER *et al*., 2014).

Researchers reported that soil physical structure might be altered as a consequence of such chemical imbalance. The result is that fertirrigated areas will eventually be salinized and potassium, sulfate, nitrate, and metals might be leached through soil inner layers and contaminate superficial and groundwaters (SILVA *et al*., 2007; CASSMAN *et al*., 2018; CHRISTOFOLETTI *et al*., 2013).

SOTO *et al*. (2015) suggested that, depending on environmental conditions, soil, and vinasse characteristics, vinasse percolation might occur between one to three years after fertirrigation.

Salts have usually been the greatest concern in vinasse composition because of their potentially negative effects on soil salinization. However, recent studies have quantified greenhouse gases (GHG) emissions from vinasse fertirrigated areas. The results indicated that emissions are significant and environmental concern with vinasse should be wider than soil degradation (OLIVEIRA *et al*., 2015; CASSMAN *et al*., 2018).

Considering all these aspects, 2G vinasse might bring the same environmental concerns. As determined in our study, 2G vinasse presented as many salts and organic acids as 1G vinasse, or more. In our analyses, 2G vinasse had higher concentrations of potassium, sodium, nitrate, lactic acid, acetic acid, butyric acid, and TPC than those determined in 1G vinasse. Further studies with 2G are definitely needed, considering that more vinasses from different locations and processes should be analyzed. However, our results have already indicated that 2G vinasse might bring the same environmental problems known to be related to 1G vinasse.

There is great interest in continuing with vinasse fertirrigation because nutrients from sugarcane can be recycled to crops and have positive effects on sugarcane growth. Given the advantages of vinasse fertirrigation, more sustainable management could start by employing the biodigested vinasse.

MORAES *et al*. (2017) have compared the GHG emissions during the transportation of in natura and biodigested vinasses for fertirrigation. Their results showed that biodigested vinasse did not show any detectable CH4 emissions. As for N2O emissions, they observed a decrease of about 48% to 78% in comparison to in natura vinasse.

The biodigested vinasse means the product of anaerobic digestion (AD), which is the bioprocess that consumes dissolved carbon compounds, converting them into biogas. The main product of AD is the biomethane, which can be further purified and used in energy generation. Still, in an ethanol distillery scenario, the biodigested wastewater would also have an important role as a fertilizer, since potassium, magnesium, calcium, and other salts are not significantly removed during biogas production (BARROS *et al*., 2017; LÓPEZ-LÓPEZ *et al*., 2015).

Fertirrigation and biogas production have important features that make them very interesting alternatives for vinasse management in the Brazilian industry. However, vinasse is generated in very large volumes and, despite AD being a very efficient and well-established technology, multiple strategies are needed.

AD with concentrated vinasses has been previously investigated as a more efficient alternative for vinasses (NACHEVA *et al*., 2009). And recently, many studies have been expanding knowledge about wastewaters' valorization. For that reason, many residues from different processes have been studied as potential culture media components. Among those, dairy effluents, molasses, paper mill effluents, winery wastewaters, food processing wastes, whey thin stillage, crude glycerol, and many others have been investigated (RATHIKA *et al*., 2018; KADIER *et al*., 2014; REVIN *et al*., 2018; SANTOS *et al*., 2016).

Table 2 provides some information from studies in which agroindustrial wastes, including sugarcane vinasse (1G), were evaluated as a component of culture medium for biofuel or bioproducts synthesis.

These bioprocesses employ bacteria or a consortium of microorganisms. In Table 2 there are bioprocesses with Pseudomonas spp. and Bacillus spp., for biosurfactant production (NASPOLINI *et al*., 2017; MD, 2012), Xanthomonas campestris for xanthan gum production (non-food applications) (BECKER *et al*., 1998), Bacillus spp. for bioplastic

production (RATHIKA *et al*., 2018; DESOUKY *et al*., 2017), Acetobacter spp. and Gluconacetobacter spp. for bacterial cellulose (BC) production (REVIN *et al*., 2018; ESA *et al*., 2014) and Corynebacterium glutamicum for amino acids production (animal feed) (BECKER *et al*., 2011). These species are able to consume saccharides, but glycerol and organic acids as well, making them potentially suitable for growth and biosynthesis in vinasse.

Some bioprocesses in Table 2 are already well-established in the industry, as AD, xanthan gum, and amino acids production. Other bioprocesses, such as biosurfactants, bioplastics, BC production, and microbial electrolysis cell (MCE) are not yet well-established in large scales, so they are majorly in early stages and/or scaling up to pilot scale studies.

Table 2. Bioprocesses, nutrient requirement, products and application.

Bioprocess	Nutrient requirements for culture medium	Product	Product's applications	Current state of technology development	Does it need supplementation?	Complex substrates previously tested	Challenges in using vinasse	References
Anaerobic digestion	COD 12,100 - 44,500 mgO_2 L^{-1}; Potassium (0.149 - 7.2 g L^{-1}), Calcium (0.005 - 0.32 g L^{-1}); Sodium (0.16 g L^{-1}); sulfate (1.0 - 5.336 g L^{-1}); phosphates (0.141 g L^{-1}); Magnesium (0.18 g L^{-1}) acetic acid (2.237 g L^{-1}); propionic acid (4.3 g L^{-1})	CH_4	Biofuel	Well established in industry	No	Sugarcane vinasse, tequila vinasse, swine wastewater, domestic wastewater	High sulfate concentrations might lead to H_2S production, which is a corrosive gas	ESPAÑA-GAMBOA *et al*., 2012; BARROS *et al*., 2017; LÓPEZ-LÓPEZ *et al*., 2015
Biosurfactant production	Glycerol (30 g L^{-1}); Sodium nitrate (1.2 - 4.0 g L^{-1}); phosphates (0.5 - 10 g L^{-1}); Magnesium sulfate (0.2 - 0.5 g L^{-1}); Potassium chloride (0.1 g L^{-1}); ferrous sulphate II (0.01 g L^{-1}); Calcium chloride (0.01 g L^{-1}); yeast extract (0.01 g L^{-1})	Glycolipids rhamnolipids	Mobilizing agent in agriculture; detergent; antimicrobial; biopesticide; applied in crude oil and hydrocarbons degradation	Laboratory scale	Glycerol or other carbon source supplementation; phosphates; trace elements, such as B, Cu, Mn, Mo, Zn	Sugarcane vinasse, molasses, crude oils, corn steep liquor	Studies on standardization of vinasse application are still required	NASPOLINI *et al*., 2017; BECKER *et al*., 1998; BENINCASA *et al*., 2002

Xanthan gum production	Sucrose or glycerol (20 g L^{-1}); urea (0.1 g L^{-1}); phosphates (1- 3 g L^{-1}); yeast extract (3 g L^{-1}); sulphate ammonium (1.5 g L-1); Magnesium sulphate (0.3 g L^{-1})	Acidic polysaccharide	Thickener and emulsifier for food, oil, pharmaceutical, cosmetic, paper, paint and textile industries; gelling and suspending agent	Well established in industry	Carbon (sucrose or glycerol) and nitrogen (urea or yeast extract);	Sugarcane molasse; glycerin; stach hydrolysates; green coconut shell hydrolysate; straw hydrolysate	In industry, xanthan gum is produced from sugarcane juices or molasses, which have a very similar composition in comparison to sugarcane vinasse, except for the carbon source composition. Using vinasse could decrease production costs	SANTOS *et al*., 2016; BECKER *et al*., 1998; BRANDÃO *et al*., 2013; RONCEVIC *et al*., 2014
Bioplastic production	Glucose, sucrose or glycerol (10 - 20 g L^{-1}); ammonium (0.6 - 1.5 g L^{-1}); phosphates (7 g L^{-1}); sulphates (0.72 g L^{-1}); Calcium (0.084 g L^{-1})	Polyhydroxyalkanoates	Packaging, moulded goods, coatings, adhesives, films	Mainly laboratory and pilot scale, still emerging in industry	Carbon source (sucrose or glycerol); nitrogen source (ammonium); phosphates	Sugarcane molasse; cheese whey; wheat bran; paper mill effluent; dairy whey	Studies have already evaluated sugarcane molasse, which is a substrate with similar composition in comparison to vinasse, except for the carbon source composition.	RATHIKA *et al*., 2018; DESOUKY *et al*., 2017; KHIYAMI *et al*., 2011
Bacterial cellulose	Glycerol (2.39 - 7,87 g L^{-1}); lactic acid (5.07 - 7.41 g L^{-1}); acetic acid (0.56 - 2.72 g L^{-1}); succinic	Cellulose	Food packaging; reinforcement material for electronic and biomedical materials; paper restoration	Laboratory scale	Nitrogen sources might be required.	Wheat thin stillage; cheese whey; waste beer yeast; grape skin; oil mill residue	Studies on stardadization of vinasse use are still required; using vinasse could decrease	REVIN *et al*., 2018; ESA *et al*., 2014; RATANAPARIYANUCH *et al*., 2011

	acid (0.63 - 0.93 g L^{-1})						production costs.	
Amino acids	Sucrose (10 - 30 g L^{-1}); yeast extract (5 g L^{-1}); ammonium (10 - 36 g L^{-1}); Magnesium sulphate (0.2 - 1.5 g L^{-1}); phosphates (0.25 - 3.0 g L^{-1})	Lysine, threonine, tryptophan	Animal feed supplementation	Well stablished in industry	Carbon (sucrose), nitrogen (ammonium) sources; phosphates	Sugarcane, beet molasses; starch hydrolysates	Amino acids production with complex culture media is well established, including sugarcane molasses, which have a similar composition in comparison to sugarcane vinasse, except for the carbon source composition.	BECKER *et al*., 2011; YING *et al*., 2014; LIU *et al*., 2016
Microbial electrolysis cells (MEC)	COD 6,500 mgO_2 L^{-1}; residual reducing sugars (0.2 g L^{-1}); acetic acid (0.74 g L^{-1}); propionic acid (0.35 g L^{-1}); butyric acid (0.2 g L^{-1})	H_2	Biofuel	Laboratory scale	No	Lignocellulosic biomass wastes; molasses; domestic wastewater; fermentation effluents in general; food processing wastewaters	MEC is a recently developed technology and it is not yet ready for large scale implementation. Most studies still focus on higher yields, suitable material for electrodes, costs and substrates optimization in general.	LU *et al*., 2009; KADIER *et al*., 2014

Regardless of the current state of technology development, vinasses have potential as nutrient sources for biofuels and bioproducts generation, providing a wider view for vinasse application.

Although many factors contribute to the exact determination of culture medium composition for a specific bioprocess, such as the microorganism strain, bioproduct characteristics, biosynthesis conditions, downstream operations, and others, some general information about the main microbial nutrient requirements are presented (Table 2).

As mentioned above, the employed bacteria are capable of consuming different types of carbon sources. Glycerol might be the chosen carbon source for biosurfactant, xanthan gum, and BC production. Despite not being well established, amino acid production by Corynebacterium glutamicum is also possible through glycerol consumption by engineering strains for that purpose (RITTMANN *et al*., 2008). Besides, organic acids found in vinasse might be important carbon sources in BC and MCE processes. As for AD, the bioprocess is carried out by a consortium of microorganisms, involving many bacterial and archea species, which consume a wide variety of carbohydrates and organic acids, especially acetic acid (STAMS, 1994).

The vinasses we characterized in our study had an important carbon concentration considering glycerol, mannitol, residual sugars, and organic acids altogether. For 1G vinasse, we determined over 4,800 mg L-1 of carbon sources, and for 2G vinasse, over 23,050 mg L-1. Thus, both vinasses, but most importantly 2G vinasse, have interesting carbon sources concentrations to be exploited, meaning energetic resources to be seized by industrial microorganisms.

For all the bioprocesses listed in Table 2, there are possible challenges in applying vinasse. High sulfate concentrations (AD), the lack of studies on the specific usage of vinasse as substrate (xanthan gum, bioplastic, BC and amino acids production), replacing the use of sugarcane molasse by sugarcane vinasse (biosurfactant, xanthan gum, bioplastic, and amino acids) or the technological development itself (MCE). On the other hand, all these bioprocesses have been extensively studied with complex components for culture media, including some agro-industrial wastes.

Finally, taking into consideration the biorefinery concept, the use of vinasse in other bioprocesses could also mean water recycling. Different from for water treatment technologies that employ resins and membranes, with high investment and maintenance

costs, keeping vinasse inside a production facility and employing it in the production of bioproducts could optimize logistics and resources use.

Expanding studies on vinasse application in bioprocesses could turn a wastewater, with high pollutant potential, into a nutrient source for processes with economic and environmental benefits.

4 CONCLUSIONS

Some typical compounds obtained from lignocellulosic physical-chemical and enzymatic pretreatments were found at very high concentrations in 2G vinasse, namely acetic acid, and total phenolic compounds.

Contents of potassium, sodium, calcium, magnesium, nitrate, sulfate, and other organic acids were higher than those we determined for 1G vinasse. These compounds might bring risks to the environment once vinasse is commonly employed in fertirrigation. Therefore, 2G vinasse might bring the same risks for soil acidification and leaching.

However, these same compounds are needed in other bioprocesses. That makes both 1G and 2G vinasses interesting materials as a potential source of nutrients for biotechnological applications.

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RESUMO

O Brasil é o segundo maior produtor de etanol e os resíduos gerados pela fermentação alcoólica têm levantado questões econômicas e ambientais. Recentemente a indústria brasileira implantou o processo de segunda geração (2G) para atender às crescentes demandas pelo biocombustível. Neste estudo, foi investigado se a vinhaça 2G tem potencial para trazer os mesmos desafios ambientais associados à vinhaça de primeira geração (1G), isto é, aquela proveniente dos processos de etanol a partir de caldo e/ou melaço de cana-de-açúcar. Foi coletada vinhaça de uma das unidades de etanol 2G recentemente instaladas no Estado de São Paulo e, então, caracterizada quimicamente. Considerando-se glicerol, manitol, açúcares residuais e ácidos orgânicos, determinou-se que a vinhaça 2G possui um total de fonte de carbono de 23.050 mg L-1 (comparado a 4.800 mg L-1 na vinhaça 1G). Magnésio, cálcio, potássio e outros sais também foram determinados. Com base na composição química, vinhaças podem ser consideradas fontes de nutrientes para bioprocessos. Finalmente, foram abordados bioprocessos cujas demandas nutricionais podem ser completa ou parcialmente garantidas pelas vinhaças, resultando na produção de bioenergia ou bioprodutos.

Palavras-chave: Setor sucroenergético. Análises cromatográficas. Valorização de resíduos. Biorrefinarias. Vinhaça 2G.

RESUMEN

Brasil es el segundo más grande productor de etanol y los residuos generados por la fermentación alcohólica han levantado cuestiones económicas y ambientales. Recientemente la industria brasileña implanto el proceso de segunda generación (2G) para atender a las crecientes demandas por el biocombustible. En este estudio, fue investigado se la vinaza 2G tiene potencial para traer los mismos desafíos ambientales asociados a la vinaza de primera generación (1G), aquella proveniente de los procesos de etanol a partir de caldo y/o melaza de caña de azúcar. Fue colectada vinaza de una de las unidades de etanol 2G recientemente instaladas en el Estado de São Paulo y, así, caracterizada químicamente. Considerándose glicerol, manitol, azúcares residuales y ácidos orgánicos, se determinó que la vinaza 2G posee un total de fuente de carbono de 23.050 mg L^{-1} (comparado a 4.800 mg L^{-1} en la vinaza 1G). Magnesio, calcio, potasio y otras sales también se determinaron. Con base en la composición química, vinazas pueden ser consideradas fuentes de nutrientes para bio-procesos. Finalmente se abordaron bio-procesos cuyas demandas nutricionales pueden ser completa o parcialmente garantidas por las vinazas, resultando en la producción de bioenergía o bio-productos.

Palabras clave: Sector sucroenergético. Análisis cromatográficas. Valorización de residuos. Biorrefinerías. Vinaza 2G.

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NOTAS EDITORIAIS

CONFLITO DE INTERESSES

Os autores declaram que não há conflito de interesses neste trabalho.

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RESPONSABILIBADE EDITORIAL

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