



Sustainable sugarcane vinasse biorefinement for *trans*-aconitic acid-based biopolymer synthesis and bioenergy generation

Guillermo Montoya^{a,1}, Maria I. Gutierrez^{c,1}, Juan D. Giraldo^e, Luis D. Jaramillo^b,
Jeyson Ruiz-Sandoval^c, Sandra Orozco^a, Ferley Orozco^c, John Ward^d, Giovanni Rojas^c,
Maria F. Villegas-Torres^{c,*}

^a Departamento de Ciencias Farmacéuticas, Universidad Icesi, Calle 18 No. 122-135, Cali, Valle del Cauca 760031, Colombia

^b Departamento de Ciencias Biológicas, Universidad Icesi, Calle 18 No. 122-135, Cali, Valle del Cauca 760031, Colombia

^c Departamento de Ciencias Químicas, Universidad Icesi, Calle 18 No. 122-135, Cali, Valle del Cauca 760031, Colombia

^d The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, UCL, Gower Street, WC1E 6BT, United Kingdom

^e Servicio Nacional de Aprendizaje, Centro para la formación Cafetera, Regional Caldas, Manizales, Colombia

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ABSTRACT

This study aimed at developing a multiproduct biorefinery scheme for vinasse valorization. It involved the extraction of *trans*-aconitic acid, its transformation into biopolymers using microwave irradiation and the use of the raffinated-vinasse for biogas production. Each technology was assessed individually, followed by a process integration at bench scale, from which a mass balance was calculated, supporting the feasibility of the approach. As a renewable source derived from a residue, these bioproducts can replace crude-oil-based materials in various chemical industrial processes; however, a detailed techno-economic and life-cycle analysis are required to defined the sustainability of the process.

1. Introduction

Bioethanol production generates large volumes of vinasse after alcoholic distillation (Aparicio et al., 2017; Costa, 2018). Its main features are its dark brown color, acidic pH (3.5–5.0), high temperature (80 °C–100 °C), high organic-matter concentration (COD: 50–150 g/L), and high salinity (K, Ca, and Mg) (Naspolini et al., 2017). It has been reported that the average production volume of vinasse is approximately 9–14 L/L of ethanol produced (Aparicio et al., 2017; Devia-Orjuela et al., 2019); thus, it is one of the main waste materials discharged by biofuel distilleries in India, South America and Europe (Suhaili et al., 2019).

The current practices for vinasse management involve composting, land application via fertirrigation (Christofoletti et al., 2013; Devia-Orjuela et al., 2019; Ortegón et al., 2016; Suhaili et al., 2019), and as a feedstock for renewable energy generation in the form of biogas through anaerobic digestion, a process well established in Brazil (Parsaee et al., 2019). The composting application is associated with high energetic costs and an extensive land area, fertirrigation practice can result in soil and groundwater pollution from hydro-chemical changes (Christofoletti

et al., 2013; Ortegón et al., 2016), and the implementation of anaerobic digestion is not economically feasible at scales below 25 m³ biogas/h (Canizalez et al., 2020). Therefore, there is an increasing interest in the development of sustainable processing schemes economically feasible with zero environmental. Previous studies have shown that a multi-product scheme targeting high-value chemical products together with energy generation to develop a fully sustainable process could be a feasible strategy for vinasse management (Bernal et al., 2017).

A detailed analysis of sugarcane-based vinasse shows that it contains a wide variety of organic acids, of which *trans*-aconitic acid (TAA) with the highest content is attracting significant interest because of its versatile biofunctions such as nematocidal (Du et al., 2017; Montoya et al., 2014; Parnaudeau et al., 2008) and anti-edematogenic activities (Garcia et al., 2010), and it can be used as a food additive, a bulk chemical in polymer chemistry (Cao et al., 2011), and a precursor for itaconic acid synthesis (Kuenz and Krull, 2018).

TAA extraction and purification from molasses have been previously described (Azzam and Radwan, 1986; Barnes et al., 2000; Gil, 2007; Kanitkar and Madsen, 2013; Malmay et al., 1995), for which biphasic

* Corresponding author.

E-mail address: mfvillegas@icesi.edu.co (M.F. Villegas-Torres).

¹ BioInc. Research Center for Biomass Valorization. Universidad Icesi, Calle 18 No. 122-135, Cali, Valle del Cauca, 760,031, Colombia.

extraction solvent systems are commonly employed. Gil (2007) concluded that ethyl acetate (EtOAc) was the most selective of the solvents tested for TAA extraction in molasses, with a yield of 34.0%–69.0% and a TAA purity of 99.9% (Gil, 2007). Similarly, Kanitkar and Madsen (2013) validated the high selectivity of EtOAc toward TAA extraction, achieving a yield of 26%–62% from vinasses, which is slightly lower than that from molasses, and no loss of TAA was observed after the fermentation process (Kanitkar and Madsen, 2013).

Hyperbranched, crosslinked, and linear polymers containing TAA have been synthesized via a traditional wet, one-step polymerization process (Cao et al., 2011). However, this method involves contaminating solvents and high-energy consumption, which are detrimental to the environment (Dubé and Salehpour, 2014). Alternative polymerization strategies can be conventional heating and microwave irradiation, which have been used to produce poly(xylitol sebacate), an elastomer/nano-hydroxyapatite bionanocomposite (Ma et al., 2014), and poly (butylene succinate) (Komorowska et al., 2009; Velmathi et al., 2005; Wiesbrock et al., 2004) respectively.

Polymers from wastes are obtained mainly from vegetable-based agricultural residue rich in polysaccharides, such as cellulose and starch (Di Donato et al., 2020; Heredia-Guerrero et al., 2017). However, their applications are still limited, and because of the nature of the feedstock, a reduced number of agricultural waste-types can be employed (Maraveas, 2020). Furthermore, the development of low-cost and scalable technologies for establishing a sustainable process and the identification of potential applications for complex products, such as thermoplastics, thermosets, and composite materials, are still in progress (National Academy of Science, Engineering and Medicine, 2020). Ferri et al. (2020) successfully obtained phenol complex mixtures that could be valorized for use as additives in polymer formulations to obtain materials with antioxidant and antibacterial properties. The mixtures were extracted from red pomace residues by a solvent-based pressurized liquid-extraction technique, developing a zero-waste valorization cascading approach (Ferri et al., 2020). In the present work, a multi-product process is proposed as a vinasse-valorization strategy for the extraction and polymerization of TAA and the subsequent biogas generation from the resulting raffinate of the extraction process to establish sustainable circular economies centered on the sugarcane industry.

2. Materials and methods

All chromatographic solvents (acetonitrile and methanol) were purchased from Merck, and reagents, such as pH modifiers and polymeric resins (hydrochloric acid, sulfuric acid, formic acid, reference *trans*-aconitic acid, Diaion® HP-20 resin, and Amberlite® IRA-400 in chloride form), were acquired from Sigma-Aldrich. Sucroal S.A (Colombia) supplied commercial-grade ethyl acetate. Ultrapure water for chromatographic purposes was obtained using an Arium® pro-VF (Sartorius Stedim) with a resistivity of 15 M Λ .cm (at 25 °C). Ethylene glycol and tetrahydrofuran were purchased from Merck and used without further purification.

The activated sludge for the biomethane tests was obtained from a wastewater treatment plant in Cali, Colombia. A 40-L sample was collected, transported, and stored at room temperature to minimize ecological disturbances. Physicochemical characterization was performed on the fresh sample, and all biomethane tests were conducted 2 days after sampling. Concentrated vinasse (30–40°Bx) was provided by Manuelita S.A. as part of an academic cooperation agreement. The samples were freshly collected after distillation, packed in 20 kg reservoirs, and transported to our facilities.

2.1. Vinasse preparation and TAA quantitation

Two analytical chromatographic methods were used to characterize the raw materials and raffinate. In the first method, which is widely known for organic acid identification/quantitation, a Rezex ROA-

Organic acid H+ (8%) crosslinked sulfonated styrene-divinylbenzene ion exclusion column (300 mm \times 7.8 mm) from Phenomenex is used. The protocol was performed at 40 °C at a 0.5-mL flow rate, with isocratic 0.005 N H₂SO₄ as the eluent and using spectrophotometric detection at 210 nm. The procedure was necessary for determining the purity and characterizing the other volatile organic acids accompanying the recovered TAA. Notwithstanding, this method requires long column preparation times and long chromatographic runs, making it unfavorable for routine work. A second, quick, and simple 5-min method was validated using a Kinetex C18 2.6 μ m 100 mm \times 4.6 mm column at a 0.8 mL/min flow rate and an isocratic solvent system consisting of water/acetonitrile (95/5), with formic acid used as a modifier in the water with a pH of 2.5 and detection at 240 nm.

2.2. Validation of the quantitative chromatographic method

The validated parameters include acid degradation, linearity range, specificity, precision (repeatability and ruggedness), method accuracy, quantitation and detection limits. All data processing was carried out with the EZchrom Elite software. The calibration curves were analyzed by a linear equation with a correlation coefficient of >0.99994, using a five-level calibration in triplicate (between 20 and 200 mg/L). A Cochran G-test determined that the variances of each concentration level were homogeneous (G_{exp} : 0.626, and G_{theo} : 0.683, with $\alpha = 0.05$). The limit of detection (LOD) and limit of quantitation (LOQ) were determined as 3 and 10 times the signal-to-noise ratio using a low-range calibration curve in triplicate (between 2 and 20 mg/L). The LOD and LOQ of the method were 0.93 mg/L and 10 mg/L, respectively. The accuracy was determined using sample spiking with recoveries of 100.4%, 99.2%, and 100.9% for 80, 100, and 120 mg/L respectively. A 12 h stability test was carried out to determine if the TAA content in the vinasse was as stable as that of the prepared standards. A total run time of 4 min per sample was established to quickly measure the TAA content.

2.3. Optimization of the biphasic extraction solvent system

An experimental design comprising 45 experiments was run to determine the optimal conditions for extraction at the bench scale. Three factors at three levels were assessed using the TAA yield as a response: pH between 2.0 and 2.6; extraction times between 0.5 and 3.0 h; and temperature between 20 °C and 60 °C.

A semi-pilot extraction protocol was defined according to a bench-scale optimization study as follows: a 1:5 (vinasse: EtOAc) proportion was added to a percolation tank for biphasic liquid-liquid extraction using concentrated vinasse at pH 2. The optimum proportion was determined in a previous study (Apache, 2018). The extraction process time was established according to the results of Ruiz (2016) with constant agitation. The organic phase (OP) was transferred after phase separation to a concentrating tank for solvent recycling. The aqueous phase (extract) was used for preparative ion-exchange chromatography. EtOAc was chosen instead of other reported diluents (Nimmakayala and Pal, 2019) because it has been used previously for TAA extraction with high selectivity (Gil, 2007).

2.4. TAA purification

The Isolera one flash system was used for preparative anion-exchange chromatography in a 750-g bed using empty cartridges packed with Amberlite® IRA-400 in the chloride form. The use of this polymeric resin has been reported for acid recovery from complex matrices (Han et al., 2016).

The dried OP obtained from the biphasic extraction at the semi-pilot plant was dissolved in ultrapure water, and the pH was adjusted to 7.0 to assure the full ionization of the carboxylic acids present in the sample (pKa: ~5.0). Thereafter, 49-g pH-conditioned OP was transferred to a 750-g bed with Amberlite® IRA-400 and rinsed with enough water (pH:

7.0) to elute all colored compounds from the chromatographic bed. Subsequently, the bed was eluted with water; however, this time, the pH was adjusted to 2.0 using hydrochloric acid, to recover all the TAA attached to the chromatographic bed. Afterward, the rapid high-performance liquid chromatography (HPLC)-validated method was performed. The water used was concentrated by sublimation using a freeze dryer (Eyela FDU-1110 Tokyo, Japan) to obtain a white-brownish powder at a 10% yield. A second purification step was carried out with a Diaion® HP-20 resin, which is a non-polar copolymer styrene-divinylbenzene adsorbent resin in a 400-g bed. This stage removed the remaining color, affording a purity level greater than 90%.

2.5. Microwave-assisted polymerization of the recovered TAA

Since polymerization proceeds by a step-growth mechanism, high-purity monomers are essential. High-purity monomers were obtained by converting the TAA to the corresponding trimethyl ester using Fisher esterification with an excess of acidic methanol. The product was purified through a chromatography column using silica and hexane/EtOAc as eluents. Trimethyl aconitate was hydrolyzed with NaOH to obtain pure *trans*-aconitic acid (99% purity).

Polyesters were produced in a synthetic microwave (Discover SP) from CEM Corporation, equipped with a camera for monitoring the reaction. Crosslinked materials were synthesized under specific time and temperature conditions. The reactor utilized standard vessels for different amounts of reactants, closed by septa to permit adequate pressure control. A dynamic method was used; the desired temperature was set (150 °C, 160 °C, or 170 °C); the power was set to 280 W; and the durations of microwave exposure were set to 7, 12, 15, 17, 20, and 30 min. The temperature was monitored by an infrared (IR) sensor to prevent explosions. The power, time, and agitation speed were set using established methods in the equipment software according to the reaction conditions and the desired product characteristics. Fourier transform infrared (FT-IR) spectroscopy was performed using a Nicolet iS10 FT-IR spectrometer in the attenuated total reflection (ATR) mode. Nuclear magnetic resonance spectra (¹H and ¹³C NMR) were recorded in D₂O using a Bruker instrument at 400 MHz for protons. Chemical shifts were reported in ppm relative to D₂O (4.82 ppm) with tetramethylsilane as the internal reference. The thermal transitions and stability were determined by differential scanning calorimetry (DSC, Q2000 V24.11) and thermogravimetric analysis (TGA, Q50 V20.10) using TA instruments. The DSC analysis was performed using an instrument equipped with a controlled cooling accessory at a heating rate of 10 °C/min. Calibrations were performed using indium and freshly distilled n-octane as the standards for peak temperature transitions and indium for the enthalpy standard. All samples were prepared in hermetically sealed pans (5–10 mg/sample) and were run using an empty pan as a reference and empty cells as the subtracted baseline. The samples were scanned for multiple cycles to remove the recrystallization differences between the samples, and the results reported are for the third scan in the cycle.

2.6. Physicochemical characterization

Activated sludge, vinasse, and raffinated-vinasse (VR) were characterized in terms of the percentage of total solids (%TS), volatile solids (%VS), and humidity (%H), following the National Renewable Energy Laboratory (NREL) standard protocols (NREL/TP-510-42,621) (Sluiter et al., 2008).

2.7. Biomethane potential batch test

A 1-L reactor from batch bioreactors (Anaero Technologies, UK), held at 35 °C and continuously stirred, was used for the biogas tests. Each unit was connected to a biogas flow meter, an Arduino device for data acquisition, and a Tedlar® (Dupont, USA) bag for biogas collection. The biogas composition was analyzed with a portable BIOGAS 5000

(Geotech, USA). The biogas production was evaluated continuously for 13 days, and all analyses were performed in duplicate.

The biomethane potential batch (BMP) test was performed to determine the amount of biogas produced per gram of volatile solids of the substrate (VS), employing a %VS substrate/%VS seeding sludge ratio of 0.17 following the VDI 4630 Standard Protocol (Ingenieure und Deutscher, 2006). Crude vinasse, VR, EtOAc, cellulose, and pretreated VR were used as substrates. Pretreated VR was obtained by evaporation using a rotary evaporator (Hei-VAP, Heidolph, Germany) to remove any residual EtOAc.

2.8. Statistical data analysis

Statistical analysis was performed using the MiniTab® 17 software for Windows. A Mann–Whitney test was conducted to determine the statistical differences between the BMP results among the substrates with a 95% confidence level. A Box–Behnken design with three factors using three replicates was established for a superficial response experiment to establish the optimal extraction conditions in a bench scale. The experimental design comprised 45 experiments to determine the optimum conditions for extracting TAA from vinasse. We identified temperature, extraction time, and pH as the most important factors in our biphasic extraction system. Once defined, the optimal conditions were used for semi-pilot scale extractions.

3. Results and discussions

3.1. TAA extraction and purification

To establish a multiproduct process to obtain TAA-based polymers and bioenergy, an analytical method was developed for the rapid quantitation of TAA in different matrices. This strategy allowed the identification of the optimum extraction conditions during the design of the experiments, e.g., to define the water desorption volume when TAA was eluted from the ion bed or to characterize vinasse at the starting point. The method was developed using a LaChrom elite Merck–Hitachi liquid chromatograph, equipped with a diode array detector. The selectivity was evaluated using *cis*-aconitic acid obtained from Sigma-Aldrich and TAA in refined white sugar to prepare spiked samples (See Supplementary Material). After proving the performance of the method, the analytical method was used for decision-making purposes. A system suitability test was always carried out before quantitation using the parameters of retention time, peak asymmetry, theoretical plates, and resolution (Fig. S2).

Optimal conditions for extraction were established by a response surface method with a total of 45 experiments, assessing three variables over three levels: pH at 2.0, 2.3, and 2.6; extraction time for 0.5, 1.45, and 3.0 h; and temperature at 60 °C, 40 °C, and room temperature (close to 20 °C). The choice of conditions mentioned above was based on the partition coefficients of the ionizable molecules that undergo a dramatic change in their protonation state depending on the pH. The target molecule has three carboxylic groups with three pK_a values, and the lowest value is 2.8. By decreasing the pH value, we can maintain the completely protonated state of the compound, increasing its non-polarity and favoring the extraction with the organic phase (OP) (Hu et al., 2017). The OP always yields TAA; however, the best TAA extraction yields were achieved when the tested factors were controlled. Fig. 1a shows the extraction yield at different pH values and extraction temperatures, with the best extraction yield achieved at the lowest pH values, regardless of the extraction temperature. Fig. 1b correlates the extraction time with the extraction temperature at pH 2.0. According to the results, TAA extraction does not vary after 2 h, and at a shorter extraction time, relatively high extraction temperatures negatively impact the extraction yield. Fig. 1c correlates the extraction pH with the extraction time, indicating a two-factor interaction with an optimum condition of pH 2.0–2.08 and extraction time between 2 and 3 h. The pH

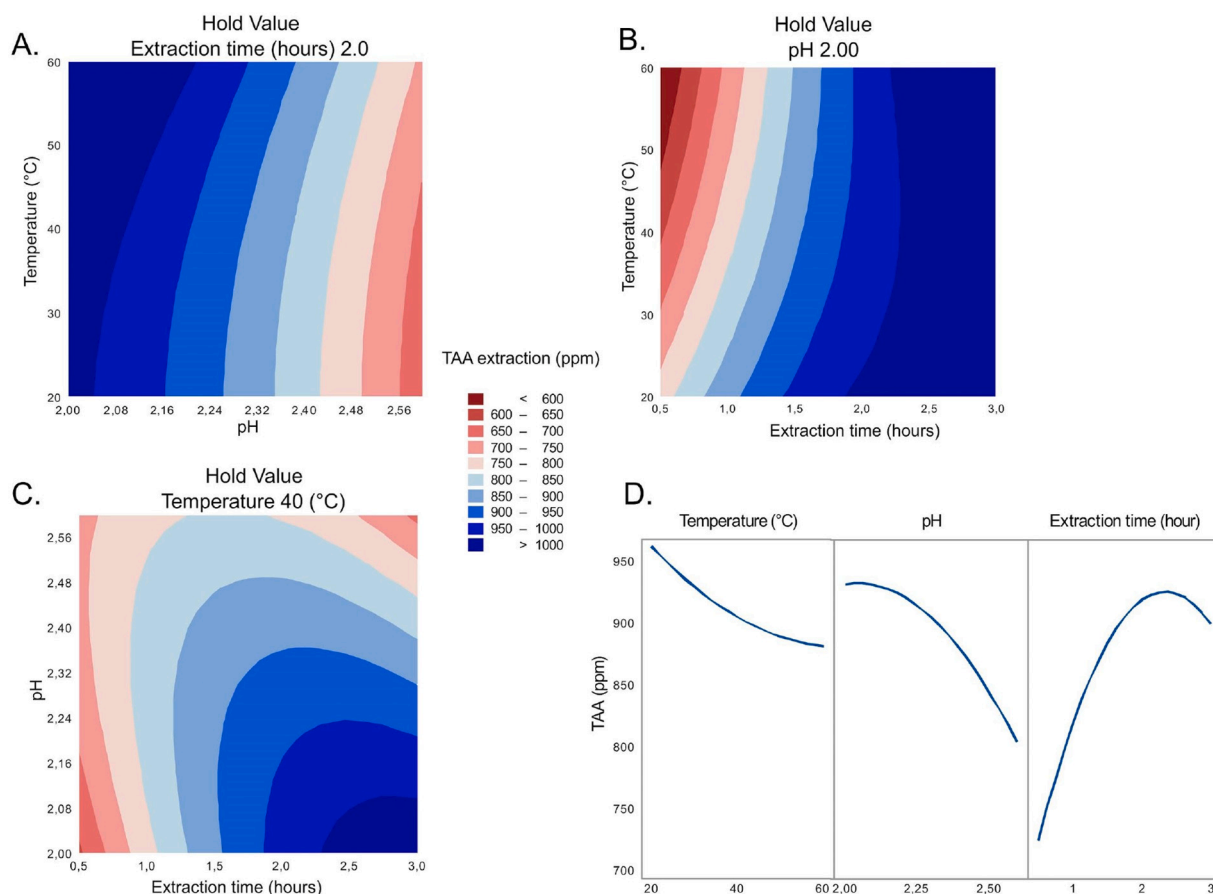


Fig. 1. Contour plot (surface response methodology) evaluating the optimum extraction conditions based on the extracted TAA at different pH values, extraction times, and temperatures. A. pH vs temperature with an extraction time of 2 h; B. extraction time vs temperature at pH 2.0; C. extraction time vs pH at 40 °C. D. The main effect plot showing the effect of the selected variables on the TAA extraction.

is tremendously important because TAA dissociates at high pH values. In summary, the pH value and extraction time were statistically significant factors, which allowed us to set the optimum extraction conditions at: pH of 2.0, extraction time of 2 h, and temperature of 20 °C (Fig. 1d). These conditions were corroborated on a semi-pilot plant using up to 2 L of conditioned vinasse (pH: 2.0) treated with 10 L of EtOAc. During this stage, no heat was used during the 2-h extraction. Once extracted, the OP was transferred to a concentrating tank to recover the EtOAc by evaporation. The extraction process increased the TAA content from close to $1.39 \pm 0.17\%$ in the raw vinasse to $33.8 \pm 1.05\%$ in the concentrated OP extract. Thereafter, the OP extract was dissolved in ultrapure water at pH 7.0, guaranteeing the deprotonation of all carboxylic acids and allowing retention on the Amberlite anion exchange resin. This polymeric resin has been widely used for acid extraction (Han et al., 2016). As the process did not remove all the color, we included an additional purification step using Dianion® HP-20.

3.2. TAA polymerization

TAA is a versatile molecule with various applications, such as polymer synthesis via a traditional wet, one-step polymerization process (Cao et al., 2011) that involves contaminating solvents and high-energy consumption. Thus, implementing sustainable polymerization process, such as microwave irradiation, are needed.

The polymerization of TAA followed a classic esterification mechanism, where a carboxylic acid reacts with alcohol to produce the respective ester (Flory, 1986; Knani, 1993; Li et al., 2020; Moore and Stupp, 1990). Based on the reaction conditions, e.g., temperature, time, and molar mass, the esterification of the TAA could occur at all the three

carboxylic acid groups, yielding water as a byproduct. The same reaction proceeds several times until a high molecular weight is achieved via a step-growth polymerization mechanism.

The polymerization was carried out by controlling the following parameters: polymerization temperature of 150 °C–170 °C, reaction time of 5–30 min, and monomer molar ratio (TAA:diol) of 1:1 to 1:3 (Hoogenboom and Schubert, 2007; Komorowska et al., 2009; Takasu et al., 2003; Velmathi et al., 2005). Owing to the nature of this reaction, all the polymers were crosslinked in varying degrees and as such with different solubility (Table S1). IR, NMR, and DSC were conducted to confirm the structural and thermal characteristics of the partially soluble polymers. The IR spectra (Fig. S4) of the monomers shows a stretching vibration around 1700 cm^{-1} , which belongs to the carbonyl group of TAA, and a bending vibration at 1651 cm^{-1} , which corresponds to the hydroxyl groups of ethylene glycol; while the partially soluble polymer shows an additional stretching band around $1000\text{--}1300 \text{ cm}^{-1}$, indicating the formation of an ester group and demonstrating that the polymerization between TAA extracted from vinasse and ethylene glycol occurred effectively. The ^1H NMR spectrum (Fig. S4) display a signal at 3.5–4.5 ppm and another at 6.9 ppm indicative of the methylene groups ($-\text{CH}_2-$) associated with TAA and the ethylene glycol moieties, and the vinyl protons, respectively. To explore their thermal behavior, DSC was carried out to observe the thermal transitions (Fig. S5), such as the glass transition (T_g) and melting temperatures (T_m), for the materials prepared using different polymerization times and temperatures. While crosslinked or amorphous polymers show a T_g exclusively, linear polymers can present both T_m and T_g . As shown in Table S1 and Fig. S5, all polymers, regardless of the reaction conditions, presented solely a T_g ranging from 30 °C to 61 °C, suggesting that all the polymers are

amorphous or crosslinked. Although the polymers did not present significant changes in their glass transition temperature, while varying the reaction conditions from 150 to 160 °C, it was observed that longer polymerization times and temperature produced higher crosslinking and consequently higher T_g with decreasing solubility in organic solvents, e. g., tetrahydrofuran, as shown in Table S1. The same trend was observed while varying more extensively some reaction conditions (Orozco et al., 2020). As expected, the T_g increased as the polymerization temperature was increased from 150 °C to 170 °C (Table S1). The most noteworthy change was observed in the polymers prepared at 170 °C, which exhibited the highest T_g of 61 °C, suggesting that this material had the highest degree of crosslinking, was the most amorphous, and consequently the most insoluble material. Diverse thermal properties were obtained by controlling the polymerization parameters, including time and temperature, expanding the possibility of using the materials in diverse applications.

According to the solubility tests and DSC analysis, all the polymers synthesized while varying the reaction conditions (time and temperature) are different. The polymers obtained at low temperatures and short reaction times exhibited low crosslinking distribution among the polymeric chains, thus allowing partial solvation. Conversely, the materials produced at high temperatures and long reaction times showed a high crosslinking distribution along the polymer chains, which produced materials exhibiting the highest T_g . Although Figs. S4 and S5 show the spectroscopic and thermal behavior of our best polymeric material in terms of molecular weight and solubility, a series of materials can be obtained by simply tuning the reaction conditions to achieve the desired thermal and physical properties. Our methodology permits the control of the polymerization process, which relates to the final application of the material, and, more importantly, creates polymers for desired applications from a sugarcane byproduct employing a solvent-free sustainable polymerization reaction.

3.3. Bioenergy generation from raffinated-vinasse processes

After TAA extraction and purification, an aqueous phase, named raffinated-vinasse (V_R), can be subjected to anaerobic digestion for bioenergy generation to develop a fully sustainable process toward achieving a circular economy scheme. To assess the bioenergy potential and determine the effect, from the extraction process, biogas production from crude vinasse and V_R were assessed in batch anaerobic digesters. The daily biogas production was calculated under standard temperature and pressure by subtracting the average of the biogas produced by the methanogenic inoculum without a substrate from the total biogas produced by the inoculum fed with the substrate under study per day. Thereafter, the calculated value was normalized according to the substrate volatile solids (VS) added. The cumulative biogas curves over time

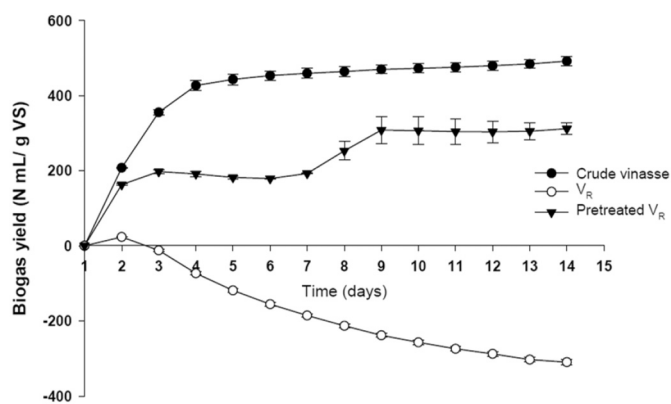


Fig. 2. Accumulated biogas yield from vinasse, raffinated-vinasse, and pretreated raffinated-vinasse. Error bars represent the standard deviation of biological triplicate values.

are shown in Fig. 2. According to the results, V_R fully inhibited the methanogenic inoculum, which did not generate intrinsic residual biogas, as demonstrated by the negative trend of the accumulated biogas over time.

To determine whether the inhibitory effect was caused by residual EtOAc from the extraction process, we evaporated the residual ethyl acetate from the V_R to obtain a pretreated V_R . No inhibition was observed with the pretreated V_R (Fig. 2, inverted triangles), although the biogas yield was significantly lower ($p \leq 0.0001$) than that generated from the crude vinasse (Fig. 2, circles), as well as the biomethane percentage (Table 1).

The degradation of ethyl esters via hydrolytic bacteria metabolism into acetate and ethanol is proposed, e.g., the degradation of ethyl butanoate and ethyl hexanoate into ethanol and butanoic and hexanoic acids, respectively (Gerardi, 2003) have been previously reported. In this case, the hydrolysis would generate ethanol and acetic acid, which would cause a pH drop, affecting the buffer capacity of the system and inhibiting the methanogenic population (Chatterjee and Mazumder, 2019). Yanti et al. (2014) evaluated the effects of various esters at different concentrations in anaerobic digestion, concluding that the minimum inhibitory concentrations were between 5 and 20 g/L, depending on the type of ester (Yanti et al., 2014). In this work, 2.50 and 7.50 g/L mixtures of EtOA and crude vinasse were used as substrates with a methanogenic microbial consortium to assess their inhibitory effect. The results showed the same inhibitory effect (Fig. S3). Vermorel (2017) observed an incomplete degradation of EtOAc at concentrations above 0.44 g/L in a batch anaerobic digestion process, attributed to the fast initial hydrolysis, followed by the inhibition of the methanogenesis (Vermorel, 2017). Therefore, the residual EtOAc must be removed from the V_R prior to any further biological processing, or depending on the EtOAc, the inhibitory effect can be overcome by alkalinity supplementation (Vermorel, 2017).

3.4. Process integration and mass balance

Following the assessment of each technology individually we integrated the whole bioprocessing scheme at bench scale and calculated the mass balance to establish the feasibility of the process (Fig. 3). The overall TAA extraction yield was $66.78 \pm 17.84\%$ from the raw vinasse (40°Bx), which is consistent with previous reports (Kanitkar and Madsen, 2013). The TAA purity was 45% in the dried OP. Therefore, a purification step with flash chromatography Amberlite® IRA-400 was added to achieve an average purity of 90%, which is the value required for a broad range of TAA applications in the pharmaceutical industry, e. g., as an anti-inflammatory or antitumoral agent, or in the bioplastic industry (Wu-tiu-yen et al., 2020). The recovery of EtOAc was 80.22%, allowing the recycling of the solvent in the process and consequently minimizing the total solvent consumption. Solvent losses were

Table 1

Physicochemical characterization of the substrates and generated biogas.

Parameter	Crude vinasse	Raffinated-vinasse (V_R)	Inoculum	Pretreated V_R
TS (%w/w)	38.21 ± 0.003	12.17 ± 0.009	4.95 ± 0.0003	31.13 ± 0.005
VS (% w/w)	28.10 ± 0.003	8.17 ± 0.004	2.3 ± 0.0002	22.14 ± 0.0001
H (%w/w)	61.79 ± 0.003	87.83 ± 0.009	95.05 ± 0.0003	68.87 ± 0.005
Biogas yield (N mL/gVS)	491.94 ± 11.55			311.85 ± 15.78
Methane (%)	63.83 ± 2			49.17 ± 0.45

Standard deviations reported for the %TS, %VS, and %H were calculated from two independent samples.

NA: Not analyzed.

ND: Not detected.

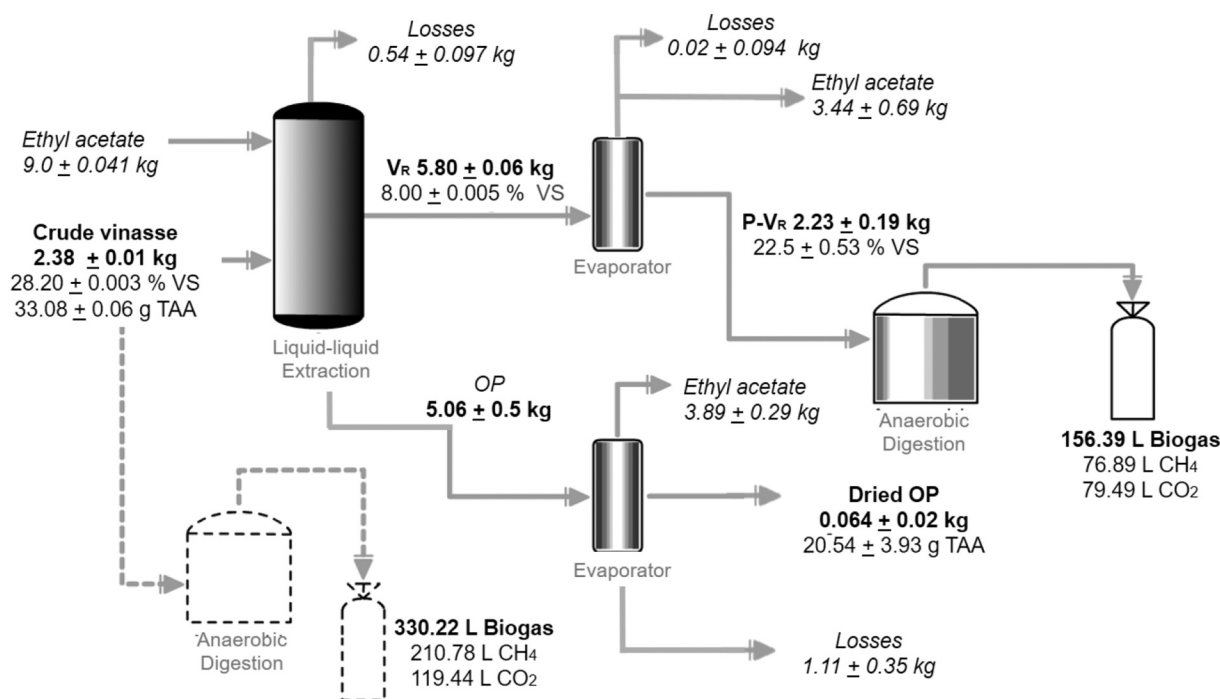


Fig. 3. Mass balance of the multiproduct scheme valorization of sugarcane vinasse. Standard deviation reported was calculated from three independent samples.

associated with operative control during the evaporation and in the remaining V_R fraction.

Based on these results and assuming an average TAA content of 1.39% in the concentrated vinasses (30–40° Bx) from annexed distilleries, this multiproduct scheme has the potential to produce 12,540.58 t of TAA from 694.5 million liters of vinasse annually obtained from 6 distilleries (Asocaña, 2020), and approximately 8374.6 t of TAA can be recovered by this process. The recovered TAA can be employed for polymerization via green chemistry methodologies, as shown here, due to the 90% purity achieved. Finally, the liquid stream after TAA extraction can be used for bioenergy generation. However, the TAA extraction process reduces the biogas yield by 37%, with an additional detrimental effect on the methane concentration, consequently reducing the energy output. Therefore, further evaluations are required to understand the change in the biogas composition to develop management strategies.

Waste biorefineries contribute to sustainable practices because they use waste as feedstock for green products and bioenergy generation. However, very few examples are economically feasible; thus, one possible strategy to overcome the economic burden is using multiproduct schemes, which combine high-value products with low-value commodities, balancing the trade-off between market share and product price. The present work successfully integrated solvent extraction processes with green chemistry technologies to obtain biobased polymers as high-value products that can potentially displace fossil-fuel-based polymers and with biodigestion to generate bioenergy as a commodity, expanding the renewable energy market share. We identified the limiting factors that influence process integration: organic acid purity, which affects the polymerization capability, and residual solvent, which strongly inhibits biogas generation. Despite the successful proof of concept for a waste biorefinery scheme reported here, further work is underway to increase the TAA recovery and biomethane enrichment and to assess the economic feasibility of the process and the environmental impacts through techno-economical and life-cycle analyses, respectively, based on the mass balance reported here.

4. Conclusions

A valorization scheme using vinasse was developed. 90% pure TAA was obtained through biphasic extraction with ethyl acetate with a resulting yield of $66.78 \pm 17.84\%$. Optimization studies highlighted pH and extraction time as the main factors influencing the TAA yield. TAA was used to synthesize crosslinked polymers of varying crosslinking degrees, achieved by different reaction temperature and time, using microwave irradiation. The remaining raffinate from the extraction was used for bioenergy generation, from which 311.85 N mL/gVS was generated. Using the resulting digestate as a fertilizer, a circular economy centered on sugarcane vinasse can be achieved.

Data availability

Datasets related to this article can be found at doi: [10.17632/4gycgck3gm.1](https://doi.org/10.17632/4gycgck3gm.1), hosted at Mendeley Data, V1.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.btr.2021.100786>.

[org/10.1016/j.biteb.2021.100786](https://doi.org/10.1016/j.biteb.2021.100786).

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