Research Article

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Valorization of cocoa's mucilage waste to ethanol and subsequent direct catalytic conversion into ethylene

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Abstract

BACKGROUND: The identification of new resources for producing biofuels and chemical-based products is crucial for processes sustainability. This study presents a valorization route to produce ethanol and ethylene using cocoa's mucilage juice (MJ) residue from cocoa farms of variety 'Arriba' (AC). The processing parameters to maximize the ethanol production and subsequent selective conversion into ethylene were determined. Ethanol production has been carried out by investigating the effect of three parameters: the temperature of fermentation, the initial fermentation pH and the addition of $(NH_4)_2SO_4$ as an N source in the presence of free *Saccharomyces cerevisiae* NCYC 366. Consecutively, the selectivity of ethanol–ethylene conversion using a zeolite-based ZSM-5 catalyst was evaluated at different temperatures and ethanol concentrations.

RESULTS: During ethanol production, the best sugar conversion was reached at 30 °C, adjusting the initial pH to 5 and without nitrogen source, resulting in 86.83% sugar conversion, the maximum ethanol concentration of 68.65 g L^{-1} and maximum ethanol production rate of 2.03 g L^{-1} h^{-1} after 168 h of fermentation. On the other hand, ethylene was produced using ZSM-5-based zeolite catalyst with >99.9% of efficiency in the temperature range 240–300 °C. In addition, selective ethylene formation was found at 240 °C and 30 g L^{-1} ethanol.

CONCLUSION: The approach hereby presented shows the valorization of MJ waste of AC variety to produce ethanol and ethylene with minimum processing input costs, demonstrating a successful route to convert a farm residue into a bio-based product with enhanced marketability.

Keywords: bioenergy; bioethanol; cocoa waste; fermentation; *Theobroma cacao*

INTRODUCTION

Cocoa beans are essential for chocolate production and are traded worldwide. According to the International Cocoa Organization, global production was around 5 million tons in 2019. During the harvesting procedure of cocoa beans, a juice is generated from the placenta or mucilage of the pods and beans. This so-called mucilage juice (MJ) is typically discarded as waste on agricultural soils. Each 100 kg of pods can generate 4–7 L of MJ.[1] MJ is made up of water (82–87%), fermentable or reducing sugars (RS) (10–15%), pentosans (2–3%), mineral salts (~0.4%), pectin (1.0–1.5%) and proteins (~0.09%), and with a pH of 3–4.[2,3] Among the applications, MJ had been used for ethanol, wine and marmalade production and as a source of bioactive agents such as succinic acid, erythritol and fumaric acid.[1,4]

The largest producers of cocoa are Africa, Latin America and Asia. Cocoa varieties of *Theobroma cacao* include Criollo, Trinitario and Forastero. Another variety is 'Arriba' (AC), also known as 'Nacional', which is categorized as protected geographical indication cocoa.[5] Cocoa

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beans are classified as *fine* or *flavor* cocoa (FC) and *bulk* or *ordinary* cocoa. AC, which is considered FC because of its fine undertone flavors, including fruit, floral, herbal and wood notes,[6] is used for manufacturing high-quality chocolate products. Nevertheless, AC is mostly produced in Ecuador, Colombia, Peru and the Dominican Republic, and represents 5–8% of global cocoa production.[7] In particular, Ecuador is the largest worldwide exporter of AC, producing 315 000 tons in 2018, covering 560 000 ha and employing 100 000 producers, of which ~90% corresponds to AC production.[8]

On the other hand, as a consequence of the growing tendency to transition from oil to sustainable resources, bioethanol has gained attraction in recent years because it can be obtained from lignocellulosic waste biomass.[9] The yield of ethanol production is directly related to the amount of fermentable sugars in the matrix. In MJ, the concentration of fermentable sugars depends on the species, growth conditions and maturity indices.[10] In general, fermentable sugar concentrations contained in this kind of waste ranges between 10% and 15%, which allows its use for ethanol production without a pre-hydrolysis step. Furthermore, the control of operational factors including pH, nutrients and fermentation temperature is crucial for reaching maximized ethanol production[11] and consequently saving process costs.

The literature reports varying amounts of ethanol produced from MJ. However, it is only the final concentrations in the fermentation broths that are given. The values range between 13 and 75 g L^{-1}, while neither the cultivated variety of cocoa is given, nor the microorganism strains, nor the isolated yield. Only Delgado *et al.*[12] specify their variety as CCN-51 (Colección Castro Naranjal variety) in their experiments, in which a final concentration of 22.06 g L^{-1} ethanol was obtained at 35 °C, but again without giving the yield.

Our research presented here focuses on the AC variety using a specified cultivated variety originating from specified geolocation (see 'Fruit collection and cocoa MJ preparation', below). These data are necessary, as there are significant differences in the chemical composition of MJ from different locations – information that is indispensable for future application on an industrial scale.

While ethanol is the typical product of biomass fermentation, other products are imaginable, too. Ethylene, for instance, is one of the most important industrial organic chemicals and is used, among other applications, for the production of polyethylene, the plastic with the highest production volume worldwide.[13] Catalytic dehydration of ethanol to ethylene enables the production of a platform chemical without additional workup of the fermentation broth. Furthermore, ethylene has a higher market price in comparison to ethanol (e.g. ~1010 USD per ton *vs.* ~750 USD per ton for ethylene and ethanol, respectively),[14,15] which increases the net overall economics of the process. Whether or not, however, this approach delivers a higher process economy depends on the final process, the overall costs of which must be considered entirely. In the same course, it is evident too that the different market prices together with the lower procedural effort of a combined fermentation/dehydration justify the initial investigation of this alternative concept.

Although various studies have been conducted addressing ethanol production from MJ, there is a considerable lack of information. This starts with the cultivated AC varieties, their varying fermentable sugar contents and MJ composition. To the best of our knowledge, there is no information available for combined ethanol/ethylene production from MJ. This work focuses on valorizing AC mucilage through (i) ethanol production and determining the optimal fermentation parameters, such as temperature, pH and $(NH_4)_2SO_4$ conditioning, and (ii) the direct use of ethanol in the selective production of ethylene by catalytic dehydration.

EXPERIMENTAL

Fruit collection and cocoa MJ preparation

AC mucilage was collected from the farm of Machala University of Technology, Ecuador (3° 17′ 30.6″ S, 79° 54′ 54.7″ W). The fruits did not present any pests and were carefully selected during harvesting. AC is a variety of *Theobroma cacao*, which is considered a particular genotype of 'fine cocoa', extensively farmed in tropical Latin American regions.

Mucilage juice from mature fruits of AC was manually extracted and filtered through a canvas filter. MJ was stored at −20 °C before characterization and experimentation.

Characterization

MJ pH was measured using an Accumet AE150 (Fisher Scientific International Inc., Pittsburgh, PA, USA) pH meter. Acidity and water content were determined by titration with 0.1 mol L^{-1} NaOH and phenolphthalein as an indicator, and by thermogravimetry, respectively. °Brix was identified by refractometry, using a digital refractometer HI 96822 (Hanna Instruments, Woonsocket, RI, USA) according to ISO 2173:1978. Ash was determined by AOAC 923.03. The elemental contents of carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) were measured in a Flash EA1112 (Thermo Fisher Scientific, Waltham, MA, USA) elemental analyzer.

Fermentable sugars and ethanol analysis

Reducing sugar (RS) content was determined by the DNS method. An aliquot of 0.5 mL of sample was added to 0.5 mL 3,5-dinitrosalicylic acid (DNS reagent), warmed in a bath at 100 °C for 5 min, and cooled before addition of 5 mL deionized water. Measurements were carried out at 540 nm in an Evolution (Thermo Fisher Scientific, Waltham, MA, USA) spectrophotometer.

Additionally, the sugars glucose, fructose, sucrose and cellobiose were determined before experimentation by high-resolution liquid chromatography (HPLC 1100, Agilent Technologies Inc., Santa Clara, CA, USA), equipped with a refraction index detector (G-1362A XR RI) using a SUPERCOGEL C-610H column. The column oven was set at 55 °C. The mobile phase consisted of 5 mmol L^{-1} H_2SO_4 at 0.6 mL min^{-1}.

Ethanol concentration was determined by gas chromatography (GC; 9790 II, Fuli-Scientific, Zhejiang, China) using a KROMAT KB column (FFAP 30 m × 0.30 mm × 100 μm) equipped with a flame ionization detector. Nitrogen at 50 mL min^{-1} was used as a carrier gas. The samples were collected every 24 h for a total of 180 h. Before GC analysis, the samples were centrifuged at 3000 rpm for 5 min and filtered through a 0.2 μm filter.

Yeast cultivation

Before each experiment, *Saccharomyces cerevisiae* Hansen 1883 (NCYC 366) was grown in a sterile medium consisting of 10 g L^{-1} yeast extract, 20 g L^{-1} peptone and 20 g L^{-1} dextrose at pH 5.2 through incubation at 37 °C for 24 h and constant agitation at 130 rpm. The yeast was harvested by centrifugation at 4800 rpm for 5 min.

Fermentation

The fermentation process was carried out under anaerobic conditions in batch reactors of 1 L, placed on an orbital shaker in an incubator at the desired temperature according to the experimental setup. Before fermentation, MJ was sterilized at 121 °C for 15 min and cooled to room temperature (25 ± 2 °C).

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The yeast culture obtained as above was suspended in tap water. Cell density was adjusted to 10^7 cells mL^{-1}. 1 mL yeast suspension was added to the batch reactor.

The fermentation experiments were carried out evaluating the effect of different parameters on the ethanol production or RS consumption, considering two stages as follows: (i) studying temperature effects at 25 and 30 °C by adding yeast; and (ii) studying the effects of initial pH and $(NH_4)_2SO_4$ concentration at the temperature optimum determined in stage (i). Spontaneous fermentation was performed as a control experiment. Table 1 shows the experimental conditions used in stage (ii). The initial pH was adjusted using calcium carbonate, and $(NH_4)_2SO_4$ was added to 1 g L^{-1}. Every 24 h the pH, RS and ethanol concentration were determined.

Modelling of ethanol production

The data collected were adjusted to the nonlinear modified model of Gompertz for kinetic studies, according to Eqn (1),[16] using MATLAB software:

$$P = P_m \exp\left\{-\exp\left[\frac{r_{pm}\exp(1)}{P_m}\right](t_L - t) + 1\right\} \quad (1)$$

where P is the experimental ethanol concentration (g L^{-1}), P_m is the ethanol potential production (g L^{-1}), r_{pm} is the maximum rate of ethanol production (g L^{-1} h^{-1}), t_L is the lag phase or the time needed for the exponential ethanol production (h) and t is the fermentation time.

The fermentation experiments were carried out in triplicate and standard deviations were determined.

Dehydration of ethanol and product characterization

Dehydration was carried out in a fixed-bed quartz glass reactor filled with dehydration catalyst ZSM-5 (H-form) that had been calcined at 300 °C for 1 h not later than 12 h before the experiment. Ethanol was transferred to the dehydration reactor by stripping the aqueous medium (37 °C) with nitrogen (0.2 L m^{-1}) and introducing the gas flow at the bottom of the column.

Both the gas and liquid samples from the ethanol dehydration experiments were analyzed by GC. The liquid samples were diluted with acetone and then analyzed by DHA (PDMS column: 100 m × 250 µm; injector and detector temperature: 250 °C; carrier gas: hydrogen (39 kPa); split rate 50; oven temperature: 0–1.5 min, 5 °C, temperature increase at 22 K min^{-1} to 48 °C, held for 29 min, temperature increase at 2.5 K min^{-1} to 150 °C and held for 15.7 min). Gas samples were injected without further pretreatment (column: Elite-1, 60 m × 320 µm; injector and detector temperature: 200 °C; carrier gas: hydrogen (60 kPa); split rate: 3.7; oven temperature: 0–7 min, 35 °C, temperature increase to 200 °C at a rate of 10 K min^{-1}, held for 2 min).

Table 1. Experimental conditions used for the fermentation experiments in stage (ii)

Label	Conditions
S	Spontaneous fermentation
T1	Yeast addition, no pH adjustment, and no addition of $(NH_4)_2SO_4$
T2	Yeast addition and adjustment of pH to 5.0 (no addition of $(NH_4)_2SO_4$)
T3	Yeast addition and $(NH_4)_2SO_4$ addition to 1 g L^{-1} (no pH adjustment)

RESULTS AND DISCUSSION

Characterization of cocoa MJ

The physical and chemical characteristics of MJ are shown in Table 2. The results are comparable to those reported in previous investigations.[17] The differences in the concentration of glucose and sucrose are attributed to maturity indices, location of the plantation, soil treatment, harvesting and post-harvest management.[8]

The RS and sucrose contents in MJ are sufficient to carry out fermentation without the requirement of adding sugar, which is beneficial as the facile treatment of MJ is a prerequisite for conducting ethanol production economically. The C/N ratio (weight basis) in MJ was 57.3, which is in accordance with literature reports, which have shown that C/N ratios > 10 offer the best conditions for sugar fermentation by yeast.[18,19]

Fermentation

Effect of the temperature in the fermentation

The fermentation process was monitored by RS concentration, ethanol concentration in the broth and medium pH. To check that the process was run with the least possible energy input, fermentation was conducted at an ambient temperature between 25 and 30 °C. The latter value is well known to be optimal for *S. cerevisiae*.[20] However, in terms of energy and resource efficiency as well as to design a process with the least possible carbon footprint, the ambient temperature in Ecuador of 25 °C on average appeared promising and worth a try.

Expectedly, different RS conversion rates were observed at these two temperature levels. At 30 °C it was 75.09%, but only 59.93% was obtained at 25 °C (Fig. 1). It turned out, though, that a fermentation temperature of 25 °C was sufficient for *S. cerevisiae* cell growth. This is in line with published knowledge.[20] However, 30 °C is clearly the better operating temperature, not only in terms of fermentation activity; the higher RS consumption also resulted in 12 g L^{-1} more ethanol than at 25 °C. This effect has already been observed for other yeast strains with different substrates.[21-23] In addition, not only was RS consumption more effective at 30 °C, but sugar conversion was also observed to proceed faster in the first 24 h of fermentation.

Note that the natural pH (3.7 ± 0.67) of MJ was not adjusted in this stage of experimentation. Potential effects of adjusting the initial pH were studied in the next stage of experimentation.

Effect of pH and ammonium sulfate on ethanol production

Our investigations started with experimentation stage i as described under 'Fermentation', above, where we studied ethanol production at T = 30 °C at MJ pH = 3.7. Subsequent experiments served to improve ethanol production. They were done at both initial and varying pH and also under addition of $(NH_4)_2SO_4$ (stage ii; Table 1).[11]

After 168 h of fermentation, the RS concentration had dropped from 170 to 76.63, 42.43, 22.42 and 35.56 g L^{-1}, reflecting 55.01%, 75.09%, 86.83% and 79.12% RS utilization during S, T1, T2 and T3 experiments, respectively (Fig. 2).

It was observed that modifying the initial pH to 5.0 (T2) turned out to be the most effective treatment, being 31.66% more effective than blank experiments (S), 11.6% more than T1 and 7.55% better than T3. This major influence in the ethanol production over N adjustment is consistent with Yu *et al.*,[24] who adjusted pH and the amount of N and P, concluding that pH is the most determining factor to produce ethanol using sweet sorghum juice with immobilized *S. cerevisiae*. On the other hand, although

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Table 2. Characterization of mucilage juice waste

Parameter	Unit	Value	Literature[26]
pH	–	3.7 ± 0.67	3.75 ± 0.81
Water content	%	81.2 ± 2.05	85.3 ± 8.60
Brix	°	17.1 ± 1.01	16.17 ± 0.74
Ash	%	1.4 ± 0.05	3.76 ± 0.84
Acidity	%	1.7 ± 0.52	1.08 ± 0.04
RS	g L^{-1}	173.6 ± 7.22	—
Glucose	g L^{-1}	62.5 ± 6.25	214.24 ± 6.42
Fructose	g L^{-1}	63.5 ± 5.23	—
Saccharose	g L^{-1}	48.9 ± 4.85	21.31 ± 3.21
Cellobiose	g L^{-1}	1.52 ± 0.02	—
C	%	45.9 ± 0.25	—
N	%	0.8 ± 0.02	—
S	%	5.3 ± 0.12	—

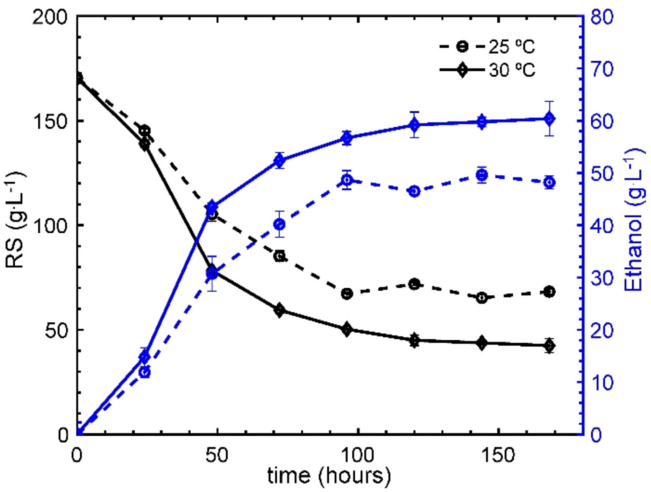

Figure 1. Effect of temperature in the fermentation process. Consumption of reducing sugars (RS) (left *y*-axis) and ethanol production (right *y*-axis) *versus* time at two temperatures.

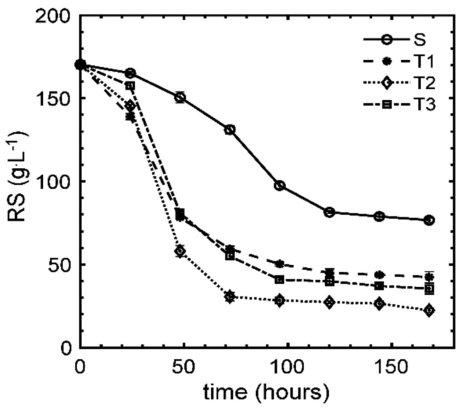

Figure 2. Effect of the treatments on consumption of reducing sugars (RS).

typical fermentations with *S. cerevisiae* are run at pH values of ~6.0–6.5,[24] elevating the pH as a standard method would imply higher costs. The question was to what extent it might be possible to combine fermentation at low pH with a reasonable fermentation performance. In addition, increasing the medium pH by adding alkali for neutralization would result in additional chemicals costs and higher ionic strength of the fermentation broth. As a consequence, a compromise situation resulted between acid stress imposed by a non-ideal fermentation pH and salt stress caused by the higher ionic strength as an outcome of neutralization. Therefore, it was decided to elevate the pH only minimally in order to find the optimal conditions.

On the other hand, the addition of $(NH_4)_2SO_4$ (T3) improves RS consumption compared with S and T1 experiments (Fig. 2). T3 resulted in 4.04% and 24.11% more RS conversion than in T1 and S experiments, respectively. $(NH_4)_2SO_4$ served as a source of nutrients, including N and SO_4^-, as well as to modify the C/N ratio.[25] Nitrogen promotes yeast growth and thus favors ethanol production as well as ethanol tolerance. Ammonium ions are rapidly taken up by yeasts and converted directly into amino acids. These, in turn, are key components in the nitrogen and carbon metabolism of the yeast cell. Concomitantly, the addition of ammonium sulfate renders RS degradation much faster and more efficient. This is in line with experiments by Saita and Slaughter,[26] who showed that supplementation of ammonium ions supports the glycolytic rate, which in turn resulted in a faster uptake of RS.

The amount of $(NH_4)_2SO_4$ added was calculated to give a final concentration of 2.5 g L^{-1}, which was selected because it had been reported as the optimum condition for fermentation under similar conditions.[25] Furthermore, this $(NH_4)_2SO_4$ added gives a C/N ratio of ~36, which is considered an appropriate C/N ratio in fermentation with *S. cerevisiae*.[27-29] Nevertheless, the effect of adding nitrogen source ($(NH_4)_2SO_4$) was far less pronounced. With a generation time of 90 min on average, there apparently is always sufficient detritus present in the fermentation broth. The malnutrition with N appears negligible here, given that the cells metabolize cell debris sufficiently rapidly, which is a clear benefit in terms of process engineering.

Mucilage juice represents a potential bio-source for ethanol production, which can be used without the need for pre-hydrolysis steps, with a slight pH conditioning and without adding an extra N source, contributing to the profitability of the process without sacrificing RS consumption. In addition, MJ valorization into ethanol contributes to green energy solutions and the circular economy of the cocoa market.

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Kinetics of ethanol production

The kinetic parameters related to ethanol production were determined by applying the modified nonlinear model of Gompertz (Eqn (1)). The results of the fitting are shown in Table 3, while the experimental data and simulated model are given in Fig. 3. The data were adjusted with regression coefficients R^2 up to 0.992 (Table 3), which confirms that the modified Gompertz model describes the kinetics of the experimental data properly.

The production of ethanol follows the same trend as the consumption of RS. The T2 experiments, in which pH was adjusted to 5.0, obtained 21.55 g L^{-1} ethanol more than the spontaneous fermentation (S) and were also slightly better than T3 and T1. There was only poor ethanol production for the first 6 h of fermentation, which is a typical lag phase. After 6 h, exponential ethanol production set in until the maximum rate was reached after ~90 h. Under S conditions, the fermentation set in far more slowly after 23 h and reached maximal ethanol production after 180 h, when the experiment was stopped.

The supplementation of ammonium sulfate as a nitrogen source as well as the pH adjustment serve to improve the cells' response to cell stress. While pH stress can easily be compensated for by exchanging H^+ against K^+, the N metabolism may be affected much more. Under cell stress conditions the Ehrlich pathway is activated, which means that there is an elevated level of amino acid catabolism. The cell fulfills its need for N sources to compensate for these losses by activating amino acid anabolism.[30] As this latter pathway means consuming RS in the service of producing amino acids instead of ethanol, it is evident that cell stress is at the expense of ethanol yield. A high ethanol concentration, in turn, is essential for process economy. However, ethanol imposes cell stress itself with increasing concentration. In its actual use, which is in producing beer, yeast generates approximately 44 g L^{-1} ethanol.[31] This largely corresponds to experiment S without nitrogen addition and pH adjustment. Both pH adjustment (T2) and the addition of ammonium sulfate (T3) improved yeast cell tolerance towards ethanol.

Kinetic parametrization determines the engineering aspects of the process towards the reactor design at a real scale. The kinetic behavior observed in the current work (Fig. 3) is in agreement with data from Bacelar *et al.*,[18] who reported elevated sugar consumption after 24 h. Lagunes *et al.* observed broth pH to decline to 4.5 after 144 h of fermentation,[32] which is in full accordance with the optimal parameters determined for T2 process conditions.

The nonlinear modified Gompertz model calculated the potential production of ethanol (P_m), the maximum ethanol production rate (r_{pm}) and the time it took to reach the exponential production of ethanol (t_L). Of the variants tested, the T2 treatment reached the highest values of P_m and r_{pm}, which were ~1.45 and 3.5 times higher, respectively, than under S conditions.

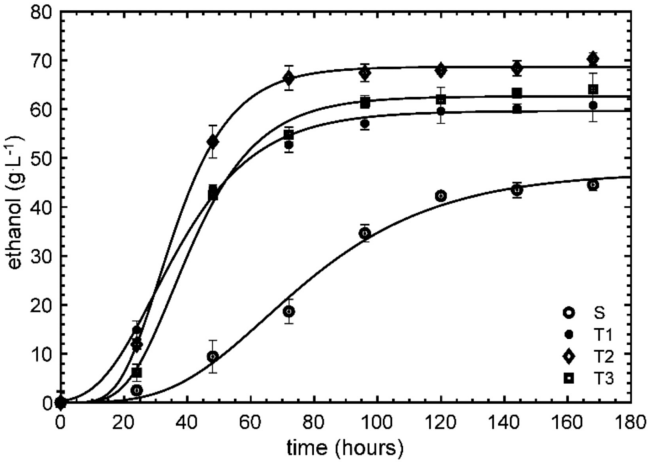

Figure 3. Modeling of ethanol production.

Ethanol production observed in the current work was comparable to studies using similar substrates based on cocoa MJ (Table 4). The highest ethanol production was reported by Anvoh *et al.*,[33] who obtained 72.33 g L^{-1} at 30 °C, and Takrama *et al.*,[34] who obtained 72.41 g L^{-1}, which is roughly the same. In both cases, the strain of *S. cerevisiae* was not further specified and there is no information about the cocoa cultivated variety used. However, ethanol concentration in this study (68.65 g L^{-1}) is similar to the values reported in the literature, with the advantage that the variety of both yeast and cocoa are specified. Other studies report far lower values. For instance, MJ from CCN-51 was reported to concentrate 22.06 g L^{-1} ethanol at 35 °C and pH 4.[12] With *Pichia kudriavzevii*, 13 g L^{-1} ethanol was obtained from MJ from a non-specified cocoa variety.[1,35]

In terms of ethanol production, MJ fermentation can compete with other substrates such as sugar cane syrup (41 g L^{-1})[36] or molasses (67 g L^{-1}).[37] The production rate of our study was r_{pm} = 2.03 (T2; Table 3) and considerably exceeds those obtained for sweet sorghum juice (1.67 g L^{-1} h^{-1})[38] or sugar beet molasses (1.07 g L^{-1} h^{-1}).[39]

Complete consumption of RS was not achieved. A possible reason for the incomplete degradation of RS may be the inhibition of yeast by the high ethanol concentration. Zhang *et al.*[40] observed a 50% decrease in yeast concentration from an ethanol concentration of 50 g L^{-1}. This problem can be solved by continuous removal of the formed ethanol from the fermentation broth (e.g. stripping). In addition to avoiding product inhibition, this has the advantage that subsequent catalytic conversion to higher-value products such as ethylene is possible without further workup.

Table 3. Kinetic parameters calculated by the nonlinear modified Gompertz model

Calculated parameter	Unit	S	T1	T2	T3
P_m	g L^{-1}	47.1	59.62	68.65	62.6
r_{pm}	g L^{-1} h^{-1}	0.58	1.24	2.03	1.57
t_L	h	35	11.75	18.56	20.83
R^2		0.992	0.997	0.999	0.997

Abbreviations: S, spontaneous fermentation; T1, yeast addition; T2, yeast addition and pH adjusted; T3, yeast addition and nutrient addition with $(NH_4)_2SO_4$; P_m, potential production of ethanol; r_{pm}, maximum ethanol production rate; t_L, time to reach exponential production of ethanol.

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Table 4. Comparison of ethanol production using MJ of cocoa

Yeast strain	Cocoa variety	Fermentation conditions	Maximum ethanol concentration	Reference
Saccharomyces cerevisiae	Non-specified	T = 30 °C, pH = 4.5–9, non-adjusted pH (spontaneous)	72.33 g L^{-1}	33
S. cerevisiae and *Issatchenkia orientalis* (*Candida krusei*)	Non-specified	T = 28.7–34.9 °C, pH = 4.8, T and pH non-adjusted (spontaneous) different yeasts	72.41 g L^{-1} (*S. cerevisiae*), 64.35 g L^{-1} (*I. orientalis*)	34
Pichia kudriavzevii	Non-specified	T = 30 °C, pH = non-specified	13 g L^{-1}	1
S. cerevisiae	CCN-51	T = 35 °C, pH = 4, different cell densities	22.06 g L^{-1}	12
S. cerevisiae	Arriba (AC)	T = 30 °C, pH = 5, addition of $(NH_4)_2SO_4$	68.65 g L^{-1}	This study

Ecuador is the world's largest cocoa producer, with ~525 000 ha of cocoa sowing in 2019.[41] Each hectare of cocoa plantation can produce ~74 L of MJ per harvest. Thus, considering 18 harvests per year, an RS concentration of 14.27% and an RS/ethanol conversion rate of 0.41, as resulting from this study, this equals ~40 000 tons of annual ethanol production in Ecuador, with a current market value of 31 000 000 USD (July 2021). However, an accurate estimation of potential ethanol production must also take into consideration factors such as the age of the plantation, maturity index, season, region of the plantation and the number of harvestings achieved, among others. Thus, research specifically targeted in this direction is recommended and is the subject of forthcoming studies.[42]

Ethylene production

The use of ethanol as a biofuel has the major disadvantage that a time- and energy-intensive separation of the water is necessary. Catalytic dehydration of the fermentation alcohol to ethylene enables the production of a platform chemical without additional workup of the fermentation broth. Furthermore, continuous removal of ethanol from the fermentation broth by stripping can avoid inhibition of yeast by excessive ethanol concentrations, which in turn allows for complete consumption of sugars and thus improves the process economy. All in all, direct utilization of ethanol to ethylene improves process economy and efficiency.

For catalytic dehydration, the gaseous ethanol is passed over a fixed bed of HZSM-5 catalyst. In a temperature range from 240 to 300 °C, the ethanol conversion to ethylene was >99.9%. Selectivity, on the other hand, was strongly temperature dependent (Fig. 4). With increasing temperature as well as increasing ethanol concentration, selectivity decreases. The highest selectivity was observed at 240 °C and 30 g L^{-1} ethanol concentration. The main by-products are propene, propane, isobutane, *n*-butane and further C_4 species. Contrary to expectations resulting from the acidic nature of the catalyst, no diethyl ether was detected.[43] Zhang *et al.*[13] observed increasing selectivity with increasing temperature, while it decreased at T > 270 °C. Since this work was done at temperatures > 240 °C, the observations are in good agreement with literature reports. Yet, these are only initial results that demonstrate the potential of a coupled fermentation/catalytic dehydration system. Improvement of selectivity, optimized reaction conditions and catalyst properties (e.g. Si/Al ratio, surface area, pore size) will further increase the performance of this combined approach and is a matter of follow-up investigations.

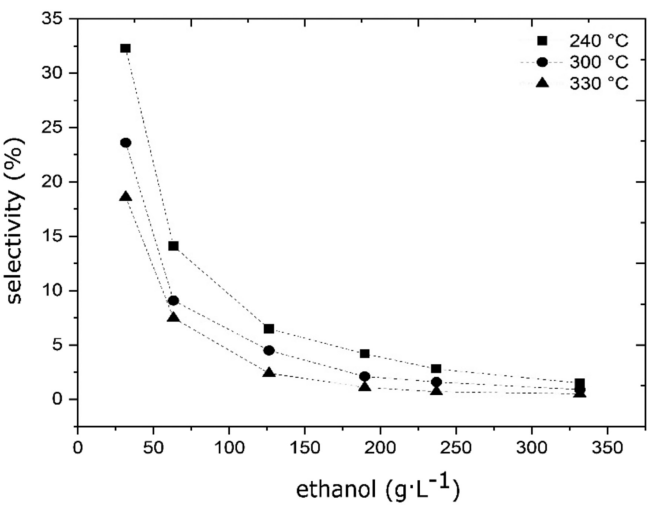

Figure 4. Dependence of selectivity of ethylene formation on ethanol concentration at different temperatures.

CONCLUSIONS

Mucilage juice from 'Arriba', which is usually discarded as waste during cocoa harvesting, has been evaluated to produce ethanol by fermentation with *S. cerevisiae* NCYC 366. The best conditions for ethanol production were carried out at a fermentation temperature of 30 °C and a slight adjustment of the MJ pH to 5, giving a maximum ethanol production of 68.65 g L^{-1}. Product quality was sufficiently high to allow for directly converting the produced ethanol into ethylene without further purification via catalytic dehydration with HZSM-5 as the catalyst. The slight modification of raw conditions of MJ for the ethanol and ethylene production process represents the lowest added costs without compromising the product yields. Moreover, this approach considerably improves the process economy since the ethylene price exceeds that of ethanol by ~1010 USD t^{-1} *versus* 750 USD t^{-1}. It has to be noted, though, that this is a rough estimate, so far not yet considering the overall process costs, the determination of which is the subject of a forthcoming study. In summary, MJ of AC represents a promising and sustainable matrix for bioenergy production and contributes to the circular economy of the cocoa market.

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CONFLICT OF INTEREST

The authors do not have any potential conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally.

CONSENT FOR PUBLICATION

All co-authors have approved the final version of the manuscript and have agreed to submit the manuscript to the Sustainable Environment Research Journal.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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