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Research article

Bioethanol production from cocoa hydrolysate and the assessment of its environmental sustainability

Dolvine Nguemfo Dongmo [a], Merveille Gwladys Nguemthe Ngouanwou [a], Cyrille Donlifack Atemkeng [a], Serges Bruno Lemoupi Ngomade [a], Junie Albine Kenfack Atangana [b], Rufis Fregue Tiegam Tagne [a,b,*], Theophile Kamgaing [a,**]

[a] Research Unit of Noxious Chemistry and Environmental Engineering, Department of Chemistry, University of Dschang, Dschang, Cameroon
[b] Department of Paper Sciences and Bioenergy, University Institute of Wood Technology, University of Yaounde I, Mbalmayo, Cameroon

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ABSTRACT

Bioethanol is recognized today as the most coveted biofuel, not only because of its tendency to reduce greenhouse gas emissions and other undesirable impacts associated with climate change, but also because of the simplicity of its methodology. This study evaluated bioethanol production from cocoa waste hydrolysates at the laboratory scale and, then evaluating the environmental impact associated with this production. Acid treatment was carried out on the hydrolysate in order to make it more accessible to ethanol-producing microorganisms. The cocoa hydrolysate was converted on a laboratory scale into bioethanol. The Ca, Mg, K and Na content of the substrate were respectively 78.4 ± 0.04; 109.59 ± 0.03; 1541.53 ± 0.08 and 195.05 ± 0.12 mg/L. The iron and total phosphorus contents were found to be at 14.06 ± 0.07 and 97.54 ± 0.01 mg/L respectively. The hydrolysate's biochemical oxygen demand (BOD 5) was 1080 ± 0.01 mg/L. A two per cent alcohol yield was obtained from 50 mL of substrate. Environmental impacts were assessed and quantified using SimaPro software version 9.1.1.1, Ecoinvent v.3.6 database, ReCiPe Midpoint v.1.04 method and openLCA sustainable development software. A total of 15 impact factors were assessed and quantified. The categories with more significant impacts in the agricultural phase were land use (1.70 E+04 m^2a crop eq), global warming (3.41 E+03 kg CO_2eq) and terrestrial ecotoxicity (7.23 E+03 kg 1,4-DCB), which were the major hotspots observed in the lab-scale biomass-to-bioethanol conversion phase due, to the use of electricity, distilled water and chemicals. The result of this work has shown that the cocoa-based hydrolysate is a suitable substrate for the sustainable production of liquid biofuels.

1. Introduction

Excessive dependence on fossil fuels and industrial development has resulted in the emission of enormous amounts of greenhouse gases (GHGs) into the environment. These anthropological activities are responsible for global warming and environmental pollution

* Corresponding author. Research unit of Noxious Chemistry and Environmental Engineering, Department of Chemistry, University of Dschang, Dschang, Cameroon.
** Corresponding author.
E-mail addresses: rufistagne@yahoo.fr (R.F. Tiegam Tagne), theokamgaing@yahoo.fr (T. Kamgaing).

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[1]. Public authorities' awareness of the environmental on the deterioration posed by fossil fuel consumption has resulted in the adoption of cleaner renewable energy sources. Among renewable source of energies, biofuels are currently the subject of, interest studies because of their utility and their low GHG emissions. Bioethanol has been recognized as the most widely used liquid biofuel made from organic waste [2]. It is mainly used as fuel and for electricity generation [2]. It is also likely to decarbonize transport cost-effectively and broaden energy diversification [3].

Currently, bioethanol is mainly produced from first-generation biomass, including food [4]. However, its development is limited due to the conflict and competition with food. The use of second-generation biomass feedstock sourced from lignocellulosic inedible agricultural and forestry waste could remove this competing barrier. The development of second-generation bioethanol production not only reduces food conflicts but also environmental pollution.

Cameroon is the leading cocoa producer in Central Africa. In 2021, the annual production was about 295,163 tons and its transformation generated 150 kilotons of waste each year [5]. These wastes are mainly composed of easily hydrolyzable sugars with low lignin fractions and high hemicellulose fractions [6]. Due to their physico-chemical characteristics, they can be good candidates for bioethanol production.

Several methods have been studied to increase the yield of bioethanol production. Lopez-Linares et al. [7] evaluated the main factors in the acid pretreatment process, to increase the yield of enzymatic hydrolysis and the sugar content. This study showed that a combination of enzymatic hydrolysis at a temperature of 202 °C for 5 min lead to optimal yield. Nascimento et al. [8] reported the effect of alkaline pretreatment with 4% NaOH for a reaction time of 60 min at 121 °C on the enzymatic hydrolysis of cocoa beans.

The economic viability of producing this biofuel using different substrates depends on variables such as local cost and feedstock composition, technological alternatives and energy cost. Bioethanol production includes several stages, namely, the pre-treatment of raw materials, fermentation and distillation. Some studies have reported that the cultivation of raw materials generates greenhouse gas emissions [9]. Thus it appears to be a compromise between the reductions in GHG emissions when biofuels are used. This trade-off requires further analysis and discussion for the development of the biofuel market. Analytical methods like environmental sustainability assessment related to bioethanol production have been carried out for some agricultural residues as feedstocks in different countries and under different conditions [1,3,9–11]. This methodology is also recognized as a standardized tool whose main objective to analyze and quantifyof the environmental impacts associated with a product by quantifying emissions and discharges that could affect the environment [11]. Previous studies were carried out with the aim of evaluating the environmental burdens of bioethanol production from different biomasses [11–14]. However, the analysis of emissions resulting from the production of bioethanol still remains a subject of debate and concern since the limits of the system vary according to researchers, thus causing variability in the results [15].

It should be noted that in most of these studies relating to the assessment of the environmental impacts associated with bioethanol production, they are most often limited to the use of already published data; not referring to experimental values for biomass production or conversion. This shows that bioethanol production in this biorefinery context is not evaluated. Considering these sustainability indicators into account requires analysing the different production phases of bioethanol obtained from various raw materials in different climates. Despite these bright prospects for bioethanol production, no work has been reported on assessing the sustainability of bioethanol production from cocoa hydrolysate. Thus, the evaluation of the sustainability of bioethanol production with this substrate is essential to help bioethanol producers in the selection of raw materials.

An evaluation study of the environmental sustainability of the production of bioethanol from lignocellulosic cocoa hydrolysates was carried out in this work. This study fills this gap by performing a comprehensive life cycle analysis (LCA) at the laboratory scale, on the production phases of bioethanol, highlighting the environmental benefits during the production process of this fuel and evaluating the potential of these residues to be considered as raw material for the production of renewable energy in Cameroon. The interest of this study is to provide a complete assessment of the life cycle of bioethanol production using empirical laboratory and/or field data and covering the different potential environmental impacts. The limits of this study are the failure to take into account transport processes from the acquisition of raw materials to the delivery and final use of bioethanol because of its many possible applications.

2. Materials and methods

2.1. Preparation of cocoa hydrolysate

Cocoa residues used to produce bioethanol were collected from an agricultural plantation in the city of Yaoundé, Center Region, Cameroon. The CellicCTec3 strains used in this study are commercial cellulolytic products (Novozymes, Bagsværd, Denmark). The hydrolysate was prepared in a 500 mL conical flask, by introducing 10 g of the cocoa pods and sulfuric acid, liquid:solid ratio 10:1 (v/w), maintained at 110 °C for 20 min in an autoclave. The liquid obtained was concentrated by heating in the rotavator before being stored in the refrigerator for analysis. The total crude fiber content was determined according to the Foss Tecator ASN 3801 method (EN ISO 13906 2008). The composition of dry matter, organic matter and cellulose was analyzed by standard NREL procedures [15]. The raw material was composed of (w/w) cellulose (18.6 ± 0.01%), hemicellulose (63.41 ± 0.02%), BOD (1080 ± 0.01 mg) and Organic Dry matter (63.22 ± 0.03%).

2.2. Cellulolytic enzymes and cellulosic sugar production potential

During cellulosic hydrolysis, the cellulose and hemicellulose contained in the cocoa hydrolysate were catalyzed using an enzyme called Novozymes CellicCTec3 (100% Ctec3) at an enzyme loading of 15 FPU. g^{-1} of hydrolysate. The enzyme was stored in the

refrigerator to avoid changes in its enzymatic activity which was evaluated on hydrolysis. The enzymatic hydrolysis was done in a three-necked flask containing 5% hydrolysate as source carbon and 100 mL of citrate buffer and cellulase as catalyst. Tween 80 surfactant was added to promote contact between the substrate and the enzyme. The mixture was stirred continuously at 150 rpm under aseptic conditions. Hydrolysis was carried out over a period of 48 h and the released sugars were analyzed by measuring their absorbances at 540 nm against a blank, followinga standard curve calibrated by an ultraviolet visible spectrometer (UV–Vis) and 3,5-dinitrosalicylic acid (DNS) as reagent [16].

2.3. Bioethanol production potential

The reaction mixture containing the biomass (cocoa hydrolysate), a yeast extract, polyoxyethylene sorbitan monooleate (Tween 80) and the citrate buffer were introduced into a 500 mL conical flask and autoclaved at 120 °C for 10 min for sterilization, comparatives studies based on the bioethanol production is presented in Table 1. After cooling, cellulase was added. Fermentable sugars were produced during the first phase. Then, the fermentation of the sugars released during the hydrolysis phase was carried out by adding *Saccharomyces cerevisiae* to the fermentation medium. The samples were collected daily to analyze the decrease in reducing sugars (DNS method, [16]. The laboratory setup of the equipment consisting of **(a)** fermentation system and **(b)** rotavapor for distillation is presented in Fig. 1.

2.4. Life cycle analysis

Life cycle analysis was the tool for assessing the environmental impacts associated with the production of 1 mL of ethanol. The objective of this analysis was to assess, on a laboratory scale, the main environmental impacts generated by the use of inputs in the preparation of bioethanol. This analysis took into account different processes, such as the production of energy, chemicals and the agricultural cultivation of raw materials, as well as emissions into the air. The eco-balance methodology was established by ISO standards and the guidelines of the ILCD (ILCD) manual [17–19] and aims to study the potential aspects and impacts on the environment and resources from a cradle-to- gate LCA. The results are present in the form of indicators linked to numerous environmental impact categories [20]. Known as the best tool for quantifying the environmental performance of a process, it is subdivided into four phases: 1) definition of the objective and scope; 2) inventory analysis; 3) impact evaluation and 4) interpretation.

The SimaPro software version 9.1.1.1, (https://network.simapro.com/rg), the Ecoinvent database version 3.5 [21] and the ReCiPe Midpoint (H) method v.1.03 [22] for impact assessment, with World ReCiPe Midpoint (H), 2010 standardization were used in this work for life cycle analysis. SimaPro is a widely recognized application for sustainability assessment, allowing users to model complex life cycle analyses according to ISO 14040. The LCA impact categories explored in this study are listed in Table 1.

2.4.1. Definition of objective and scope

This step consisted of evaluating the environmental impacts of cocoa in Cameroon, starting with the agricultural phase, the hydrolysate preparation, the hydrolyzate fermentation, and the liquid's conversion into bioethanol. The study was conducted on a laboratory scale, providing an idea of the main key points along the production chain. A functional unit that correctly reflects the product studied quantitatively has been established. The chosen functional unit (FU) to which all quantities have been proportioned was the production of 1 mL of bioethanol from an initial amount of 50 mL of cocoa hydrolyzate. The stages of the life cycle were the production and harvesting of cocoa on the one hand and the bioconversion process of biomass taking chemicals and electricity into account, on the other hand. Carbon sequestration processes were not taken into account in the analysis. This study adopted a perspective from the cradle to their end-of-life disposal, whose boundaries extend from the agricultural phase to the production. Fig. 2

Table 1
ReCiPe midpoint (H) impact categories.

Impact Category	Label	Unit
Fine particulate matter formation potential	PMFP	kg PM2.5 eq
Fossil resource scarcity potential	FSP	kg oil eq
Freshwater ecotoxicity potential	FETP	kg 1,4-DCB
Freshwater eutrophication potential	EFF	kg P eq
Global warming potential	GWP	kg CO_2 eq
Human carcinogenic toxicity potential	HCTP	kg 1,4-DCB
Human non-carcinogenic toxicity potential	HNTP	kg 1,4-DCB
Ionizing radiation potential	IRP	kBq Co-60 eq
Land use potential	LUP	m^2a crop eq
Marine ecotoxicity potential	METP	kg 1,4-DCB
Marine eutrophication potential	ME P	kg N eq
mineral resource scarcity potential	MSP	kg Cu eq
Ozone training, Human health potential	OFHP	kg NO_x eq
Ozone formation, Terrestrial ecosystems potential	OFTP	kg NO_x eq
Stratospheric ozone depletion potential	ODP	kg CFC11 eq
Terrestrial acidification potential	TAP	kg SO_2 eq
Terrestrial ecotoxicity potential	TETP	kg 1,4-DCB
Water consumption potential	WCP	m^3

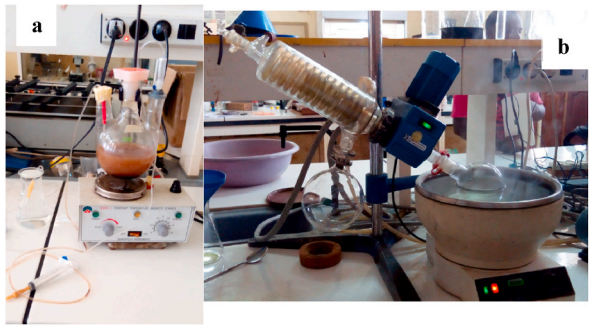

Fig. 1. Lab-scale of the, (a) fermentation; (b) distillation.

presents the system boundaries considered in this study.

2.4.2. Life cycle inventory and impacts

The life cycle inventory (LCI) was developed using primary data from companies operating in the agricultural phase (Table 2) and laboratory data collection for cocoa hydrolyzate conversion processes (Table 3). Cocoa cultivation includes the use of fertilizers, herbicides and insecticides. The units and reactions of the bioconversion of the hydrolyzate were modeled with literature values while the fermentation yield was estimated by our research team. The model reported by Nemecek et al. [23] was employed to model agricultural phase impacts and emissions, developing a method for estimating agricultural emissions to be used as part of the World Food LCA database, which adopts Ecoinvent as its base database. A physical allocation based on the amount of cocoa hydrolyzate obtained after fermentation was applied. Based on the agricultural and laboratory processes inventory, the usual life cycle impact assessment was carried out and the final impact wascritically discussed. Processes for converting cocoa hydrolyzate in the laboratory scale into bioethanol are shown in Table 2.

2.4.3. Life cycle impact assessment

The data from the inventory analysis were classified according to their potential effects on the environment [24]. The impact categories each with its unit used in this study are as follows: Abiotic depletion (kg Sb eq), Global warming (kg CO_2 eq), Ozone layer depletion (kg CFC-11 eq), Human toxicity (kg 1,4- DCB eq), Fresh water aquatic ecotoxicity (kg 1,4-DCB eq), Marine aquatic ecotoxicity (kg 1,4-DCB eq), Terrestrial ecotoxicity (kg 1,4-DCB eq), Photochemical oxidation (kg C_2H_4 eq), Acidification (kg SO_2 eq), Eutrophication (kg PO_4^{3-} eq) [24].

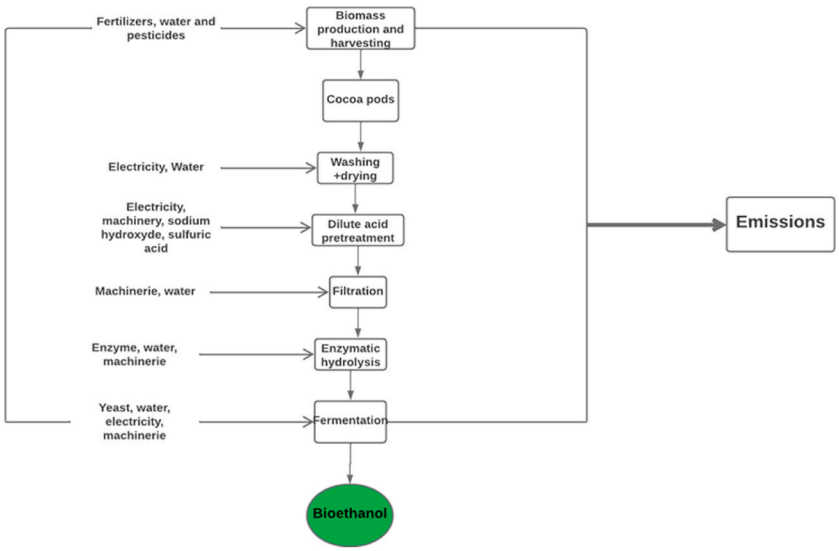

Fig. 2. System boundaries of bioethanol production from cocoa hydrolysate.

Table 2
Life cycle inventory of cocoa cultivation and fermentation (1 ha).

Item	Amount	Unit
Soil occupancy	1	ha
Water for irrigation	900	m^3
Urea (46%N)	450	kg
(20-10-10) Fertilizer	10	kg
Herbicide	5	kg
Insecticide	9	kg

Table 3
Conversion processes to bioethanol.

Processes.	Materials used.	Quantity	unit
Raw material collection	cocoa hydrolyzate	0.05	L
Washed and dried	water + sun	10	L
pretreatment	Machine	1	/
Filtration	Distilled water	3	L
	Electricity	/	/
	Machine	1	/
Hydrolysis	C6H8O7	0.042	kg
	C6H5Na3O7	0.0588	kg
	Tween 80	0.017	kg
	Cellulase	0.023	L
	Machine	2	/
	Electricity	/	/
Fermentation	$C_6H_8O_7$	0.021	kg
	$C_6H_5Na_3O_7$	0.029	kg
	Peptone	0.102	kg
	Tween 80	0.045	kg
	yeast extract	0.045	kg
	Cellulase	0.0045	L
	Electricity	/	/

3. Results and discussion

3.1. Characterization of the raw material

The Ca, Mg, K, Na, P and Fe contents of the cocoa hydrolyzate are listed in Table 4. The analysis was duplicated by the name hydrolyzate 1 and 1'.

Phosphorus, potassium, calcium, magnesium, iron and potassium are the essential elements for the growth and development of yeasts. Their deficiencies have negative repercussions on fermentation.

The data in Table 4 show sufficient values of exchangeable metals, thus showing that the cocoa hydrolyzate is a sample for fermentation because its high contents guarantee the nutritional needs of the yeast and increase its activity. These results are comparable with those reported by Acourene [25], who obtained a high yield of cellulosic bioethanol. Moreover, the results are similar to the studies carried out by Alfa et al. [26] on bioethanol production based on Cymbopogon citratus. The high nutrient content of these substrates may be due to the high capacity of some of them to absorb and store elemental nutrients in their tissues during cultivation [27]. The BOD5 (Biochemical oxygen demand for 5 days) is a physicochemical characterization of the hydrolyzate which makes it possible to evaluate the quantity of biodegradable material it contains. The analysis was performed daily for 5 days with replication. The average values obtained from this analysis are listed in Table 5. The physicochemical parameters were expressed milligrams per liter (mg/L) for lead and cadmium in water and in milligrams per kilogram (mg/kg) for compost and soil.

The hydrolysate's biochemical oxygen demand (BOD 5) was 1080 mg/L, which proved that the hydrolyzate from cocoa processing was conducive to alcoholic fermentation in an anaerobic environment [28].

Table 4
Contents of exchangeable bases (Ca, Mg, K and Na), total phosphorus and iron.

Code samples	Hydrolyzate 1	Hydrolyzate 1′	Means
Calcium (mg/L)	80.00	76.80	78,4 ± 0.04
Magnesium (mg/L)	110.81	108.38	109,59 5 ± 0.03
Potassium (mg/L)	1541.53	1541.53	1541,53 ± 0.08
Sodium (mg/L)	195.05	195.05	195,05 ± 0.12
Iron (mg/L)	14.28	13.84	14,06 ± 0.07
Total phosphorus (mg/L)	97.22	97.87	97,545 ± 0.01

Table 5
B0D5 of cocoa hydrolyzate (mg/L).

Days	BOD per day (mg/L)
Day 1	150.00
Day 2	230.00
Day 3	235.00
Day 4	235.00
Day 5	230.00
Total value BOD 5	1080 ± 0.01

3.2. Evaluation of the production potential of bioethanol

The sugars released (glucose and xylose) during hydrolysis were transformed into bioethanol by the action of the yeast *Saccharomyces cerevisiae* present in the fermentative medium at a temperature of 32 °C. Fig. 3 depicts the production of bioethanol during its three days of fermentation.

The results in this figure (Fig. 3) show that the quantity of glucose increases progressively in the reaction medium until it reaches a maximum value of 10.5 × 106 μg/L within 48 h of hydrolysis. This increase was a result of maximum cellulose degradation. After 48 h, we observed a slight decrease in glucose with a value of 4.5 × 106 μg/L, reflecting almost total decomposition of the biomass during the hydrolytic phase. On the other hand, during fermentation, we observe a process opposite to hydrolysis, which can be justified by the fact that the sugars produced during hydrolysis are transformed into bioethanol during fermentation. These results are in agreement with those obtained by Shruti et al. [29], who worked on the production of bioethanol from newspaper waste. After purification of the bioethanol obtained during the fermentation, 2 mL of bioethanol was obtained, yielding of 0.02% starting from 50 mL of the cocoa suspension. This yield is explained by the calorific character of the substrate which contains a large quantity of heavy metals thus making the fermentation more complete. Consideringthe annual production of biomass, a production potential of approximately 91,500 kg per year is estimated. These results are comparable with those reported by Vintila et al. [30] on bioethanol production, based on microwaved and alkaline treated cocoa pods.

3.3. Characterization of bioethanol by FT-IR spectroscopy

The product finally obtained was characterized by Fourier transform infrared spectroscopy in order to identify the functional groups comparable to that of fossil ethanol. Fig. 4 shows the IR-TF spectrum of bioethanol.

Fig. 4 shows the characteristic absorption peaks of the functional groups present in the compound. The absorption peak appearing at 3310.21 cm^{-1} corresponds to a band characteristic of the stretching vibration of the free OH bond. The one appearing at 2818.94 cm^{-1} of low intensity is attributed to a vibration of the methyl group (CH bond). The stretching vibration of the H–OH bond appears at 1658.6 cm^{-1} with a more or less intense peak. At 1005.58 cm^{-1}, a peak of great intensity characteristic of the stretching vibration of the CO group of the alcohol was observed.

The appearance of the H–OH bond of water molecules is observed on this spectrum, which justifies the abundant use of distilled water during the synthesis process [31].

3.4. Life cycle analysis results

3.4.1. The stage of production of cocoa in the fields

The environmental impact of the production of the target biomass per hectare is presented in Table 6 and Fig. 5.

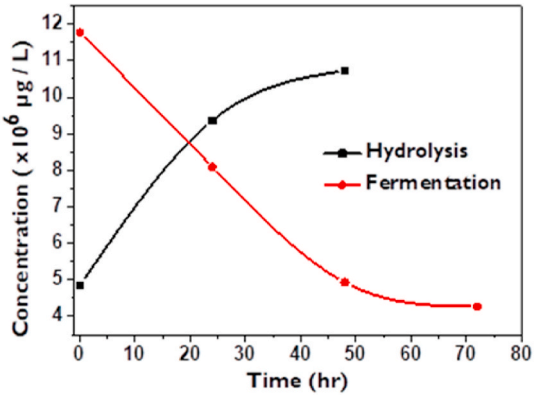

Fig. 3. Evolution of glucose during hydrolysis and fermentation.

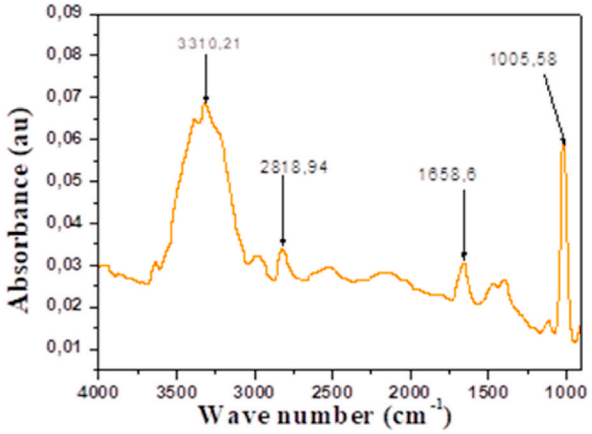

Fig. 4. FT-IR spectrum of the bioethanol produced.

Table 6
ReCiPe 2016 Midpoint v.1.04 characterized impacts for 1ha production of the considered agricultural.

Impact category	Unit	Cocoa
Global warming	kg CO_2 eq	3.41 E+03
Stratospheric ozone depletion	kg CFC11 eq	6.99E-02
Ionizing radiation	kBq Co-60 eq	9.89 E+01
Ozone training, Human health	kg NOx eq	5.30 E+00
Fine particulate matter formation	kg PM2.5 eq	1.91 E+01
Ozone formation, Terrestrial ecosystems	kg NOx eq	5.38 E+00
Terrestrial acidification	kg SO_2 eq	1.36 E+02
Freshwater eutrophication	kg P eq	6.08E-01
Marine eutrophication	kg N eq	2.82 E+01
Terrestrial ecotoxicity	kg 1,4-DCB	7.23 E+03
Freshwater ecotoxicity	kg 1,4-DCB	8.02 E+01
marine ecotoxicity	kg 1,4-DCB	1.05 E+02
Human carcinogenic toxicity	kg 1,4-DCB	1.53 E+02
Human non-carcinogenic toxicity	kg 1,4-DCB	1.47 E+03
Land use	m^2a crop eq	1.70 E+04
mineral resource scarcity	kg Cu eq	1.04 E+01
Fossil resource scarcity	kg oil eq	5.01 E+02
water consumption	m^3	5.92 E+02

The analysis of the results showed that cocoa production is mainly affected by three categories of impacts, namely land use (1.70 E+04 m^2a crop eq), terrestrial ecotoxicity (7.23 E+03 kg 1,4-DCB) and global warming (3.41 E+03 kg CO_2 eq). As mentioned in the introduction several works that have been carried out on the analysis of the environmental impact of bioethanol production. Those carried out by Morales Vera et al. [13] on the LCA of the bioethanol production from poplar biomass obtained a negative value of −1.1.10E3kg CO_2 eq. The results obtained in this study can be justified by the use of fertilizers, electrical machinery and other chemicals during the agricultural phase to boost yield, which are responsible for direct and indirect emissions into the Earth's atmosphere [32].

The contribution of the different impacts illustrated in Fig. 5 shows hot spots associated with the use of water with an average contribution of about 34%, the use of urea with an input of about 29% and emissions from the use of pesticides and fertilizers of about 33%. These results are in agreement with those obtained by Tagne et al. [9] and Tiwari et al. [14], with low variability.

3.4.2. Lab-scale bioethanol production

Table 7 and Fig. 6 present and illustrate respectively the characterized and normalized environmental impacts obtained for the production of 1 mL of bioethanol. Much more significant values of the various environmental impacts follow comparable trends depending on toxicity. The relative contributions of each factor falling into each impact category are presented in Fig. 6. The results showed that the stage of the culture was found to be the most influential point in all impact categories, but with an average contribution of 56%. These results can be justified by the use of fertilizers in the fields. Therefore, the use of fertilizers is a sensitive factor that has to be controlled and reduced as much as possible to obtain the lowest possible environmental impact, while maintaining a high yield. It is therefore a question of finding a happy medium to increase the yield of cocoa production while reducing the associated consumption. Electricity, sodium citrate and citric acid have proven to be a hot spot in higher impact categories such as global warming, terrestrial ecotoxicity, land use and fossil resources. As observed in section 3.4.1, the agricultural phase was the main

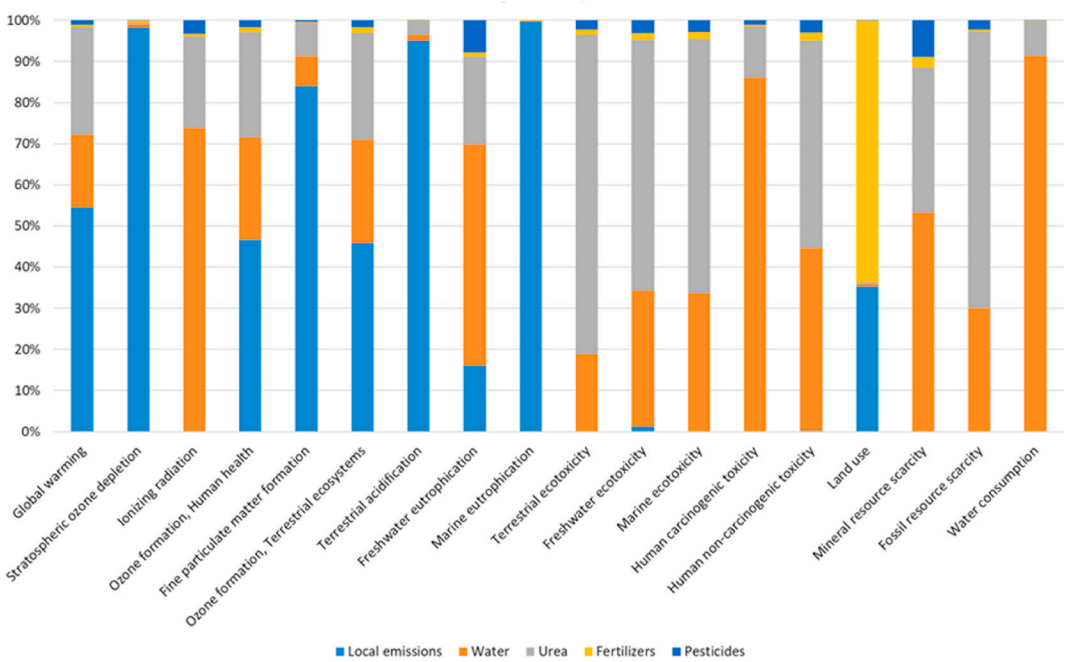

Fig. 5. Contribution to the ReCiPe 2016 Midpoint v.1.04 characterized impacts for 1ha production of Cocoa pods.

Table 7
ReCiPe 2016 Midpoint v.1.04 characterized impacts for the production of 1 mL of Bioethanol.

Impact category	Unit	Cocoa
Global warming	kg CO_2 eq	5.63E-01
Stratospheric ozone depletion	kg CFC11 eq	7.08E-06
Ionizing radiation	kBq Co-60 eq	2.23E-02
Ozone training, Human health	kg NOx eq	1.16E-03
Fine particulate matter formation	kg PM2.5 eq	2.27E-03
Ozone formation, Terrestrial ecosystems	kg NOx eq	1.18E-03
Terrestrial acidification	kg SO_2 eq	1.40E-02
Freshwater eutrophication	kg P eq	2.66E-04
marine eutrophication	kg N eq	2.70E-03
Terrestrial ecotoxicity	kg 1,4-DCB	1.70 E+00
Freshwater ecotoxicity	kg 1,4-DCB	2.28E-02
marine ecotoxicity	kg 1,4-DCB	2.86E-02
Human carcinogenic toxicity	kg 1,4-DCB	2.35E-02
Human non-carcinogenic toxicity	kg 1,4-DCB	4.37E-01
Land use	m^2a crop eq	4.99 E+00
mineral resource scarcity	kg Cu eq	2.74E-03
Fossil resource scarcity	kg oil eq	1.03E-01
water consumption	m^3	6.70E-02

contributor to environmental damage in all categories. The bioethanol production from cocoa hydrolyzate estimated a total emission of 34.11 E+2 kg CO_2 equivalent, of which 33.54E2kgCO_2 equivalent was attributed to the agricultural phase, while bioconversion of biomass contributed 0.563 kg of CO_2 equivalent. Some differences can be highlighted by comparing the environmental profile in this study with those reported in the literature. González-García et al. (2012), reported a net GWP of −0.619 kg CO_2 equivalent per kg ethanol [33]. In 2023, Tiwari et al. [14] reported a Global Warming Potential of 7.2/kg BDO including biogenic carbon emissions. The higher Global Warming Potential in our study compared to results reported in the literature could be the result of taking into account cocoa cultivation subsystems and ethanol production up to the biorefinery stage. Since the raw material was lignocellulosic biomass for which a large stock was used for its growth, the agricultural phase was the one that contributed the most to the carbon footprint. In contrast, carbon dioxide captured during biomass growth was not included in the impacts.

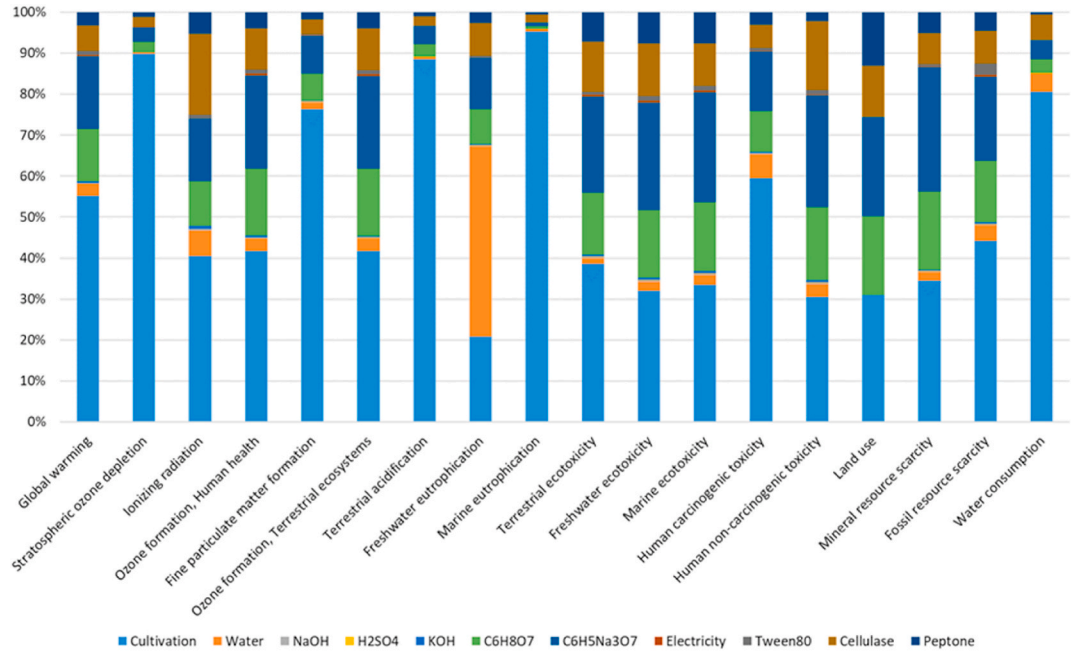

Fig. 6. Contribution to the ReCiPe 2016 Midpoint v.1.04 characterized impacts for the production of 1 mL of Bioethanol from Cocoa.

3.4.3. Significant findings

- ✓ Cocoa cultivation could be improved by reducing the use of fertilizers, pesticides and local emissions due to its environmental impact.
- ✓ The potential for climate change is the most important environmental impact, due to carbon dioxide emissions from land use, that must be considered
- ✓ The carbon sink during cocoa cultivation makes it possible to obtain an environmental gain for the climate change category, when producing 1 mL of bioethanol from its residue.
- ✓ Reducing the quantities of fertilizers and pesticides used during cocoa cultivation is a crucial parameter to improve the environmental impact linked to bioethanol production. To do this, an improved variety of disease-resistant and high-yielding cocoa is advocated. Green manures are also a way out if waste composting is popularized on a farm scale. Reducing the amount of electricity and distilled water used in different stages of the process would improve the impact of bioethanol.

The results obtained are of the order, slightly lower. Nevertheless, the level of bioethanol is of great interest for the sustainable use of cocoa residues. A comparison with similar work targeting the production of bioethanol under the influence of economic viability is presented in Table 8.

Five parameters are decisive in estimating the cost of ethanol production: raw material, pretreatment, hydrolysis, fermentation and purification. Sugar cane, beets, wheat and corn contain fermentable sugars (glucose, fructose, sucrose). The hydrolysis step which consists of depolymerizing the lignocellulosic matrix into simple sugar thanks to the action of microorganisms is most often not necessary for these raw materials, thus being able to considerably reduce the cost of producing ethanol from this type of biomass. This is not the case for certain biomasses which instead contain non-fermentable cellulose and hemicellulose. The hydrolysis step is therefore obligatory. Even after hydrolysis, the quantity of free sugars remains lower than that of non-lignocellulosic biomasses. Coupled with fermentation and distillation, they represent 77–91% of the overall cost of bioethanol production, with the raw material representing only 9–23% (Table 8). In other words, producing only ethanol from lignocellulosic biomass (cocoa for example) is not currently profitable. We need an integrated production system that makes greater use of by-products while waiting for classic enzymes and yeasts to be replaced by more affordable ones. Indeed, cultivating cocoa for specifically energy purposes (production of fuel ethanol) is not indicated. Fortunately, beyond its pulp, the cocoa bean has an appreciable market value. The labeling of this bean like that produced in Cameroon in specific agro-ecological zones would considerably limit the cost of producing bioethanol as part of an integrated system in which cocoa beans and bioethanol would constitute two main finished products [36].

Ultimately, diversification of the cocoa sector is recommended if we want to produce bioethanol from residues. Among the sectors of the future, we have the production of beans which is already mastered, the production of bioethanol which is the subject of this study, and the manufacture of activated carbon from residues. Previous work gives these coals a specific surface area of 800 m^2/g, which is considerable [32].

In the context of an abundant raw material such as cocoa residue, a part can be converted into ethanol and another into activated

Table 8
Comparative cost and impact of bioethanol production.

	Field production				Ethanol production				
Raw material Imputs	ImputsKg/ha	Yield (tone/ha)	Production cost (%)	Impacts	Reagents	Yield (m^3/ha)	Cost (%)	Impacts	Références
Sugar cane	14,620	71.19	50–83	culture and transport, emissions of pollutants	Cellulase, Yeast	7.5–11	17–50	Eutrophication, fresh water, occupation of agricultural land)	[34,35]
Beet	4938	65	50–68	emissions from fertilizers	Cellulase, Yeast	6.0–7.5	32–50	76 % impacts on boethanol (climate change and fossil resources)	[34,36])
wheat	–	–	53–87	–	–	2.7–3.5	13–47	–	[34]
corn	–	–	60–70	–	–	2.7–3.5	30–40	–	[34]
cocoa residue	1375	2.5	9–23	Land use, terrestrial ecotoxicity, global warming	Cellulase, Yeast	2% (V/V)	77–91	56% Impact on bioethanol (culture)	this work

carbon, knowing that upstream, the beans are marketed.

4. Conclusion

The objective of this study was to produce bioethanol from cocoa hydrolyzate and to assess the life cycle of processes from downstream to upstream using empirical data. The hydrolyzate was obtained by acid treatment. A notable energy conversion rate has been achieved with advanced anaerobic fermentation technology. The availability of the biomass tested in the Cameroonian economy in large quantities gives a high yield of second generation bioethanol. This abundance makes this biomass an important potential source for the production of green energy in Cameroon. In total, 17 environmental categories were evaluated to determine the main hotspots. The results showed that the agricultural phase was the main contributor to all environmental categories due to the use of electricity, distilled water and chemicals. The production of bioethanol from cocoa hydrolyzate estimated a total emission of 34.11 E+2 kg CO_2 equivalent, of which 33.54E2 kg CO_2 equivalent was attributed to the agricultural phase, while bioconversion of biomass contributed 0.563 kg of CO_2 equivalent. The agricultural phase was the one that contributed the most to the carbon footprint. Given that the agricultural phase contributes strongly to all categories, future work could examine alternative pathways in assessing the environmental and economic benefits of bioethanol production to improve the sustainability of this biofuel's production. These results indicate that bioethanol production based on cocoa hydrolyzate can serve as a roadmap for future work for a much more ecological production.

Data availability

The authors declare that data supporting the funding of this study are available within the article.

CRediT authorship contribution statement

Dolvine Nguemfo Dongmo: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Merveille Gwladys Nguemthe Ngouanwou:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Data curation, Conceptualization. **Cyrille Donlifack Atemkeng:** Software, Methodology, Data curation. **Serges Bruno Lemoupi Ngomade:** Writing – review & editing, Methodology, Investigation, Data curation. **Junie Albine Kenfack Atangana:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Rufis Fregue Tiegam Tagne:** Writing – review & editing, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Theophile Kamgaing:** Writing – review & editing, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

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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