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Production of bioethanol by hydrolysis and alcoholic fermentation of sugar present in the wood: Case of Fraké

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Abstract:
Bioethanol is one of the most promising and friendly alternatives of our environment to fossil fuels, which is produced from renewable energy sources. Although the current fuel ethanol is generated from edible sources (sugar and starched plants), the lignocellulosic biomass (LB) because of its potential, has attracted the attention of users from Central African countries who have a huge potential in trees. Fraké is one of those tropical tree species from the Combretaceae family that overloads the Congo Basin. Obtaining bioethanol by recovering Fraké wood waste was done by anaerobic fermentation process using the Saccharomyces cerevisiae yeast strain. The results achieved showed a polysaccharide rate of 55.1%; a extractable rate of 24.7%; the percentage of polysaccharide conversion was 4.6%; the analysis of the ethanol obtained gave the values of boiling point (79 ° C), the density (0.82 g / ml) as well as the relative density (0.82). These results show that waste from Fraké is a lignocellulosic biomass with little wood which takes an interesting energy benefit because of its high level of polysaccharides which can be hydrolysed to produce bioethanol, particularly in sub-Saharan Africa where the access to energy still remaining at a very low rate.

Keywords - Bioethanol, cellulase, enzymatic hydrolysis, *saccharomyces cerevisiae*.

INTRODUCTION

The world faces three major problems, whichare population increase, decrease of natural resources and climate change. As population growth being on all fours with increasing consumption needs, there is increasing pressure on natural resources **[1].** Based on this observation, it is wise to turn to lignocellulosic biomass energy valorization which is affluently available especially in sub-Saharan African countries, at low cost, easy to process especially for the production
of second-generation fuels **[2].** Like most wood producer, processor and exporter African countries, Cameroon faces an energy deficit in the transport and electricity sector **[3].** However, the country generates a significant annual quantity of lignocellulosic biomass waste from unexploited wood, i.e. about **1,130,539.9 m^3 for its East region [5]**. Many studies have shown that this waste, rich in organic matter, was an important product and constituted new raw materials for many industries.

On the other hand, their valorisations by biotechnological procedures (biochemical and thermochemical) represent a solution of choice to the problem such as that of energy deficit like fuel. Several works relating to the production of bioethanol from lignocellulosic material (wood waste, sawdust, wood chips, etc.) have been carried out in recent years through the world; such as the production of bioethanol from barley and cereal straws **[5].** The biotransformation of these lignocellulosic products like sawmill waste would potentially produce around 90 million cubic meters of bioethanol per year **[6].** Wood waste contains up to 60.63% fermentable sugars and therefore represents an excellent substratum of choice for the production of many value-added substances, including bioethanol **[7].** In addition, in wood processing sawmills, biomass is present in several forms (slabs, edgings, wood waste, sawdust, wood chips, wood scraps, core defects). A study carried out in Cameroon in 2013 reveals that for 2,500,000 m^3 of logs entered in the factory, there is approximately 1,800,000 m^3 of waste which is burnt in the open air each year **[8].**

Plant material (wood) is heterogeneous and the polysaccharides are drowned and interconnected to lignin. Themaintaining of tropical woods would be very likely different from one species to another and depends on various parameters such as structure, chemical composition as well as nature of removable/ extractable. This is how the application of some methods for processingFraké (Limba) wood is studied. To achieve this, it was necessary to highlight a few specific objectives which will facilitate a best understanding. The 4 objectives are:

- Identify wood wastes from the sawmill;
- Estimate the share of waste product destined for the dump;
- Produce the bioethanol;
- Specify the product obtained after fermentation.

II. EXPERIMENTATION

A. WOOD MATERIAL (Fraké)

Wood is a woody material produced by an organism living in an ecosystem environment. Two groups of wood can be noted, among others coniferous or wood from temperate zones and leafy or wood from tropical zones. Tropical woods are woods located between the two tropics (capricorn in the South and cancer in the North). Classes of these species are most often defined according to their physicochemical properties. They can be classified into two main categories, namely: hard class woods which are characterized by a high level of lignin in the material contrary to soft woods which have a relatively low lignin level. The objective is to produce bioethanol-fuel through an enzymatic hydrolysis and an alcoholic fermentation of the sugars present in the wood (Fraké) in the presence of a strain of yeast such as *Saccharomyces cerevisiae*. Fraké with the scientific name *Terminalia superb,* is a slightly nervous wood with undifferentiated sap-wood in creamy white colour which is generally found in the Congo Basin forests in particular in East, Center, Littoral, North-West and South of Cameroon as

indicated in figure 1. It is noted that this species is chemically made up of removable/extractable (terpenes, tannins, resins), lignin, hemicellulose as well as cellulose which is an assembly formed of several molecules of D-glucose linked between them by β,1-4 contacts ; three biopolymers which are strongly interconnected **[9].** Non-fermentable lignin plays a protective role and prevents access to cellulose by hydrolysis agents. Fraké was chosen for applying the aforementioned process due to several valid reasons including its cellulose content of up to 60% compared to dry matter, its availability in the forest, the degree of processing within the wood processing units (UTB) and also the availability of some data related to the mechanical and physical properties (odour, colour, density, porosity) of the said species.

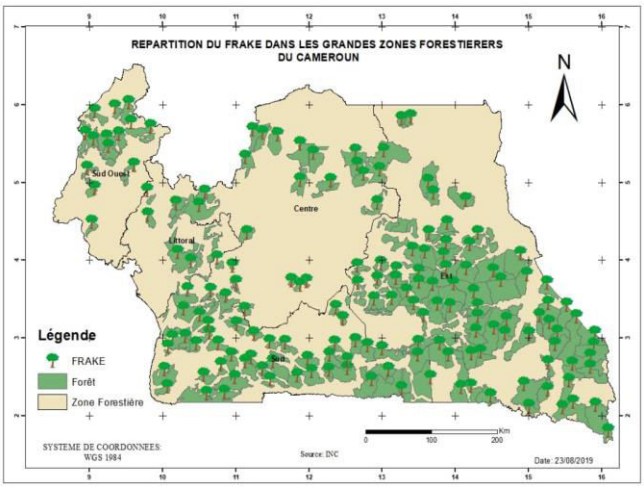


Fig. 1.1 Distribution of Fraké wood

Plant, biological and chemical products were used as well as apparatus.

A. Materials and Apparatus for Analysis

For the production of ethanol, the plant material used is the Fraké wood chip and baker's yeast (Saccharomyces cerevisiae) for biological facility. A set of laboratory equipment is used particularly for packaging. A digital thermometer for measuring temperatures, an oven with the following characteristics: T.max = 250 ° C, U = 220 V, P = 1300W for drying the sawdust. Volumetric flasks (50, 100 and 500 mL) for the preparation of the solutions; test-tubes of 10, 50,100 mL for the measurement of exact volumes, A 5 mL syringe for measuring exact volume of the enzyme; flasks for fermentation and diggers, funnels and filter papers for filtering solutions; a simple distillation assembly to separate the ethanol from the mixture, a blade mill and a sensitive scale for weighing the samples.

The reagents used are distilled water, sodium hydroxide tablet (sodium hydroxide (P≥90%; M = 40 g / mol) house: Tinyechemicals); 99.8% acetic acid (sodium acetate); sulfuric acid H2SO4: (98%; d = 1.84; M = 98.07g / mol), Potassium dichromate: K2Cr2O7: (99.5%); 95% ethanol and concentrated nitric acid: HNO3.

B. Data Collection

Fraké wood chip was collected in the Yaoundé wood processing units. It is a junction for the woods coming from the South and the East forests for Douala (port). Our study was carried out in the laboratory of applied inorganic chemistry unit of macromolecular chemistry research of the University of Yaoundé 1

C. Method / Experimental Procedure

After being collected, weighed, sorted and dried in the open air the samples of Fraké, these were dry-crushed in a blade mill in order to obtain particles of dimensions between 2 to 3 millimeters [5]. Later on, a quantity of the said material was weighed on a sensitive scale in order to obtain an initial mass (M_i).

The drying of the raw material in the oven is an important step in the process of producing bioethanol from biomass, because it enables to determine the moisture content [10] of the raw material by eliminating a good amount of the linked water it contains. Once the oven is on, set to a temperature of 103 ° C ± 2 ° C, and wait for the oven enclosure to stabilize, then introduce the material previously weighed inside the oven. The drying time is 24 h. After this time, remove the material from the oven and re-weigh in order to have a final constant mass (Mf)**. This dried material can then**

undergo a pre-treatment. Thus from the formula of moisturerate on an anhydrous base, the moisture rate of the material is determined by the following formula:

$$\text{Moisturerate}\ (\%) = \frac{Mi - Mf}{Mf} \times 100 \qquad (1)$$

D. Dry matter pre-treatment

The chemical pre-treatment aimed to dissolve the extracts and part of the hemicelluloses included in the material in order to facilitate the enzymatic attack [11]. This operation is divided into two stages. The first step concerns the calculation of the various elements involved in the pre-treatment process in alkaline solution. The second step is based on the preparation of the sodium hydroxide solution.

After weighing 15 g of dry matter (M_S), take a volume of 300 ml of distilled water and pour into a 500 ml beaker containing a sodium hydroxide solution with a concentration of 0.5 mol per liter. The mixture obtained (substratum + sodium hydroxide solution) is under magnetic boilingwith a shaking frequency of 500 Revolutions per/ min. Let the mixture getting cold and then filter by using a Buchner. Rinse the solid material thoroughly with distilled water to ensure complete removal of the soda.

Finally dry the solid material in an oven for 12 hrs at a temperature of 103 ° C ± 2 and weigh its mass (M_{Pr}). The said pre-treated material is now ready for hydrolysis.

The rate of extracts present in the material is determined using the following formula:

$$\text{Extract rate}\ (\%) = \frac{Ms - Mpr}{Ms} \times 100 \qquad (2)$$

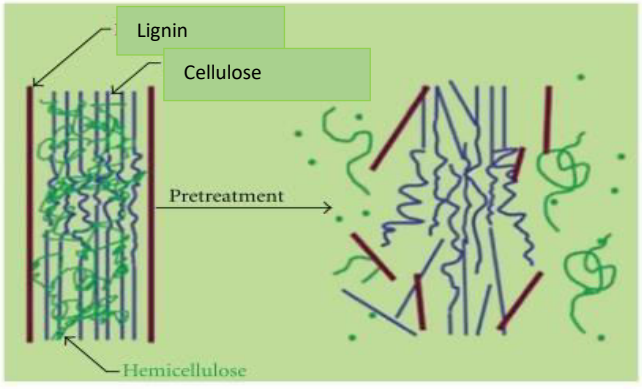


Fig. 2.1 Illustration of the pre-treatment impact on the lignocellulosic biomass

In order to have an idea on the influence of certain parameters on the material yield, this pre-processing phase was done on six (6) samples which the parameters are recorded in table 2.1.

Table 2.1: Variant and non-variant parameters of the samples

	Mass of soda (NaOH) in grams	Mass of the substratum in grams	Temperature	Reaction time in minutes
1	6,225	15	50°C	45
2	6,225	15	70°C	60
3	6,225	15	90°C	120
4	6,225	15	110°C	150
5	6,225	15	130°C	210
6	6,225	15	150°C	240

- **Determination of the cellulose polysaccharide level**

The cellulose level is determined by the Kruschner method. 2g $C_{1)}$ of dry matter was introduced into a flask containing a solution mixture of 45 ml of ethanol (95%) and 11 ml of acid. The mixture is brought to a temperature of 90 ° C at a shaking frequency of 500 rpm for 1 hour. Under reflux, the process is repeated three times to ensure complete elimination of the sugars. The filtrate obtained is subsequently dried in an oven for 12 h at a temperature of 103 ° C ± 2 ° C. After 12 hours, the residue is weighed in order to obtain the mass C_2. Thus, the level of polysaccharide (cellulose) is determined through the Kruschner method from the following formula:

$$\textbf{Polysaccharide rate}\ (\%) = \frac{C1 - C2}{C1} \times 100 \qquad (3)$$

➢ **Hydrolysis of the pre-treated material**

The cellulose and some of the hemicelluloses present in the pre-treated material must be converted into fermentable glucose by a process called enzymatic hydrolysis.

The enzymatic hydrolysis (fig. 4) is done by applying a load of 40 FPU (2 ml of enzyme solution (accellerase $^{R.}$ 1500) in 198 ml of acetic buffer solution of pH = 4.8) in 6 g of the pre-treated material. The mixture obtained is placed under magnetic shakingat a temperature of 50 ° C for 48 hours with a frequency of 500 Revolutions per / min. After hydrolysis, the mixture is filtered to remove the solid residue and then the hydrolysate obtained is heated for a few minutes to 100 ° C. in order to denature the enzyme since it does not withstand temperatures above 70 ° C. This hydrolysis operation is performed twice to reduce the margin of error. The quantity of glucose produced in each hydrolysate is measured using a spectrophotometer and the average of the two is used to calculate the percentage of saccharification which the formula is as follows:

$$\text{Saccharification rate} = \frac{\text{Mass of the reducing sugar}}{\text{Mass of the polysaccharide}} \times 100 \quad (4)$$

Fig. 3 Reactional mechanism of enzymes for the hydrolysis of cellulose (Debboub, 2012) [12]

The glucose obtained can therefore be transformed into ethanol by a process called fermentation.

➢ **Glucose fermentation**

After the pre-treatment and hydrolysis of lignocellulosic biomass, simple sugars (glucoses) are produced as a result of the depolymerization of cellulose and hemicellulose. Fermentation consists in transforming sugar (glucose) into ethanol in anaerobic conditions:

Sugars + Yeast ==> Ethanol + CO2 + Energy [13] **(5)** The hydrolyzate obtained after hydrolysis is fermented by introducing 8g of baker's yeast (*saccharomyces cerevisiae*) into a bottle containing 400 ml of hydrolyzate then close hermetically for 4 days with a temperature between 28-30 ° C [14]. After this operation, the pasteurized solution is heated to a temperature between 60 to 70 ° C for a few minutes using a hot plate to destroy the pathogenic bacteria and enable the preservation without changing the composition or the flavour of the mixture. After previously cooled it, the solution is filtered to remove the rest of the yeast. The filtrate obtained is a mixture of water and ethanol, hence the need for carrying out a distillation**.**

➢ **Distillation of bioethanol**

The distillation is a process for mixture separation of liquids with different boiling points. It enables the constituents of a homogeneous mixture to be separated. Under heat, the substances vaporize successively, and the vapour obtained is liquefied to give the distillate [15]. This is done using a distiller (simple distillation) to a temperature of about 79 ° C. The product obtained always contains a little water. Some tests have been done to see if our product actually contains alcohol. Figure 5 gives the stages of bioethanol production from forest biomass.

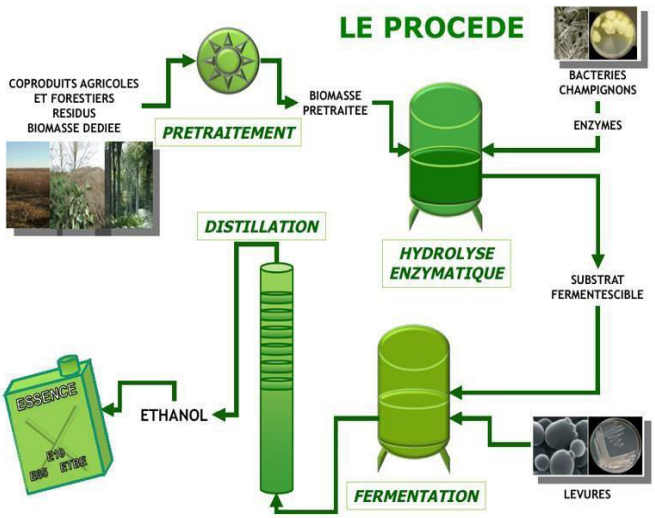


Fig. 3.2: Schematic diagram of the process for producing bioethanol from lignocellulosic biomass **[16].**

It is clear that this fig. 5 matchedup the different stages presented.

❖Characterization of the product obtained after fermentation.

The aim is to see if the characteristic values of the product obtained approximate those found by some authors who have worked on the production of bioethanol and also to see if these values are close to that of 95% ethanol.

➢Qualitative test of the functional group

The product (distillate) obtained after distillation will be subjected to thebreathalyzer test which the procedure is as follows:

1 ml of sulphuric acid (H_2SO_4) is poured into a test tube containing 5ml of distilled water and 1g of potassium dichromate ($K_2 Cr_2 O_7$) is then added to the mixture. The solution obtained is orange in colour (characteristic colour of dichromate). From the ethanol solution, a few drops of pure ethanol (95%) are introduced into the tube for a test and a change in coloration (from orange to green) has been observed, which highlights the presence of alcohol and therefore functional group[6] (-OH). The same operation is repeated, but this time with our distillate obtained after distillation by using the microchemistry kit.

➢Determination of boiling point

A quantity of the distillate is introduced into a 250 ml flask containing a small opening through which a thermometer will be introduced. The latter is therefore heated. The apparatus must be waterproof as soon as the liquid begins to be on the thermometer. It remains stable for some times.

➢Determination of density

The density is determined by pouring a known quantity or volume of bioethanol into a previously weighed test-tube. This test-tube and its content is then weighed on an electronic balance in order to have the mass of the bioethanol solution. Thus, the density (ρ) will be determined by the following formula:

$$P = \frac{\text{Mass of the bioethanol solution}}{\text{Volume of the bioethanol solution}} \times 100 \quad (6)$$

Determination of the Relative density

Therelative density is defined after determining the density of the ethanol. Therefore, the density is equal to the ratio of the density of the body (ethanol) and that of water. The following formula is used to determine the said density.

Relative density = density of ethanol (f_{body}) / density of water (f_{water})

After obtaining the product and the various tests which have been carried out, it can be affirmed that the distillate obtained contains alcohol and meets a certain number of criteria. These results enable to

think of a large-scale production and for that reason it is therefore necessary to put in place a production unit.

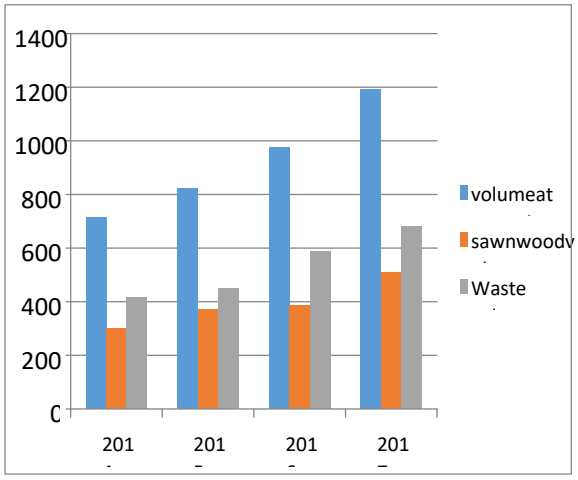
E. Results analysis tools

Once the four stages of bioethanol production process were completed, it was important to analyze some of the data collected during each stage of the process in order to highlight the impact of temperature and accelerate1500 on each sample. To do this, some data were encoded in an Excel 2016 spreadsheet to firstly highlight the waste yield produced by the CUF processing unit. This yield enables to know approximately the quantity of waste intended for the dump. In a second stage to highlight the proportion of polysaccharide, extract, saccharification as well as the characteristic values of the product among others the density, the odour, the colour, the boiling point and the density. The SPSS software version 2017 enabled to highlight graphs facilitating their interpretations. Many results were achieved via these analysis tools. It is therefore a

question in the following paragraphs to presentthe results and provide appropriate interpretations.

3. RESULTS AND DISCUSSIONS

In the volume of wood entering the factory (all species combined), about 2/5 are processed into sawn wood (i.e. an average material yield of 42.31%) and 3/5 intended for dump (i.e. a proportion of 57.69% of waste intended for the production of bioethanol). The proportion of waste



intended for the production of ethanol is slightly low compared to that of EFA'A Gilles which is 65.49%. This 65.49% results from the study conducted in 2016 on the influence of the carbonization of sawmill waste on greenhouse gas emissions at the Mbang MFID. This slight difference can be due to the log sawing equipment that the MFID uses or to a poor implementation of the machines.

A. Production of bioethanol

For producing0 bioethanol, the final mass of the material collected amount to Mf = 5 Kg.

After sterilizing the samples, the percentage of water in Fraké is presented in Table 3.1

Table 3.1: Moisture content of the Frakéwood chip

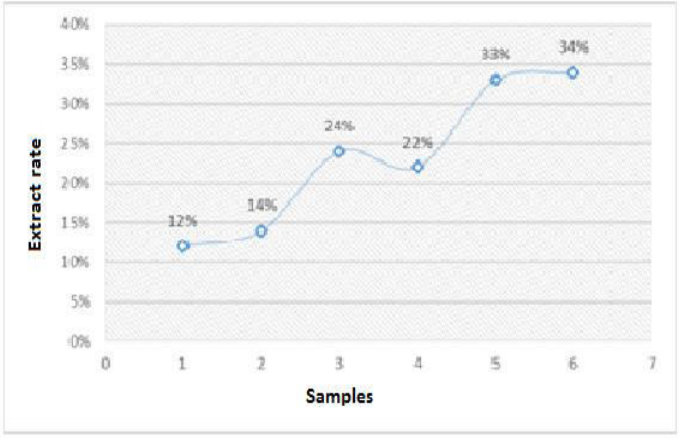
substratum	Wet mass (g)	Dry mass (G)	Mass of water (G)	T (%)
Fraké Wood	160	147.2	12.8	8.69

The low moisture rate (8.69%) shows that the Fraké wood used was sufficiently dry. Wood stabilized under room conditions generally has a moisturecontent between 8 - 12%. A quantity of this dry matter will be used for the pre-treatment. The moisture content of Fraké wood obtained is very different from that of ESSOMBA Paul which is 85.53%, obtained in 2012 during the study on the production of bioethanol from palm tree trunk. This difference in value simply means that the trunk of a palm tree would constitute a large reserve of water to supply the entire plant.

Dry matter pre-treatment

The main purpose of the pre-treatment of dry matter is to dissolve the extracts, and part of the hemicelluloses included in the wood in the presence of an alkaline solution (NaOH). The levels of extracted materials from the 6 samples are shown in Table 3.2.

Table 3.2: Extract rate in Fraké wood



	Wet mass (Mi) in grams	Anhydrous mass (Mf) in grams	Extract rate in grams
1	15	13,1	12%

2	15	12,8	14%
3	15	11,4	24%
4	15	11,6	22%
5	15	10	33%
6	15	9,8	34%
Final Extract rate = 23%			

This extract rate was calculated by averaging the extracts within the range of the lignin rate of tropical woods (15-25%). The result achieved shows that the treatment applied eliminated practically 23% of the base product. Pre-treatment removes extracts made of low molecular weight that are stuck to the cell side of the wood capable of impeding the development of microorganisms. It thus releases the cellulose for enzyme attack. The sodium hydroxide solution also has the capacity to rise the cellulose, a phenomenon which in no doubt frees up spaces for the infiltration of hydrolysis agents. The extract rate (23%) obtained is close to that of KLASON in 1984 which is 21.2%. These two rates fall within an interval [15-25] which corresponds to the variation of lignin rate of tropical woods. It should be noted that the temperature as well as the duration of the reaction strongly influence the level of lignin. The following graph shows some samples whereby the extract rate is either below or above what is expected.

We can therefore conclude by saying that the most satisfactory results are those of which the temperature of the medium rises to 90 ° C and a reaction time of two hours. Once the dry matter has been pre-treated, it can then be hydrolyzed. Only samples with an extract rate of 24 and 22% will be hydrolyzed.

Kruschner's method is used to determine the level of cellulose present in the wood. Let C_1 be the mass before treatment and C_2 the mass after treatment. The results are shown in the table below:

Table 3.3: Cellulose (polysaccharide) level in wood

Substratum	**C1 (g)**	**C2 (g)**	**Tc (%)**
Fraké wood	2	0.898	55.1
Mass of polysaccharide (g) = 3.306			

The method used to determine the cellulose content is very reliable (Kruschner method) because the mass of polysaccharide represents almost half of the raw material. We can therefore say that the material is a good reservoir of cellulose that can be processed by hydrolysis method into bioethanol. The cellulose level (55.1%) obtained is close to that of ZE Wilfrid which is 60%, result achieved during the study on the bioethanol production from corn stalks.

Hydrolysis of pre-treated material

- **Saccharification rate**

The hydrolysis of 2 samples, starting from the same mass (6g), and the same enzymatic charge (40 FPU/g) and under the same conditions, namely: hydrolysis time = 48 hours, buffer solution, pH = 4.8 and temperature = 50 ° C. The analysis of the reducing sugars produced was measured in a medical analysis laboratory (GT Labo located at the former Tsinga council in Yaoundé) using a spectrophotometer. The quantity of sugars produced is the average of the two. This value will be used to calculate the saccharification rate. The results are shown in the table below:

Table 3.4: Concentration of reducing sugar

Hydrolyzate	1	2	Average of sugar
Concentration of reducing sugar (mg/ml)	0.64	0.89	0.765

From the table above we can deduce the following table corresponding to the different masses, knowing the volume of each solution which is 198 ml.

Table 3.5: Masses of reducing sugars

hydrolyzate	1	2	Average mass
Mass of reducing sugar (g)	0.12672	0.17622	0.15147

This result shows that a very small quantity of polysaccharides has been processed into glucose. The rate of saccharification is determined by taking the ratio between the concentration of glucose obtained and the concentration theoretically expected. The results obtained are shown in Table 3.6.

Table 3.6: Saccharification rate

Mass of Polysaccharides (g)	Mass of sugars obtained (G)	Saccharification rate (%)
3.306	0.15147	4.58

The rate of saccharification is very unsatisfactory, because in 55.1% of cellulose, only 1/12 of this quantity is transformed into glucose. Firstly, this low rate may be due to the duration of hydrolysis because in some articles, the hydrolysis time can be up to 96 hours. Secondly, the enzymatic charge used during the hydrolysis phase was insufficient because in some articles, this charge went up to 45 FPU. The average of reducing sugars (0.765 mg / ml) obtained in our study is much lower than that of FENNOUCHE Ibtissem (36.5 mg / ml), result achieved in 2017 during the study on the production of bioethanol from agricultural residues. This difference in results is due to the use of date as a raw material because it contains enough sugar.

For physical characterization of ethanol, the functional group (-OH) test was done by using microchemistry hardware kit. The change in colour from orange to green during the test highlights the presence of the OH group in ethanol. To confirm that the final solution obtained after distillation is indeed bioethanol; physical properties such as: boiling point, density and relative density have been determined.

It is found that the experimental value of the density of the ethanol obtained (0.82 g / ml) is in agreement with that of 95% ethanol (0.79 g / ml). This small difference could be further clarified by the presence of impurities in the ethanol solution obtained

Table 3.7: Boiling point of the ethanol solution obtained

Boiling point of the ethanol solution	Boiling point of 95% ethanol
79 ° C	78 ° C

The value of the density (0.82 g / ml) found in Table 3.8 is close to that found by FENNOUCHE Ibtissem (0.86 g/ml).

Table 3.8: Density of bioethanol

Mass of test – tube and ethanol solution (g)	Volume of ethanol solution (ml)	Density of ethanol solution (g / ml)
16.3	4	0.82

The density was calculated from Table 3.9. The high density value (0.82) shows that the ethanol obtained still contains a small quantity of water

Table 3.9: Relative density of bioethanol

Mass of test tube and ethanol solution (g)	Volume of ethanol solution (Ml)	Density of ethanol solution (G / ml)	Relative density of ethanol
16.3	4	0.82	0.82

After presenting the different results, despite the low rate of saccharification, it is clear that the bioethanol obtained from Frakéwood chips has characteristics very close to that of ethanol at 95% given in literature. In addition, our product actually has a pungent alcoholic smell and is highly inflammable (Figure 3.3).

Fig. 3.3 Flame obtained after burning bioethanol

The flame is very intense but not long lasting and reminds of the one obtained with petrol.

Other analyzes such as octane rating and the flash point would have given more information on the quality of this alcohol.

3. CONCLUSION

The overall objective of this study was to produce bioethanol from Fraké waste through an enzymatic hydrolysis of the sugars therein and by alcoholic fermentation. A pre-treatment of Fraké wood in a basic medium was completed (24% of extracts), the solid residue obtained was hydrolyzed in the presence of cellulase (Accellerase ® 1500). The sugar solution obtained was fermented with baker's yeast (*Saccharomyces cerevisiae*) to produce ethanol. From the qualitative and quantitative analyzes made, we can conclude by saying that the bioethanol obtained is a quality product and that the Fraké woodchips constitute a potential source of raw material in the production process of bioethanol. The low rate of saccharification (4.6%) can be explained by the fact that the conditions of extraction, enzymatic hydrolysis and alcoholic fermentation were not optimal.

Since scientific research in the bioethanol productiondomain as fuel increased these days, and since enzymatic hydrolysis gives a low saccharification percentage, it is important to think of hydrolysis. This percentage can also be enhanced by the use of catalyzed steam explosion pre-treatment which increases the efficiency of enzymatic hydrolysis and decreases the production of inhibitory compounds or to experiment with biological pre-treatment in order to reduce the cost of production. This is why the future objective may be to produce ethanol by going through a physical pre-treatment and by acid hydrolysis after taking into account some of its weaknesses.