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Optimization of alkaline pretreatment of Yagoua rice straw for bioethanol production

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Key words

Rice's straw,
Bioethanol,
Alkaline Pretreatment,
Alkaline process,
Optimum,
Enzymatic hydrolysis.

Abstract

The aim of this work is to optimize the alkaline hydrolysis of rice straw lignocellulose using kanwa, with the goal of producing bioethanol. The alkaline pretreatment was carried out using a saline rock from North Cameroon (kanwa) under the following experimental conditions: temperature between 170 and 200°C, and duration between 20 and 60 minutes. Characterization revealed that the local material contained 91.75 ± 0.48% dry matter, 8.25 ± 0.48% water, 31.22 ± 2.04% cellulose, 9.92 ± 2.46% lignin, and 17.72 ± 0.51% hemicellulose. The optimization design (central composite design) used to validate the reducing sugar models was based on the following indicators: Adjusted R^2 (99.36), AADM (12%), Bf (1.004), and Af (1.049). The optimal pretreatment conditions were 1.681, -1, and 0.808 in terms of temperature, time, and kanwa concentration, resulting in the maximum extraction of 49% cellulose, 2.4% hemicellulose, and 19.3% lignin. A concentration of 4.50 ± 0.02 mg/mL of reducing sugars was obtained under the conditions of 55°C for 72 hours, followed by centrifugation at 4000 rpm for 15 minutes. This resulted in an ethanol yield of 32% and a lower calorific value of 9184 ± 0.01 J/g.

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1. Introduction

Recent technological advancements have enabled the development of sustainable and efficient energy production methods, including liquid, solid, and gaseous fuels, through biomass recovery processes [1, 2, 3]. These technologies are employed by both developed and developing countries, driven by rapid population growth, the depletion of natural resources (e.g., fossil fuels), environmental pollution (e.g., greenhouse gas emissions), and climate change (e.g., ozone layer depletion) [2, 4], challenges that are also evident in Africa [5]. The Sustainable Development Goals (SDGs) present both opportunities and challenges for developing nations [6]. Although implementing these goals can be costly, they offer long-term benefits. Each country has its own specificities and unique perspectives. It is essential that nations adapt each relevant SDG into national action plans, including economic, social, and environmental indicators [7, 8].

Among biomass subcategories, lignocellulosic biomass stands out as one of the most abundant renewable resources on Earth, making it a viable option for meeting global energy demands through the production of bioethanol, biosyngas, biodiesel, biogas, biochar, and biohydrogen [9, 10]. The range of substrates is diverse and includes woody materials (hardwood and softwood), agricultural residues (such as straw), and industrial lignocellulosic waste [11]. Rice straw is one of the most abundant lignocellulosic resources worldwide, yet it remains largely underutilized due to its low nutrient content [9, 12, 13]. Nevertheless, because of its rich carbohydrate composition, it is considered a promising feedstock for biofuel production [14, 15].

The lignocellulosic structure of rice straw—composed of cellulose, hemicellulose, and lignin—is highly rigid [9, 15, 16]. To better exploit the hemicellulose fraction, pre-treatment methods are used to break down the complex lignocellulosic matrix, thereby facilitating hydrolysis for optimal release of fermentable carbon [1, 17, 18, 19]. Several pre-treatment technologies are available to deconstruct the lignocellulosic matrix. These include chemical pre-treatments (e.g., alkali, acid, organosolv, ozonolysis, and ionic liquids), physical pre-treatments (e.g., grinding and milling, microwave, and extrusion), physico-chemical pre-treatments (e.g., steam explosion, liquid hot water, ammonia fiber explosion, wet oxidation, and CO explosion), and biological pre-treatments (involving microorganisms) [20].

Chemical pre-treatment primarily involves the use of acids and bases to hydrolyze biomass [21]. This method is particularly promising due to its ability to effectively break down the substrate's complex structure [22]. It is also economically viable, requiring less energy input, and is environmentally friendly, with faster reaction rates and greater ease of technology transfer compared to physical and biological approaches [23, 24]. Acid hydrolysis typically uses concentrated or diluted H_2SO_4 or HCl to treat lignocellulosic biomass, although it may result in enzymatic and/or microbial inhibition [20, 22].

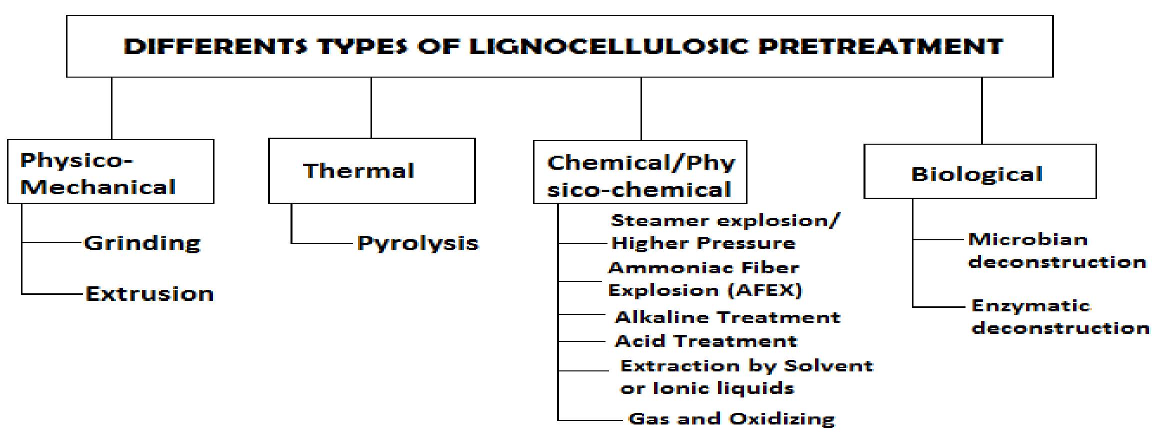

Figure 1. Pre-treatment processes for the lignocellulose degradation [22].

Alkaline pre-treatment involves the use of relatively low temperatures and pressures in combination with alkaline agents such as calcium, sodium, potassium, and ammonium hydroxides [11]. Typical conditions for NaOH-based pre-treatment include 8–12% NaOH (w/w, dry basis), applied for 30 to 60 minutes at temperatures ranging from 80°C to 120°C. During this process, lignin is almost completely solubilized, along with a portion of the hemicellulose fraction [16, 25]. However, for residues like straw, temperatures between 180°C and 200°C can yield enzymatic hydrolysis efficiencies of cellulose that approach the theoretical maximum [26]. This process offers the advantage of dissolving nearly all lignin while simultaneously reducing the degree of cellulose polymerization [27]. Previous studies have shown that the efficiency of this pre-treatment depends on the concentration of sodium hydroxide, which also facilitates the extraction of pentoses [28]. Nevertheless, the use of chemical reagents is often costly.

Kanwa, a naturally occurring alkaline rock, contains trace elements such as calcium, magnesium, sulfur, chlorine, silicon, phosphorus, and potassium [29], and presents a promising alternative for alkaline pre-treatment.

The objective of this study is to optimize the alkaline hydrolysis of rice straw lignocellulose using kanwa, with the aim of producing bioethanol

l.2.Materials and Methods

2.1. Study site and period

Our study site is located in the Far North region of Cameroon, specifically in the Yagoua area, which serves as the capital of the Mayo-Danay department. The site is managed by SEMRY (Société d'Expansion et de Modernisation de la Riziculture de Yagoua), headquartered in Yagoua. The company operates an annual irrigated double cropping system covering 11,500 hectares, including 5,300 hectares in the Yagoua area. The cultivation season begins in July and extends until August 15. Harvesting starts in late September and continues through mid-October, typically lasting up to 30 days.

2.2. Plant material

The rice straw used as the substrate material belongs to the species *Oryza sativa*, variety IR46. The sample was sourced from the Yagoua Rice Farming Expansion and Modernization Company (SEMRY), located in the Far North Region of Cameroon.

2.3. Halite rock (Kanwa)

The white halite rock used in this study is abundantly found in Cameroon, where it is known by various names such as *akanwu, kanwa, kaun, dalang, kawe,* and lake salt. It serves a wide range of purposes, including use as natural potash [30]. This natural product is relatively inexpensive and less hazardous, requiring minimal safety precautions for handling. It is a naturally occurring alkaline rock salt commonly used as a meat tenderizer, an ingredient in traditional medicinal preparations, a purgative when added to livestock drinking water, and a body purifier [29]. *Kanwa* contains chlorides, sulfates, and carbonates of sodium, calcium, and potassium [31]. It is composed of approximately 40% sodium and 60% chlorine, with a boiling point of 1412°C [31].

2.4. Chemical and reagents

The reagents used are of analytical type and are listed according to the different analyses carried out. For cellulose, hemicellulose and lignin determination: the chemicals used in this work are of analytical grade. Sulfuric acid (98%), acetone (98%), sodium borate decahydrate (97%), neutral sodium ethylene sulfate (98%), 2-ethoxyethanol (98.5%), anhydrous sodium sulfite (98%), anhydrous disodium phosphate (98%), ethanol (95%) and nitric acid (98%), D-xylose, orcinol, hydrochloric acid, ethanol and iron (III) chloride (96.5%) were used. Zinc sulfate and potassium ferrocyanide were obtained from BDH Chemicals. For enzymatic hydrolysis: citric acid monohydrate and sodium azide were used. For fermentation: ammonium sulfate, calcium chloride dihydrate, yeast extract, D-glucose, potassium hydrogen phosphate, magnesium sulfate.

2.4. Characterization of raw material

Rice straw used in this study was partially characterized. The characteristics assessed, according to the compendium of routine methods and food analyses, were dry matter, cellulose, lignin, and hemicellulose contents [9, 31]. Figure 2 illustrates the lignocellulosic biomass and the different structural modifications that occur during the alkaline pretreatment [2].

The grinding operation was carried out to reduce the size or increase the contact surface of solid particles. After grinding, the rice straw powder obtained was sieved [15, 28].

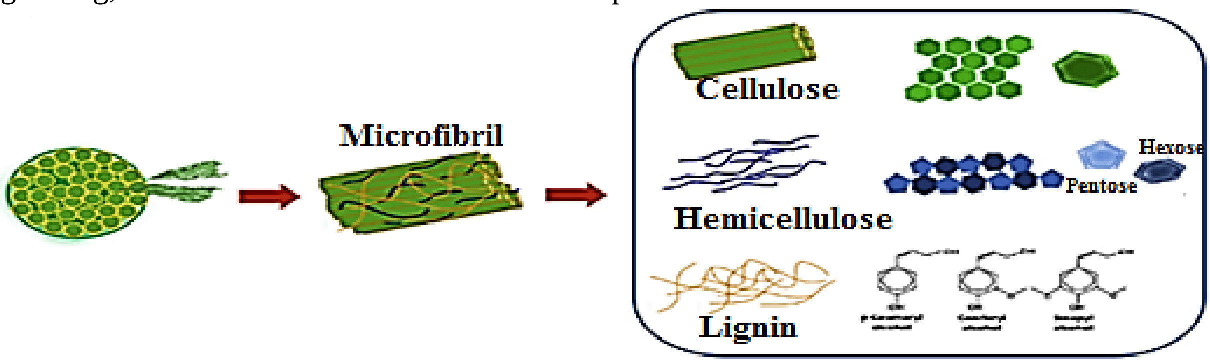

Figure 2 Structure of lignocellulosic biomass before and after pre-treatment [2]

2.5. Optimization of the alkaline pretreatment of rice straw

The alkaline pre-treatment was carried out in a pre-treatment reactor of 1 L volume, radius 6 cm and height 17.7 cm. With supporting temperatures going beyond 250°C, a mass of 10 g of rice straw was used for a volume of 62.5 mL of water. This mixture was introduced into the pre-treatment reactor with varied proportions of kanwa and brought to a temperature range varying from 170°C to 200°C during a time variation ranging from 20 to 60 min. After the alkaline pre-treatment phase, the liquid fraction obtained was collected and separated for the determination of reducing sugars and pre-treatment inhibiting compounds. Improved modeling and analysis of the various variables needed to obtain the expected responses, an optimization was carried out by the response surface methodology (RSM) [32, 33]. The Design Expert software version 13.0.5.0 was used for the realization of a central composite design on two levels, with three factors: temperature and time and concentration of kanwa, where the minimum coded value is -1 and the maximum +1 for a total number of 18 tests as presented in Table 1 below.

Table 1 : Coded and uncoded values for independent variables

Uncoded values			Coded values		
Temp (X_1) °C	**Time (X_2)** Min	**Ratio (X_3)** g/mL	**Temp (X_1)** °C	**Time (X_2)** min	**Ratio (X_3)** g/mL
185.0	40.0	1.75	0.0	0.0	0.0
185.0	40.0	1.75	0.0	0.0	0.0
185.0	40.0	1.75	0.0	0.0	0.0
185.0	40.0	1.75	0.0	0.0	0.0
170.0	20.0	1.0	-1.0	-1.0	-1.0
200.0	20.0	1.0	+1.0	-1.0	-1.0
170.0	60.0	1.0	-1.0	+1.0	-1.0
200.0	60.0	1.0	+1.0	+1.0	-1.0

170.0	20.0	2.5	-1.0	-1.0	+1.0
200.0	20.0	2.5	+1.0	-1.0	+1.0
170.0	60.0	2.5	-1.0	+1.0	+1.0
200.0	60.0	2.5	+1.0	+1.0	+1.0
159.77	40.0	1.75	-1.68	0.0	0.0
210.23	40.0	1.75	+1.68	0.0	0.0
185.0	6.36	1.75	0.0	-1.68	0.0
185.0	73.64	1.75	0.0	+1.68	0.0
185.0	40.0	0.48	0.0	0.0	-1.68
185.0	40.0	3.01	0.0	0.0	+1.68

The content of phenolic compounds, pentoses and reduced sugar obtained after a series of experiments are the expected responses given by the following polynomial model[33]:

$$Y = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i^2=1}^{k} a_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} a_k x_i x_j + \varepsilon \quad (1)$$

where Y is the predicted response, $x_i and\ x_j$ the independent variables, a_0 the constant coefficient and a_i a_{ii} a_k, the coefficients giving the existing interaction between different variables, and Ɛ the error [12]

After the optimization of alkaline pre-treatment, Field emission SEM combined with EDX analysis was used to assess the elemental composition and surface morphological characteristics. This was accomplished using a JEOL-JSM-6390A (Tokyo, Japan) device that had an accelerating voltage of 10 kV and a magnification range of 25x to 1000kx. XRD data using a PAN XPERT Pro powder X-ray diffractometer (Germany) were used to estimate phase crytallinity. Using a Cu-Kα1 radiation with a wave length of 1.54056 Å and a scanning rate of 5°/min in the 2θ range between 10° and 90°, the device was run at 30 mA and 40 kV. The materials' surface functional groups were identified using FT-IR spectroscopy and a Nicolet IS5 Thermoscientific Infrared Spectrometer. The Atenauted Total Reflection (ATR) technique was used to record the spectra in transmission mode between 4000 and 500 cm-1. A spectrum of the radiation intensity transmitted by the sample as a function of wave number was recorded after the infrared radiation had gone through it.

2.7. Production and characterization of bioethanol

2.7.1. Enzymatic hydrolysis protocol

Enzymatic hydrolysis is a specified method, carried out under relatively mild conditions (50 °C) [35]. The enzyme-substrate solutions were prepared at 10% (mass/volume) of dry plant material using cellulase [26, 35]. When the reaction time was reached, the reaction was stopped in a boiling bath. Then, 0.5 mL of the solution was taken for the determination of reducing sugars [36, 37].
At the end of the hydrolysis, a test must be carried out to identify the product of the reaction. For this, the Fehling liquor test was performed to highlight the sugars obtained.

2.7.2. Experimental protocol

Fehling's liquor test shows the presence of glucose. Fehling's solution (or Fehling's liquor) is a basic complex of cupric ions and tartrate ions. During the reaction, the copper oxidizes the aldehyde to give an acid according to the following redox reaction:

$$R - CHO + 2CU_{(aq)}^{2+} + 5HO_{(aq)}^{-} \longrightarrow RCOO^{-} + CU_2O_{(S)} + 3H_2O \quad (2)$$

The experimental protocol consists of putting the substance to be tested in solution in a test tube with distilled water, adding a few drops of Fehling's liquor (blue color); heat in a Bunsen burner or a water bath at 100°C for a few minutes, then observe the coloration.

2.7.3. Fermentation's realization

To conduct the fermentation, 1.03 g *of saccharomyces cerevisae* was used to ferment 100 mL of hydrolysates from enzymatic hydrolysis in accordance with National Renewable Energy Laboratory analytical procedure [39]. It was provided by Chemical engineering and industrial bio-processes laboratory of National Higher Polytechnic School of Douala. The ethanol yield was calculated by dividing the total amount of ethanol produced in the fermentation broth by the initial mass of reducing sugars (g). It can also be expressed as a percentage of ethanol yield compared to the theoretical value using the Eq (3).

$$Y_{Eth} = \frac{Ethanol\ (g)}{0{,}511 \times glucose\ (g)} \quad (3)$$

Where 0.511 represents the theoretical yield of ethanol and YEth the yield produced per gram of glucose. In order to determine the yield of ethanol produced in Gay-Lussac degrees, the distilled ethanol is weighed into a pre-weighed beaker (this consists of cancelling out the mass of the beaker so that only the mass of the ethanol is used), and the operation is the same for the hydrolysate from the enzymatic hydrolysis [40].

2.7.4. Characterization of the produced bioethanol

The calorimetric experiment involves loading a known mass of sample into a closed container called a bomb. The bomb is filled with oxygen at around 30 bar. The loaded bomb is placed in a bucket of water, where a thermometer records the temperature of the sample. The bucket containing the bomb (the system) is surrounded by tubes of water with a fixed temperature, commonly referred to as the envelope. The envelope guarantees a fixed heat transfer between the system and its environment [41].

2.8. Statistical analysis

The quality of fit of the model was expressed by the coefficients of determination R^2 and adjusted R^2. This mathematical tool not being sufficient to validate a model, other tools and methods were used to judge the quality of the adequacy of the models. For this purpose, the Absolute Analysis of Mean Deviation (AADM), between the calculated and observed values was performed which defines the true behavior of the system in the domain of the experimental data, the Bias Factor (*Bf*) and the Accuracy Factor (*Af*)[14].

$$Bf = 10^{B} \quad (4)$$

$$B = \frac{1}{n}\sum log\frac{Y_{the}}{Y_{obs}} \quad (5)$$

$$Af = 10^{A} \text{ with } A = \frac{1}{n}\sum \left| \left(\log\frac{Y_{the}}{Y_{obs}}\right) \right| \quad (6)$$

$$AADM = \frac{\sum_{i=1}^{n}\left(\left|\frac{Y_{obs} - Y_{the}}{Y_{obs}}\right|\right)}{n} \quad (7)$$

3. Results and discussion

3.1.Characteristics of rice straw

The results of the physico-chemical analysis are shown in the Table 2.

Table 2 : Characterization of Rice straw.

Chemical composition	Contents (%)			
Dry matter	91.75 ± 0.48	-	-	-
Cellulose	31.22 ± 2.04	65.50	41	29
Lignin	9.92 ± 2.46	11.60	25	11.60
Hemicellulose	17.72 ± 0.51	14.50	25	30.80
Reference	Local rice straw	[14]	[42]	[43]

Table 2 presents a series of data from the characterization of locally obtained rice straw. These results differ from the values presented in the literature. However, they show that the plant material obtained locally falls within the range of lignocellulosic resources (around 29% cellulose and 27.5% hemicellulose) [43] and therefore can be used in the production of bioethanol.

3.2.Optimization of alkaline pretreatment of rice straw

The matrix obtained from the different input variables enabled us to determine the different responses, namely the extraction of pentoses, reducing sugars and phenolic compounds. Thus, to the estimation of a response surface for each parameter, the relationship between the different input and response variables is given by the following equations:

$$\boldsymbol{Y_{PC}} = 0.01515 + 0.00110\boldsymbol{X_1} + 0.00688\boldsymbol{X_2} + 0.00376\boldsymbol{X_3} + 0.00821\boldsymbol{X_1^2} + 0.00891\boldsymbol{X_2^2} + 0.00333\boldsymbol{X_3^2} - 0.00235\boldsymbol{X_1X_2} + 0.00152\boldsymbol{X_1}X_3 - 0.00063\boldsymbol{X_2X_3} \quad (8)$$

$$\boldsymbol{Y_P} = 0.1377 - 0.0129\boldsymbol{X_1} + 0.0141\boldsymbol{X_2} + 0.0448\boldsymbol{X_3} - 0.0406\boldsymbol{X_1^2} + 0.0258\boldsymbol{X_2^2} + 0.0403\boldsymbol{X_3^2} + 0.0256\boldsymbol{X_1X_2} - 0.0092\boldsymbol{X_1}X_3 - 0.0126\boldsymbol{X_2X_3} \quad (9)$$

$$\boldsymbol{Y_{RS}} = 0.1026 + 0.02380\boldsymbol{X_1} + 0.04306\boldsymbol{X_2} + 0.01571\boldsymbol{X_3} + 0.01251\boldsymbol{X_1^2} + 0.00827\boldsymbol{X_2^2} + 0.00735\boldsymbol{X_3^2} + 0.02109\boldsymbol{X_1X_2} + 0.00952\boldsymbol{X_1}X_3 + 0.02058\boldsymbol{X_2X_3} \quad (10)$$

$\boldsymbol{Y_{RS}}$, $\boldsymbol{Y_P}$, $\boldsymbol{Y_{PC}}$, $\boldsymbol{X_1}$, $\boldsymbol{X_2}$ **and** $\boldsymbol{X_3}$ are respectively, the content of reducing sugars (RS), pentoses, phenolic compounds, the temperature, the time and the variation of *kanwa*. The pretreatment indicators reflecting the various acceptable models are indicated in Table 3.

Table 3 : Model validation indicator

Indicator's validation	Y_{PC}	Y_P	Y_{RS}	Standard values
R^2	88.33	70.66	99.86	-
R^2 adjusted	72.78	30.62	99.36	80
AADM	0.17	0.30	0.12	[0 - 0.3]
Bias Factors	1.01	0.93	1.01	[0.75 - 1.25]
Accuracy Factors	1.07	1.22	1.05	[0.75 - 1.75]

The indicators presented in Table 3 show that the different models are acceptable because they meet up with the required indicative conditions [44]. However, only the reducing sugars model is fully validated, with response variability (R^2 and adjusted R^2) being within the margin (99.86 and 99.36). This result is also supported by Rehman et al[45] who for a (R^2 = 0.909; R_a^2 = 0.745), concluded the validation of the reducing sugars response mode [43, 45]. The table 4 presents the analysis of the variance and the statistical parameters of the different models.

Table 4 : Analysis of variance and statistical parameters of the different models

	Coefficient			P value		
Terms	Y_{PC}	Y_P	Y_{RS}	Y_{PC}	Y_P	Y_{RS}
X_1	0.0011	−0.0129	0.0238	0.584	0.610	**0.003***
X_2	0.0069	0.0141	0.0431	**0.007***	0.575	**0.001****
X_3	0.0038	0.0448	0.0157	0.087	0.101	**0.026***
X_1^2	0.0082	−0.0406	0.0125	**0.003***	0.145	0.069
X_2^2	0.0089	0.0256	0.0083	**0.002***	0.335	0.203
X_3^2	0.0033	0.0403	−0.0074	0.135	0.147	0.254
X_1X_2	−0.0024	0.0256	0.0211	0.379	0.442	**0.023***
X_1X_3	0.0015	−0.0092	0.0095	0.564	0.777	0.241
X_2X_3	−0.0006	−0.0126	0.0206	0.807	0.700	**0.025***

**Highly significant; *Significant.

The table 4 reveals that the linear, quadratic and interaction effects in red are significant because the values of P are less than 0.05% [14]. This contribution is quantified respectively for the case of sugars at 14.70%, 26.59% and 9.70% for the direct effect of temperature, time and *kanwa* ratio. These results are similar to those of Sato et al [43] who observed a positive effect of time and temperature on the cellulosic fraction (35%-60%). The quadratics and interaction effects with P values greater than 0.05% are not significant and will simply being removed from the different models [14]. The new models therefore become:

$$\boldsymbol{Y_{PC}} = 0.01515 + 0.00688\boldsymbol{X_2} + 0.00821\boldsymbol{X_1^2} + 0.00891\boldsymbol{X_2^2} \quad (11)$$

$$\boldsymbol{Y_{RS}} = 0.1026 + 0.02380\boldsymbol{X_1} + 0.04306\boldsymbol{X_2} + 0.01571\boldsymbol{X_3} + 0.02109\boldsymbol{X_1X_2} + 0.02058\boldsymbol{X_2X_3} \quad (12)$$

In the Eq. (11)**,** the absence of quadratic effects and interaction effect between temperature and mass-water ratio proves the absence of synergistic effects between them. The pentose disappeared after comparisons with the model's validation indicators. This means that the hemicellulose fraction is totally gone. This result was also observed by Sun et al [22] who mentioned that the alkaline treatment has a strong impact on the elimination of hemicelluloses and a moderate impact on the recovery of lignin. The analysis variance of the models in table 5 shows the model which are significant.

Table 5 : Analysis of variance and statistics of the different models

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model of Reducing Sugars	0.0026	11	0.0002	198.02	0.0005	Significant
Model of phenolic Compounds	0.0357	8	0.0045	5.68	0.0242	Significant
Model of Pentose	0.1421	12	0.0118	0.4013	0.8756	Not significant

Fourier transform infrared spectroscopy (FTIR)

The characterization of the biomass in terms of functional group was done by Fourier transform infrared spectroscopy and the results are presented in Figure 3.

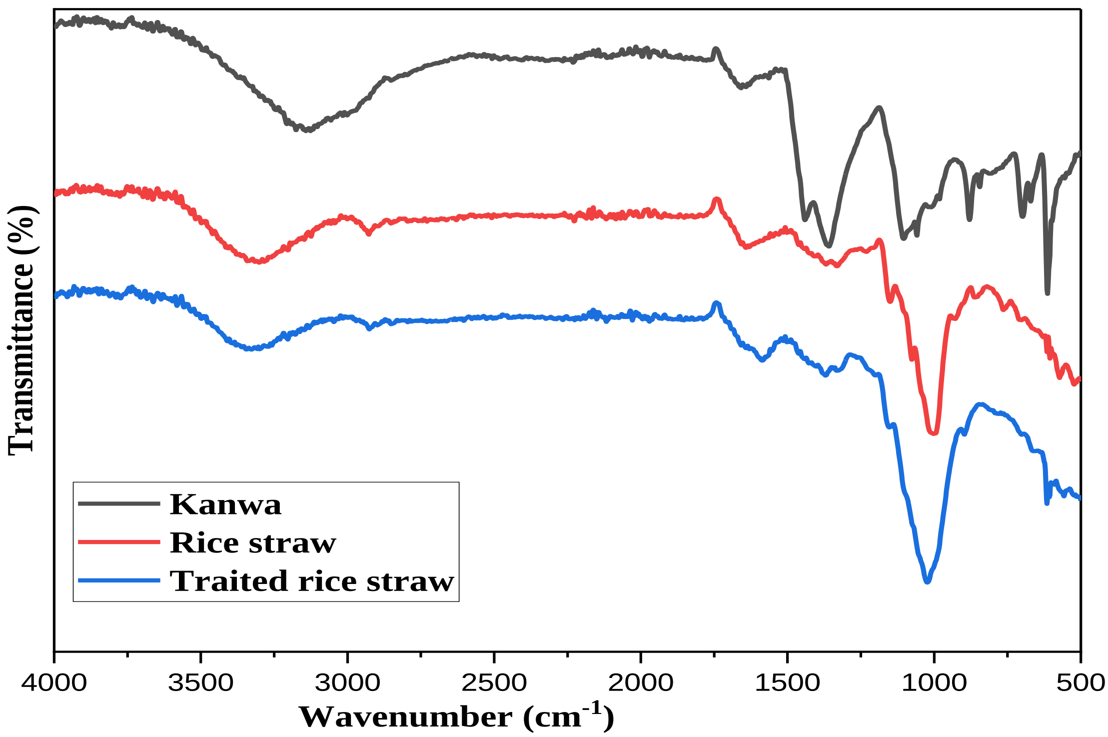

Figure 3: FT-IR spectra of *kanwa*, Rice straw, traited rice straw

The figure 3 shows two zones, zone 1 extending from 4000 cm^{-1} to 1300 cm^{-1} and zone 2 extending from 1300 cm^{-1} to 500 cm^{-1}. In zone 1, the compounds show a broad band ranging from 3250 to 3000 cm^{-1} for Halite rock with a transmittance of 97%, and from 3300 to 3500 cm^{-1} for pretreated rice straw with a transmittance of 75% and non-pretreated rice straw with a transmittance close to 50%. Note that these observed transmittance peaks correspond to the vibration of a bond (O-H). These observations do not corroborate those of Aziz et al [47]who obtained significant peaks at 2918, 2916, 2914, 2851, 1634, 1034, and 1023 cm^{-1} in particular for acid-treated rice straw samples, and alkali-treated rice straw samples [47]. It shows how the treatment has modified the structure of the lignocellulosic rice straws between treated and untreated materials.

Energy dispersive X-ray (EDX) spectroscopy coupled with scanning electron microscopy (SEM)

Figure 4 shows the scanning electron microscopy analysis of biomass

The micrographs showed that the texture and morphology of the surface after the two pre-treatments were markedly different from those of the untreated products. The SEM micrograph of the untreated sample shows a non-porous, granulated and compact surface, while porosity is visible on the pre-treated sample. The size and number of pores are larger in the treated substrate, indicating that much of the lignin and hemicellulose can be removed by the pre-treatment. The untreated substrate shows noticeable characteristics/changes in morphology compared to the treated samples. This can be explained by the breakdown of the lignin xylan bond caused by the alkaline pre-treatment. These observations were also made by Aziz et al, who observed that acid/base pre-treatment resulted in visible porosity and elimination of lignin and hemicellulose from rice straw [47].

EDX analysis being the most preferred method was performed to obtain the detailed information on the chemical composition of the samples. Figure 5 presents the elemental composition of halite rock, rice straw and traited rice straw

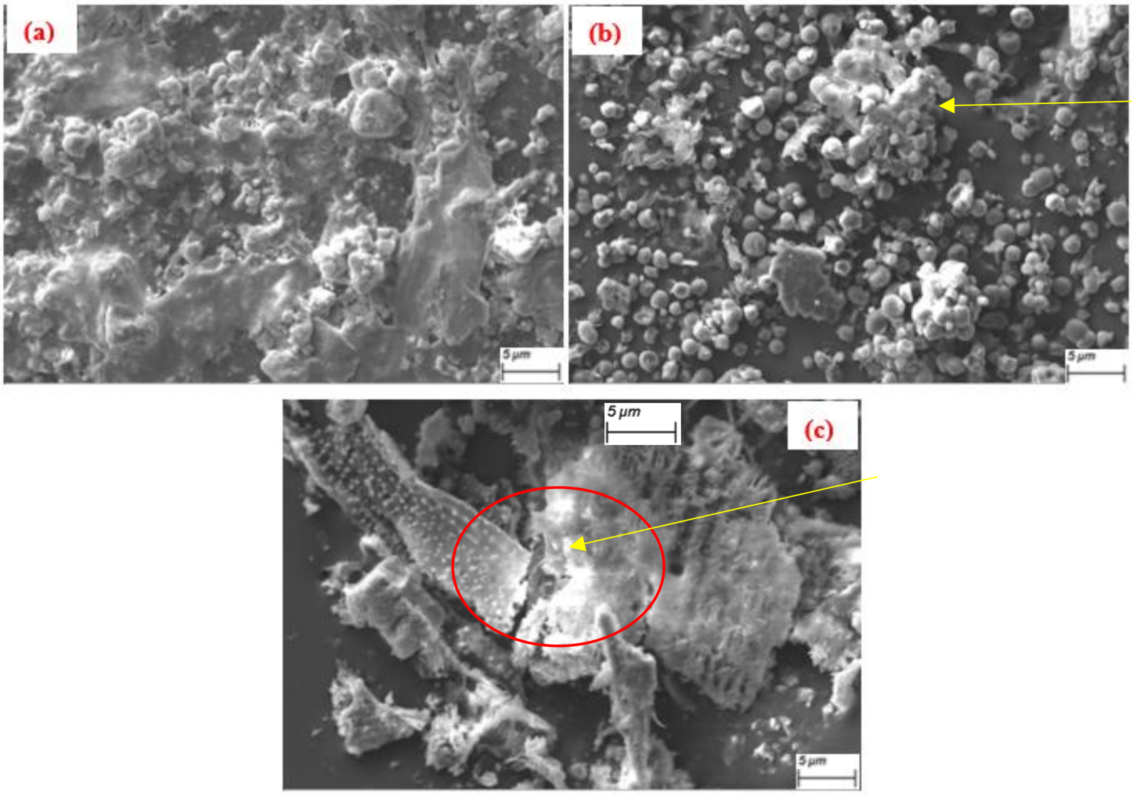

Figure 4: SEM images of *kanwa* (a), Rice straw (b), traited rice straw (c)

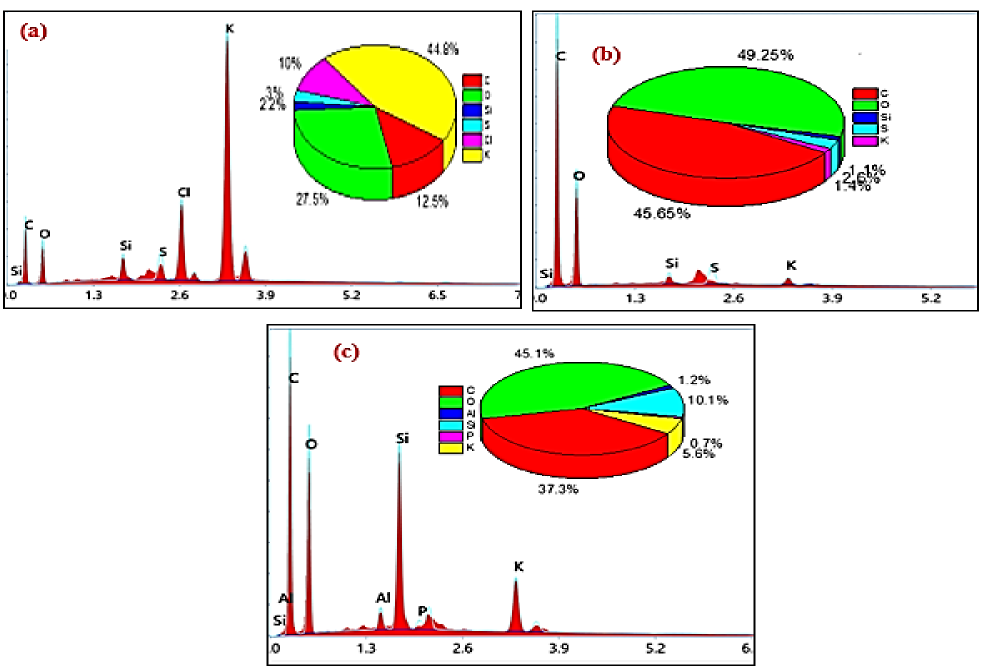

Figure 5: EDX analysis of *kanwa* (a), Rice straw (b), traited rice straw (c)

The observation made from Figure 5b and c shows that untreated and treated rice straw is that these samples are predominantly composed of carbon and oxygen. This figure 5a shows that the halite rock is essentially rich in potassium, which gives it its basic character, with a potassium content of 44.8%. Straw has a high percentage of oxygen element, 45.1% for pretreated straw and 49.25% for non-pretreated straw. The increase in potassium in the case of pre-treated straw reflects the destructuring of the lignocellulose fraction to bring out the cellulose.

As for figure 6, it presents the distribution of the different elements in the structures of the materials, figure 6c presents the combination of the materials halite rock and rice straw which testifies to a successful modification.

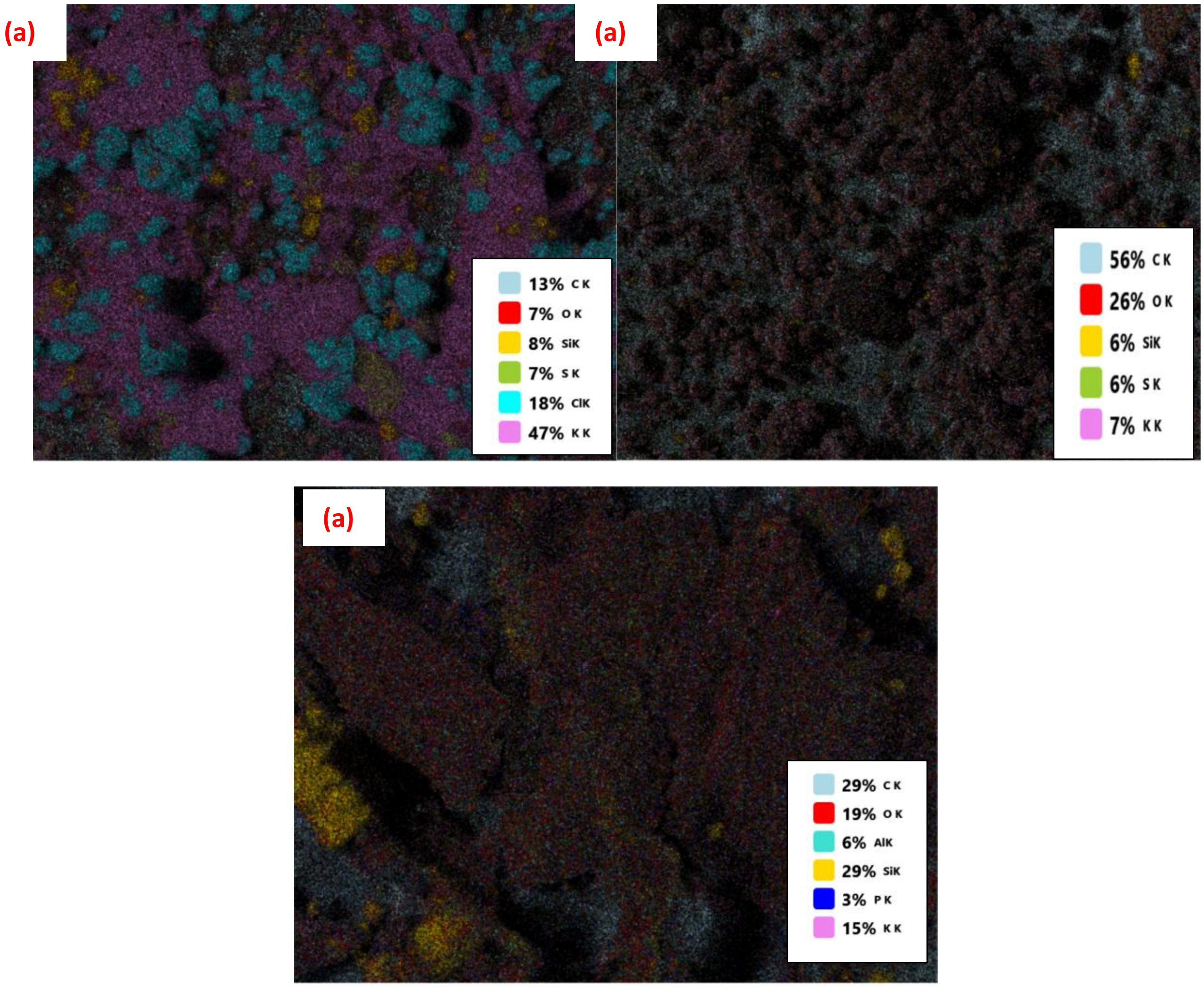

Figure 6: EDX-mapping of *kanwa* (a), Rice straw (b), traited rice straw (c)

X-ray diffraction analysis

Figure 7 shows the XRD pattern analysis of Kanwa, rice straw, and treated rice straw. The analysis of this diffractogram shows that the rice straw and treated rice straw have a predominantly amorphous structure indicating the non-arrangement of the molecular chain characteristic of the low degree of polymerization and a minority crystalline structure while that of *kanwa* (halite rock) presents a high crystallinity. X-ray diffraction analysis of the treated and untreated rice straw samples reveals a major peak located at $2\theta = 23°$, corresponding to the (002) crystallographic plane of cellulose II. This indicates that cellulose is a major component of rice straw. These observations were also made by Sato et al, who concluded that NaOH treatment had no effect on cellulose crystals [27].

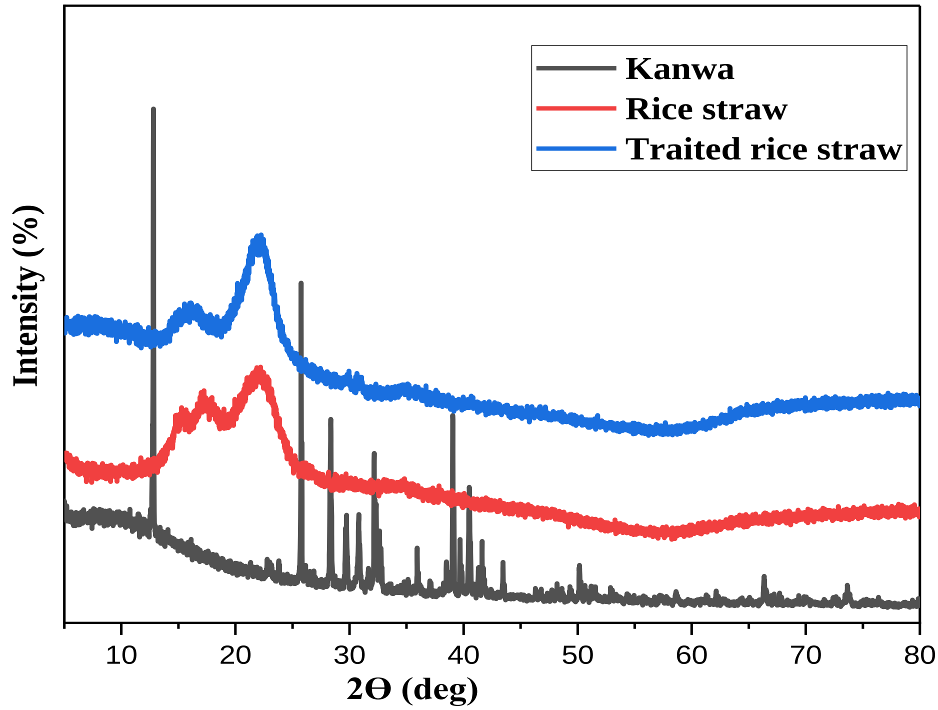

Figure 7: XRD patterns analysis of *Kanwa* (a), Rice straw (b), traited rice straw (c)

3.2.1. Response contour plots

Contour plots are used to study responses as a function of operating conditions. They represent the response surface with a three-dimensional (3D) view where points with the same response are connected to produce contour lines of constant responses, which gives the response intervals that vary depending on operating conditions.

The contour plot shows the increase of the temperature, time and the *kanwa* in the axes. The vertical graduation reveals the variation of the phenolic compound. For lignin, the values which are presented in the green zone will give a better extraction of the phenolic compounds. Figure 8 shows the increase in lignin extraction values as *kanwa* concentration decreases and temperature and time increase. In this section, we aim to minimize the effect of lignin. In other words, the phenolic compounds extraction reaction should be carried out by moderately increasing the values of the time parameter.

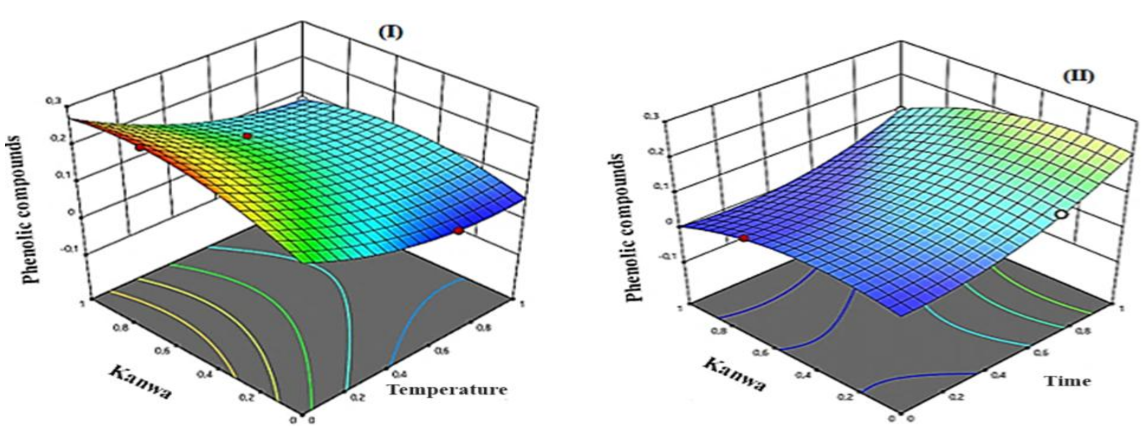

Figure 8: Phenolic compounds extraction 3D contour plot depending of Temperature and Time parameters

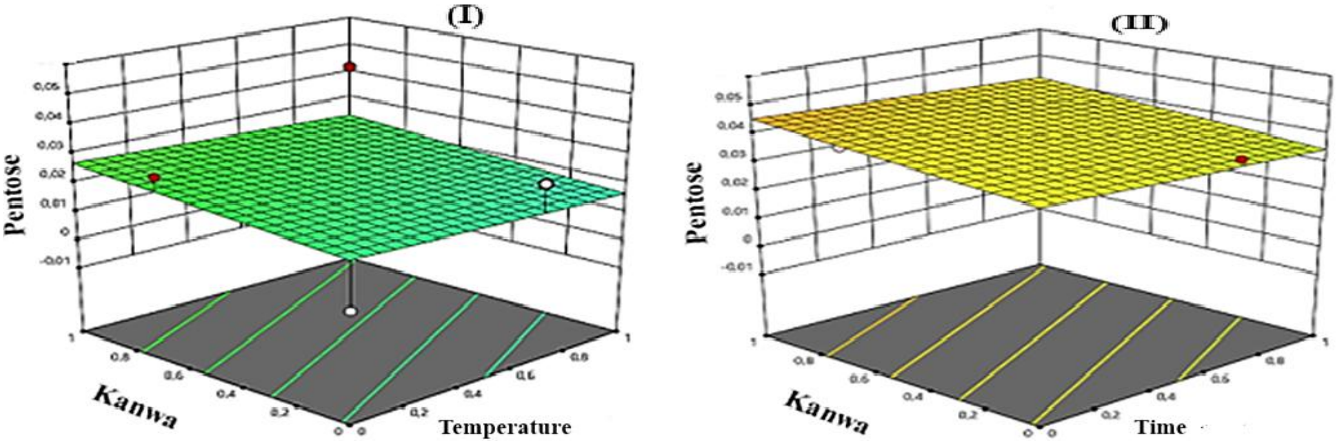

Figure 9: Pentose extraction 3D contour plot depending of Temperature and Time parameters.

For the 3D contour plots above, all areas showing responses in Figure 9 show that the hemicellulose phase drives pentose extraction. The absence of the curvilinear effect on the plot reflects the fact that the factors did not have a significant effect on pentose extraction.

The graphs in figure 10 show reducing sugar yield as a function of temperature *kanwa* and time. The graph shows that both parameters have a significant influence on cellulose extraction. The blue zone represents the minimum amount of reducing sugar extraction. The curve shows that the interactions are significant. This shows that for good exploitation of reducing sugars, the reaction must be carried out with a temperature and stirring time in the higher values and a low *kanwa* concentration. Observation of the graphs shows that for alkaline pre-treatment, the effect of temperature and time leads to almost total solubilization of lignin and part of the hemicellulose.

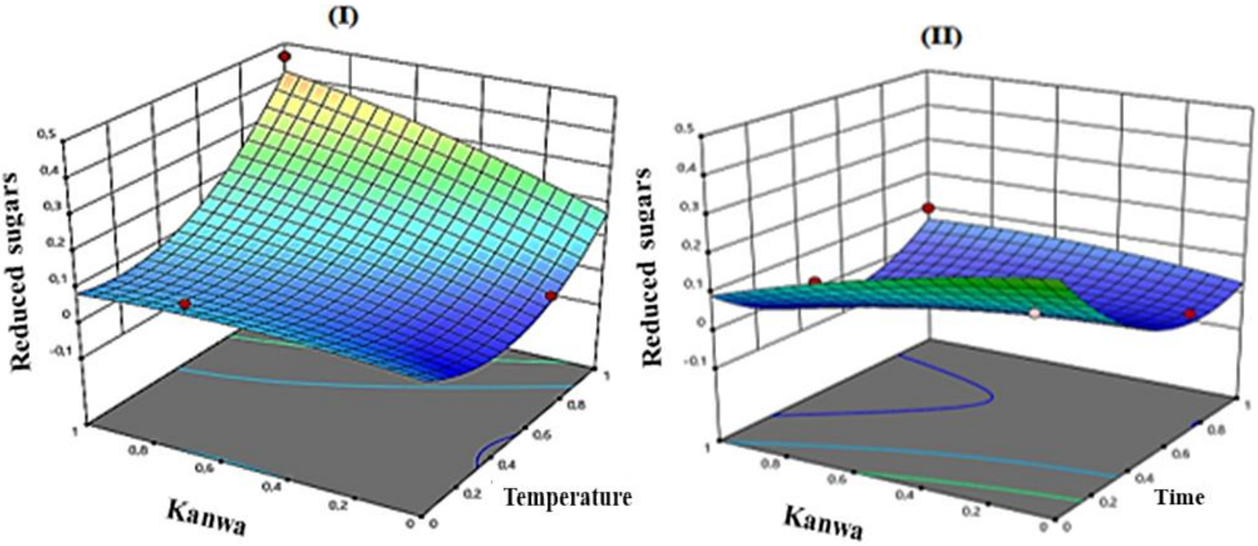

Figure 10: Reducing sugars extraction contour plot depending on temperature and time parameter

3.2.2. Optimization of preprocessing parameter

The table 6 shows the coded points which can be taken like an optimal and alternative solution points.

Table 6 : Optimal and alternative solution point in coded values

N°	Rice straw	*kanwa*	Temperature	Time	Reduced sugar %	Phenolic compound %	Pentose %	Desirability
1	0.192	0.808	-1.681	-1.000	0.49	0.193	0.024	0.953
2	0.192	0.808	0.992	-1.000	0.49	0.193	0.024	0.953

3	0.191	0.809	0.981	-1.000	0.49	0.192	0.024	0.952
4	0.191	0.809	0.966	-1.000	0.49	0.192	0.024	0.952
5	0.190	0.810	1.000	-0.981	0.49	0.192	0.024	0.952
6	0.191	0.809	0.956	-1.000	0.49	0.192	0.024	0.952
7	0.190	0.810	0.947	-1.000	0.49	0.191	0.024	0.952
8	0.189	0.811	1.000	-0.972	0.49	0.191	0.024	0.952
9	0.190	0.810	0.921	-1.000	0.49	0.191	0.024	0.951
10	0.187	0.813	1.000	-0.958	0.49	0.190	0.024	0.951

In this part, we aimed to determine the best combination of parameters for a good production of reducing sugars favorable for bioethanol production. We used the desirability method to optimize the parameters. The Design Expert and Minitab software allowed us to estimate the values predicted by the value of Desirability varying from 0 to 1 with perfect values when d=1, and acceptable values when d > 0.7. The optimization results shown in table 5 give the optimal temperature, time, mass of rice straw and mass of *kanwa* corresponding to the coded values with a desirability of 0.953. This table reveals that the optimal conditions (Desirability 0.9–1.0) correspond to the high dosage of *kanwa* and temperature. The best point corresponds to 49% of reduced sugar (coded variable +1.681) for the temperature and the time (coded variable -1). These observed values approximate those observed by Sato who had an optimum temperature of 140°C and a treatment time to a substrate ratio of 8 :1[43].

The interaction effect is given in Figure 12:

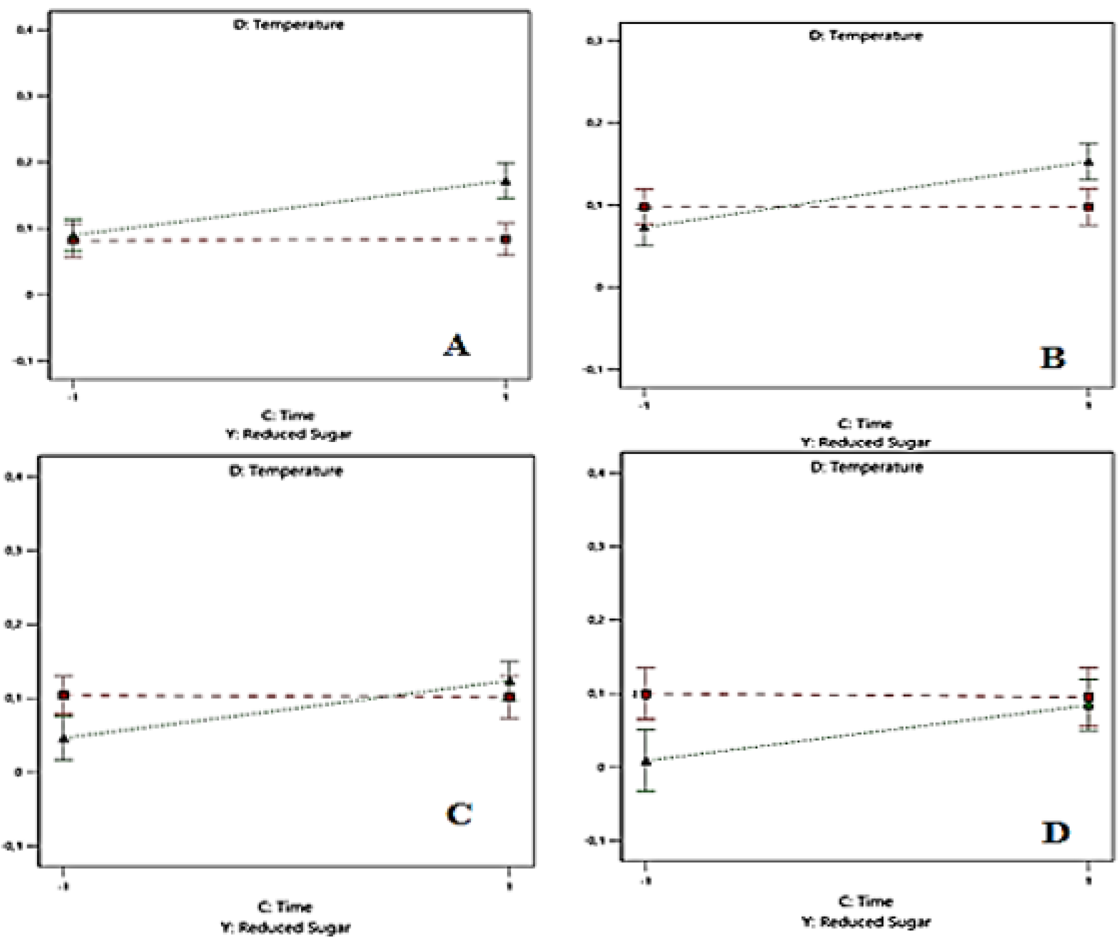

Figure 11: Interaction effect report of glucose average

The Figure 11 presents the interaction between time and temperature parameters. In the continuous factors A and B which represent the rice straw load and *kanwa* in the range (0.25 and 1), we observe that the interaction is very small. When the percentage of realization is in the range of (0.5 to 0.75), the interaction is very observable. We can conclude that when the continuous factors range is (0 to 0 other 1-0) there is no interaction between time and temperatures parameters. Both factors produce an increase of glucose and degradation products in the liquid[12]. Only glucose is affected by the interaction between categories factors. Quadratic effects on glucose and the basic solution in the *kanwa* indicate that the values of the variables are stabilized. That is, there is a point at which there is no further change due to this factor. These results can be observed by Dagnino and collaborators who realized optimization with acid pre-treatment of rice hulls [48].

3.3. Production of bioethanol

3.3.1. Enzymatic hydrolysis

To determine the production of reducing sugars during enzymatic hydrolysis, samples were incubated at 55°C for 72 hours. The sampled solution was centrifuged at 4000 rpm for 15 minutes. An amount of 4.50±0.02 mg/mL of reducing sugar was found in the supernatant or pellet depending on what was collected after centrifugation. This result differs than the 30.1 g/L of reducing sugars reported by Takano et al whose protocol was carried out with a pH of 5.5 and a reaction time of 96 hours for a straw content of 100 g/L, or from the results of Areepak and associate referenced by El Hage et al [42] who obtained a result varying between 0.52 and 0.63 g who obtained a result varying between 0.52 and 0.63 g under hydrolysis conditions of pH 5, 50°C and 72 hours.

3.3.2. Fermentation

For fermentation of hydrolysates from alkaline pre-treatment, the yield in g ethanol/g reducing sugars of 0.32 obtained from hydrolysates from pre-treatment. This result does not the work of Dagnino et al [48] who obtained a yield of 84% with the dilute acid pre-treatment.

3.3.3. Characterization of the resulting bioethanol

In order to characterize the bioethanol obtained, an experiment was conducted using an oxygen bomb calorimeter to determine the internal calorific value. For this purpose, 1.25 g of bioethanol was introduced into the bomb. After analysis, it was found that the bioethanol obtained from rice straw has a lower calorific value of 9184 ± 0.01 J/g.

Conclusion

The aim of this study was to optimize the alkaline pre-treatment of rice straw in view of producing bioethanol. Rice straw with contents of cellulose at 31.22 ± 2.04%, lignin at 9.92 ± 2.46%, and hemicellulose at 17.72 ± 0.51% is a potential substrate for bioethanol. The novelty of our work lies in the alkaline pre-treatment based on a purely saline halite rock (kanwa) with a significant natural base potential (Na). Optimization studies led to the development of different models, which were validated according to the hypotheses of extraction of reducing sugars, phenolic compounds, and pentoses. The reducing sugar model was validated based on the following criteria: R^2 (99.86%); adjusted R^2 (99.36%); AADM (0.12); bias factor (1.004); and accuracy factor (1.049). Optimum conditions for maximum cellulose release during alkaline pre-treatment are 1.681; -1; 0.808 as a function of temperature, time, and kanwa concentration, for maximum extraction of 49% cellulose, 2.4% hemicellulose, and 19.3% lignin. The conditions for producing fermentable hydrolysates are 55 °C for 72 hours. The sampled solution was centrifuged at 4000 rpm for 15 minutes for a concentration of 4.50 ± 0.02 mg/mL, which gives an ethanol yield of 32% and a lower calorific value of 9184 ± 0.01 J/g.

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Competing interests

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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