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Estimation of Reducing Sugars Released from Solvent-Treated Green Amaranth and Jute Sticks

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ABSTRACT

The sticks generated from the highly consumed green amaranth and jute plants in South-West Nigeria contain trapped polysaccharide resources that can serve as a source of reducing sugars required in the production of enzymes or biofuel. These waste resources can be harnessed as resources with no food value. To estimate the reducing sugars released from the green amaranth and jute sticks, this study employed four solvents (sodium hydroxide, ethanol, hydrogen peroxide and citrate-phosphate buffer) to harness the trapped sugar resources. The sticks were collected and processed. The components of the processed green amaranth and jute sticks were ascertained by proximate analysis. Interaction effects of solvents, time and temperature on the release of reducing sugars from the processed samples were estimated using the Box-Behnken design and the two-level factorial design. Despite the low protein, fat and moisture content, oven-dried samples showed a higher carbohydrate content. The Box-Behnken design revealed the released reducing sugars using 1.25N sodium hydroxide (2.393 mg/ml ± 0.494), 1.25N hydrogen peroxide (1.240 mg/ml ± 0.093) at 50 ºC and 2N ethanol (1.780 mg/ml ± 0.008) at 28 ºC for 60 minutes favour oven-dried green stick wastes (OGSW) over oven-dried jute stick wastes (OJSW) by a difference of 61.92%, 73.28% and 31.92%. The ability of citrate-phosphate buffer to release reducing sugars favours OGSW over OJSW with a difference of 37.3% at the factor level of pH 6, 50 ºC, and 60 minutes. With the significant interaction effect of variables, the citrate-phosphate buffer was considered a greener and more suitable option.

Keywords: Citrate-phosphate buffer, ethanol, hydrogen peroxide, oven-dried green stick wastes (OGSW), oven-dried jute stick wastes (OJSW), reducing sugars, sodium hydroxide.

Introduction

A significant drawback associated with the increasing unsustainable consumption potential of the growing population of most African nations is waste generation, which has been recognized as a menace. [1] Obviously, these wastes are concerns that need to be addressed from environmental and health perspectives as their accumulation led to subsequent blockage of water channels, creating an environment suitable for insects (houseflies and mosquitoes) and microorganisms with the potential to cause disease. [1] At the same time, the subjection of these wastes to activities such as burning has been recognized as a practice in most African nations. This activity increases the emission of greenhouse gases with negative climate feedback, which has economic, health, and environmental implications. [2,3] The need to manage the generated wastes to avert its adverse effects on human health and environmental quality is crucial and must be considered a priority. [1,3,4]

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Previously, waste management options have consigned waste to bins. [1] The current perspective has shifted to the promising fact that lignocellulose-based waste contains resources within its structure that can serve as a medium for cultivating microorganisms and the production of diverse biochemicals. [1,3] This, in turn, offers benefits that are not limited to preserving the quality of the environment; it also ensures economic prosperity through the development of technologies that allow energy availability, resource utilization and value addition.

Among these agro-processing wastes, lignocellulosic wastes obtained from the primary processing of agro-produce have gained attention in the research community.[2,5] This resource contains complex polysaccharides and non-polysaccharides closely packed within its structure. [2,5] Their renewable potential and non-significant impact on food production make them an attractive resource for industrial and sustainable biochemical production (enzymes and biofuel). [2,3,6–7]

In South-West Nigeria, the consumption of nutrient-rich vegetables, green amaranth or green (*Amaranthus hybridus) and* jute plant (*Corchorus olitorius)* usually results in the discarding of the chunky stem and sticks. Much of the generated wastes have been tossed into the bin because they have no commercial value in Nigeria. [1,3] The interest in exploring these agro-wastes to generate products requires appropriate knowledge on how to adequately access/extract resources within the selected wastes to find utilization and enhance its valorization. [2,3,5]

Sugars are one of the significant resources valuable in the fermentation industry. Fermentable sugars are considered carbon sources and substrates used by different microorganism strains to achieve an output that can be beneficial in value addition.[8,9] These outputs include different biochemicals that range from enzymes to biofuel. [2,3,6,9] As sugars are considered fuels and substrates that support fermentation-based biochemical production, access to the polysaccharide component of lignocellulosic biomass should be a priority. [2,3,5,7,8,9] Biochemically,

fermentable sugars can also be reducing sugars (monosaccharides and some disaccharides) with hemiacetal groups capable of functioning as reducing agents or non-reducing sugars (sucrose), which are locked in their cyclic form due to anomeric carbons involved in glycosidic bond linkage. [10]

Since the interest is in reducing sugars, access to the resource of interest from lignocellulosic wastes requires the breakdown and separation of resource structure with the subsequent release of sugars.[5] However, no uniform method suitable for the release of sugars from lignocellulose-based waste has been reported. Hence, finding an economically feasible method with no impact on the environment (green solution) is essential . [1,5]

Despite studies have reported the role of enzymes in achieving depolymerization, the cost of the enzyme and the amount required for depolymerization of large amounts of lignocellulose waste may be expensive for most developing countries. [5,11] Hence, the solvent extraction approach may be considerably more affordable. In this, the nature of the resource, the cost of the solvent and the ease of recovery of the resource of interest with its impact on the environment are factors to be considered before the selection of solvent . [12]

The awareness that the combination of appropriate waste recycling/reusing approaches to produce biochemicals will not only find utilization for wastes but will allow integration of SDG 12.5, which involves minimization of waste generation via recycling, prevention, reuse and reduction. [13,14] This has created an interest that draws attention to the need to find a cost-effective, green and suitable means to extract the trapped resources embedded within the wastes. Thus, it is against this background that the potential of green amaranth and jute sticks as sources of reducing sugars was ascertained. The use of green amaranth and jute sticks as suitable sources of reducing sugars involves procedures that include the collection and processing of the wastes by a short-term retting process where the stem is separated from the fiber. This is followed by sun drying and oven drying of the stem before subjection to mechanical treatment. The proximate analysis was conducted to estimate the biochemical components of processed sticks. The effect of solvents (sodium hydroxide, hydrogen peroxide, ethanol and citrate-phosphate buffer), time and temperature on the release of reducing sugars (reducing sugar concentration) were estimated using the Box-Behnken design (BBD) and the regular two-level factor design. The morphological character of the processed sticks was also presented using scanning electron microscopy (SEM).

Material and Methods

Collection and Processing of the Sticks

Sticks of green amaranth and jute plant were obtained from the household dustbin and the attached debris was removed by hand picking. The debris-free sticks were rinsed under a running tap. A fiber separation process that employs the use of moisture to soften the stem cellular tissue around the fiber bundle with a subsequent release of fiber from the stem was employed. [15] The clean sticks were submerged in a bowl until the ribbon-like fiber was easily separated from the stem. [15] The oven-drying of the exposed stem was performed using a laboratory oven (New Brunswick Scientific, USA) at a constant temperature of 100 ^{o}C for 24 hours. [16] The sample was checked randomly to ensure the effect of the drying was whole. After drying, each sample was then milled with an electrical commercial blender (Silver Crest, China) and was preserved in a clean container.

The exposed stem was also subjected to sun drying by placing them in the tray and laid directly under the sun. The sun drying process was conducted between 9 a.m. and 5 p.m. for 5 days at the average temperature of 33 ℃. Each dried sample was milled using an electric commercial blender and preserved in a clean container till further use.

Proximate Composition of the Processed Sticks

According to the A.O.A.C. method, the proximate analysis (moisture content, ash content, fat content, protein content, and total carbohydrate content) of the processed was sticks was conducted. [17]

Solvent Extraction of the Processed Sticks

The effect of solvents (sodium hydroxide (NaOH) solution (0.5N,1N & 2N), ethanol solution (0.5N,1N & 2N), and hydrogen peroxide (H_2O_2) solution (0.5N,1N & 2N)) on the release of reducing sugars from each processed stick was determined at the temperature of 28 ^{o}C, 50 ^{o}C and 100 ^{o}C. The stick with the highest carbohydrate content was subjected to citrate-phosphate buffer (pH 5, pH 6 & pH 7) treatment.

Spectrophotometric Estimation of Reducing Sugars from Solvent-Treated Extracts

The reducing sugar concentration of the solvent-treated processed stick extracts was estimated using the dinitrosalicylic acid method. In a ratio of 3:6, sodium potassium tartrate (Loba Chemie, India) was dissolved in distilled water. The resulting solution was transferred into a mixture (40 ml) containing sodium hydroxide (20 ml of 2N) (Loba Chemie, India) and 1g of 3,5-dinitrosalicylic acid (SCP, Canada) to constitute 3,5- dinitrosalicylic acid reagent. Due to the effect of light on the prepared solution, the prepared reagent was stored in an amber-coloured bottle. An equal amount of each solvent-treated extract (1 ml) and 1 ml of 3,5- dinitrosalicylic acid reagent was dispensed and mixed in the test tube. To achieve colour development, this mixture was subjected to boiling at 100 ^{o}C for 10 minutes in the water bath (DK-420, England). Absorbance was spectrophotometrically (Visible Spectrophotometer 721, China) measured at 540 nm after cooling. [3]

SEM analysis of processed sticks

The morphological image of the processed sticks with higher carbohydrate content were taken at a magnification of 10000X using SEM (Phenom world PRO: X:800-07334, Switzerland).

Statistical Analysis

Box-Behnken design was adopted to explore the effect of variables including solvents (NaOH, ethanol and H_2O_2), time and temperature on reducing sugar concentration. As shown in Table 1 -Table 3, the variables studied were at three different levels (-1, 0, +1). The regular two-level factorial design was further employed to estimate the interaction effect of each variable represented at two levels for citrate-phosphate buffer (Table 4). Using Microsoft Excel 365, data were analyzed and expressed as mean ± standard deviation (SD). The data obtained were further analyzed using Stat-Ease 23.1.0.0 and Stat-Ease 23.1.8.0 (verification) (Stat-Ease, Inc.; Minneapolis, USA). The suitability and statistical validation of the model were based on parameters generated by the software.[4, 18] The statistical significance was defined when $p<0.005$. Surface plots were used to visualize the interaction between the outcome and the predictor factor/variable.

The statistical software was also used to fit the second-order model to describe the interaction between the variables/factors and the response using Eq. (1):

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad \ldots\ldots (1)$$

In equation (1), outcome variable (Y), predictor variables (A, B, and C), the intercept (βo), the regression coefficients (β_1, β_2, β_3), the interactions between factor/variables (AB, AC, and BC), the quadratic effect (A^2, B^2, C^2) and the sign associated with each estimate (signifies the interacting effect between an independent/predictor factor and the outcome) are expressed.

Results and Discussion

Due to their abundance, availability, cheapness, organic nature, and renewability, agricultural lignocellulosic resources have emerged as an alternative feedstock that is sustainable for second-generation biofuel production. [2,5,7,19] As the waste (sticks and chunky stems) generated

from the consumed vegetables accounts for a good portion of food waste, the need to prevent the negative impacts of its accumulation by finding its utilization cannot be overemphasized.[1]

Processing of green amaranth and jute sticks

Short-term water retting was the submerged process employed to softening the stem cellular tissue around the fiber bundle with the subsequent release of fiber from the stem. [15,20] The separation of fiber from the stem was achieved at 48 hours for the green amaranth stick with the colour change of the fiber. From 24 to 48 hours, no colour change was observed when the jute stick was steeped in water compared to the green colour fading off from the green stick. At 48 hours, the ribbon-like fiber was easy to remove, with the stem retaining its intactness. This processing results in the generation of sun-dried jute stick waste (SJSW), sun-dried green amaranth stick waste (SGSW), OJSW and OGSW. (Figure 1 and Figure 2).

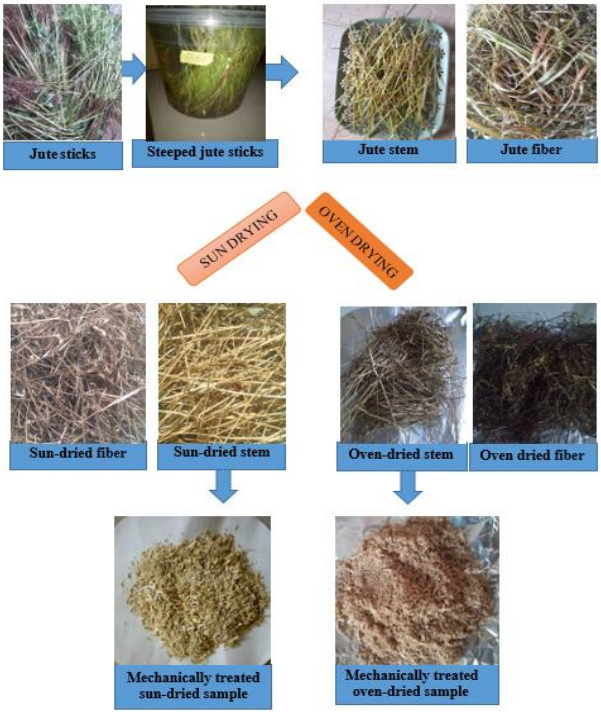

Figure 1: Processing of sun-dried and oven-dried jute sticks.

Proximate Analysis of Processed Green Amaranth and Jute Sticks

A very significant criterion in the application of lignocellulose biomass is to determine its biochemical components and their suitability for industrial purposes. [21] SGSW had higher moisture content, fat content and lower protein content compared to the oven-dried samples (Table 5). Green amaranth samples had higher ash content. The observed high carbohydrate content of over 85% makes oven-dried and sun-dried samples desirable sources of sugars that can be suitable for the production of sugar-based biochemical such as enzymes (cellulase, amylase) and biofuel (bioethanol). Despite the high carbohydrate content of the processed sticks, oven-dried samples showed a higher content and hence were selected for further studies. As their chemical composition varies considerably, lignocellulose resources contain highly compact components with cellulose as a major component. [19] With the reported thermal stability of cellulose (200-300 °C) and the need to prevent observed microbial degradation in the absence of sun drying, the sticks were dried at 105 °C. [16] Cellulose content was slightly higher with oven-dried samples (OJSW: 24.395% and OGSW: 24.43%) than with sun-dried samples (SJSW:23.93% and SGSW:24.235%). With the high carbohydrate content and evidence of cellulose content, the processed sticks are considered a good source of reducing sugars.

Analysis of the sodium hydroxide (NaOH)-Treated Extracts

Successful lignocellulose depolymerization requires the accessibility of biological tools (microbial strains or saccharifying enzymes) into the biomass structure.[3] This access usually results in the conversion process where the polysaccharides trapped within the lignocellulose-based resources are released as hexoses or pentose and detected by measuring the reducing sugar concentration.[3,22] The ineffectiveness of dissolving a polysaccharide (cellulose) in a universal solvent motivated the use of other solvents for its extraction.

With NaOH-treated SJSW, spectrophotometric estimation (Figure 3A) revealed the maximum release of reducing sugars was observed at 1.25N NaOH (1.439 mg/ml $\pm$ 0.063) and 100 °C for 30 minutes. The high reducing sugar concentration observed at 120 minutes was due to the viscous nature of the sample, which was observed to be due to the low density and solvent-absorbing nature of the processed sticks. In comparison to OJSW (Figure 3B), higher reducing sugar concentration was observed when OGSW (Figure 3D) was incubated in 2N NaOH solution at the 100 °C for 60 minutes by a difference of 25.91%. At a higher time of 120 minutes, the reducing sugars released favoured NaOH-treated OJSW by a difference of 31.4%. NaOH-treated OJSW (3.133 mg/ml $\pm$ 0.019) and NaOH-treated SGSW (Figure 3C) (2.705 mg/ml $\pm$ 0.012) displayed a higher reducing sugar concentration when incubated with NaOH solution at 120 minutes. The observed deep dark colour shows the role of sodium hydroxide as a strong base that can increase accessibility to polysaccharides within the processed sticks via lignin solubilisation and suggests the possibility of aldoses transforming tautomeric enediol via non-enzymatic base catalysis. [23]

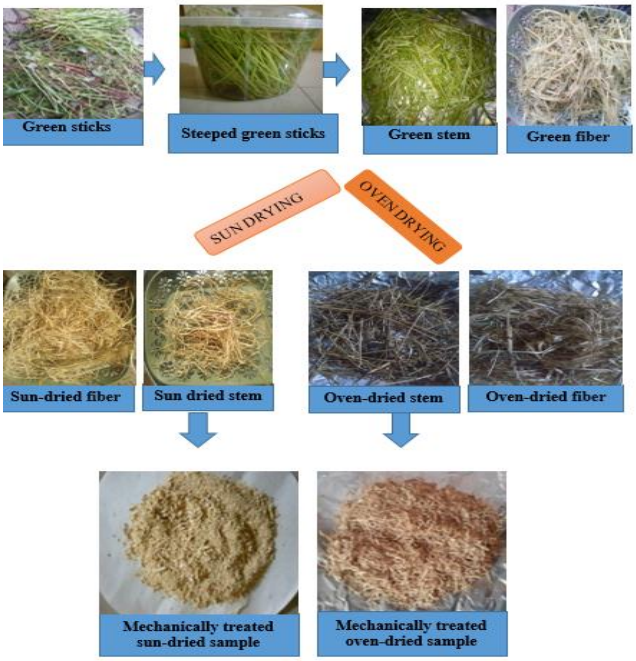

Figure 2: Processing of sundried and oven-dried green sticks.

Effect of NaOH, time, and temperature on reducing sugars released using Box-Behnken design

The response value for the analysis of reducing sugar concentration as shown in the supplementary material (Table S1, Table S2 and Table S4) for NaOH-treated SJSW, NaOH-treated SGSW and NaOH-treated OGSW reveals each significant p-value with each value less than 0.05 (Table S1.2, Table S2.2 and Table S4.2), an insignificant lack of fit, and the adequate precision (Adeq. Precision: greater than 4) indicates the model is suitable to navigate the design space.

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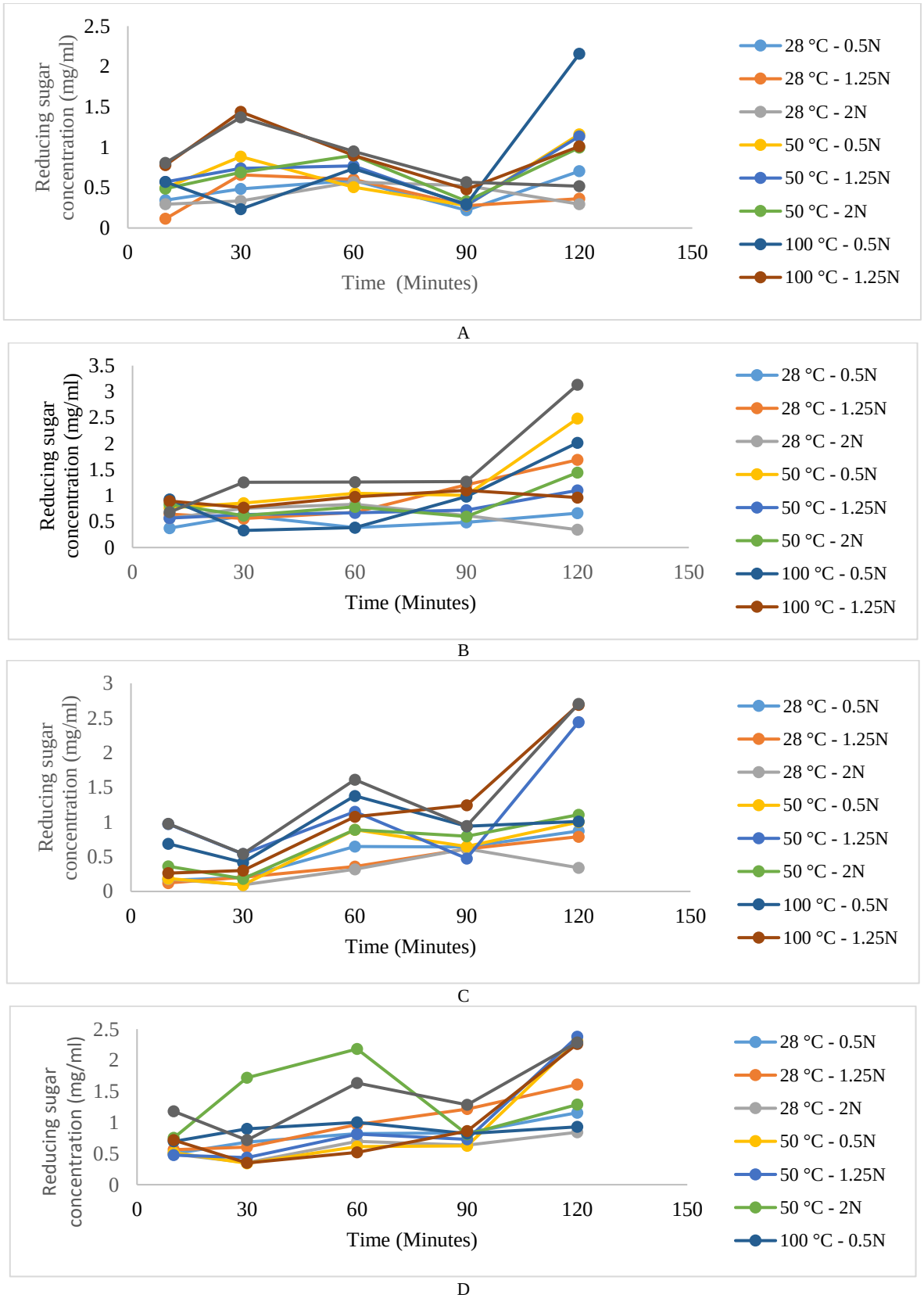

Figure 3: Effect of time and temperature on reducing sugars released from NaOH-treated SJSW (A), NaOH-treated OJSW (B), NaOH-treated SGSW (C) and NaOH-treated OGSW (D) at different concentrations.

As the line graph does not give information on the interaction effect, the 3D plot (Figure 4) shows the interaction effects of NaOH, time and temperature on the release of reducing sugars from the treated sticks. The model was able to predict the outcome as revealed in Table S1.1, Table S2.1 and Table S4.1.

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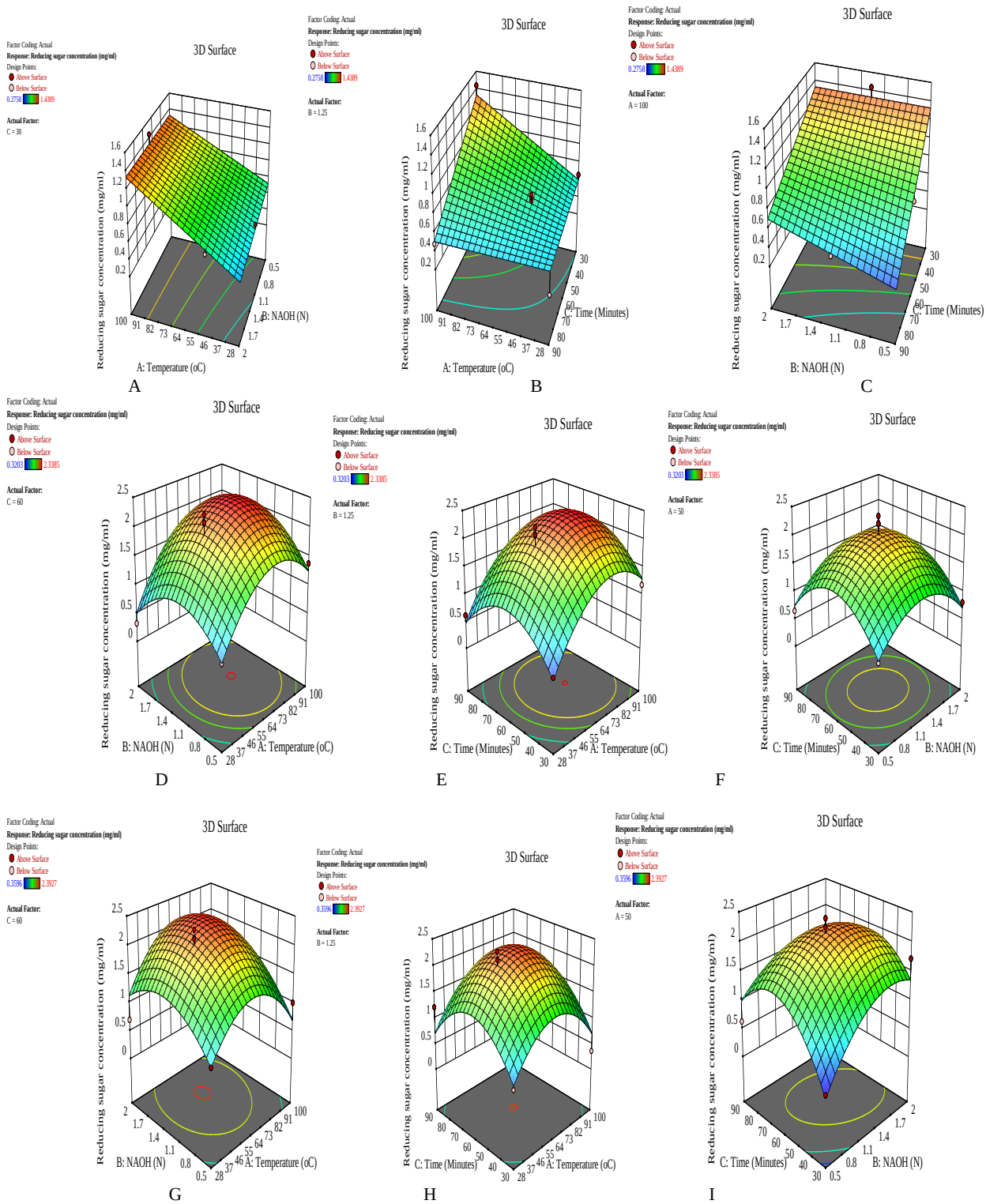

Figure 4: 3D surface plot showing the effect of variables on reducing sugar concentration from SJSW [Variables: A) NaOH and Temperature; B) Temperature and time; C) Time and NaOH], SGSW [Variables: D) Temperature and NaOH; E) Temperature and time; F) Time and NaOH] and OGSW [Variables: G) Temperature and NaOH; H) Temperature and time I) NaOH and time at different concentrations.

Analysis of reducing sugar concentration (Table S3) for NaOH-treated OJSW, reveals an insignificant p-value (0.135) with a value less than 0.05 (Table S3.1) and a low R^2 value (0.338), makes the model unsuitable for navigating the design space. The coefficient of determination (model) for reducing sugar concentration at 79.9% (NaOH-treated SJSW), 85.6% (SGSW) and 85.6%(OGSW) indicate an agreement between the results obtained and predicted outcome.

The equation below describes the interaction between the factors and the outcome for NaOH-treated SJSW (equation 2), NaOH-treated SGSW (equation 3) and NaOH-treated OGSW (equation 4):

Table 1: Summary of the Box-Behnken Design for temperature, NaOH and time

Factor	Name	Units	Minimum	Maximum	Coded Low	Coded High
A	Temperature	ºC	28.00	100.00	-1 ↔ 0.00	+1 ↔ 100.00
B	NaOH	N	0.5000	2.00	-1 ↔ 0.50	+1 ↔ 2.00
C	Time	Minutes	30.00	90.00	-1 ↔ 30.00	+1 ↔ 90.00

Table 2: Summary of the Box-Behnken Design for temperature, ethanol and time

Factor	Name	Units	Minimum	Maximum	Coded Low	Coded High
A	Temperature	ºC	28.00	100.00	-1 ↔ 0.00	+1 ↔ 100.00
B	Ethanol	N	0.5000	2.00	-1 ↔ 0.50	+1 ↔ 2.00
C	Time	Minutes	30.00	90.00	-1 ↔ 30.00	+1 ↔ 90.00

Table 3: Summary of the Box-Behnken Design for temperature, H_2O_2 and time

Factor	Name	Units	Minimum	Maximum	Coded Low	Coded High
A	Temperature	ºC	28.00	100.00	-1 ↔ 0.00	+1 ↔ 100.00
B	H_2O_2	N	0.5000	2.00	-1 ↔ 0.50	+1 ↔ 2.00
C	Time	Minutes	30.00	90.00	-1 ↔ 30.00	+1 ↔ 90.00

Reducing sugar concentration = 0.664 + 0.012*Temperature – 0.361*NaOH – 0.002*Time + 0.002*Temperature * NaOH – 0.0002 *Temperature * Time + 0.004* NaOH * Time……(2)
[p-value: 0.005, F-value: 6.610, R^2:0.799, Adjusted R^2:0.678, Predicted R^2: 0.106, Adeq Precision: 10.31]

Reducing sugar concentration = -4.934 + 0.078*Temperature + 2.407*NaOH + 0.093* Time + 0.004*Temperature * NaOH – 3.746E-06* Temperature * Time + 0.0004*NaOH * Time – 0.001*Temperature² –1.050*NaOH²–0.001*Time² ……..(3)
[p-value: 0.028, F-value: 4.610, R^2:0.856, Adjusted R^2: 0.670, Predicted R^2: 0.517, Adeq Precision: 5.303]

Reducing sugar concentration= -5.725 + 0.068*Temperature + 3.065*NaOH + 0.119*Time + 0.005*Temperature * NaOH + 0.00005*Temperature * Time – 0.013*NaOH * Time – 0.001* Temperature² – 0.887* NaOH² –0.001*Time² …… (4)
[p-value: 0.028, F-value: 4.620, R^2:0.856, Adjusted R^2: 0.671, Predicted R^2: -1.166, Adeq Precision: 5.962].

The deep dark colour observed during NaOH incubation and the release of reducing sugars shown by dinitrosalicylic reagent colour change indicates the participation of NaOH could result in the further dissolution of polysaccharides, with the possibility of generating both the products and by-products. [2,5,24] Hence, employing NaOH may not be cost-effective as additional processing would be required to separate the solvent from the sample components released (by-products and the reducing sugars). This processing, in turn, would affect the overall cost of production of any biochemical that requires the use of the reducing sugars as substrate.

Analysis of the Ethanol-Treated Extracts

Due to its polar nature and its ratio with water in the medium, ethanol is useful in dissolving hydrophilic compounds. [25,26] As presented in Figure 5A, the maximum release of reducing sugars was observed at 1.25N ethanol-treated SJSW (0.864 mg/ml ± 0.009) and 50 ºC for 120 minutes. At the same experimental conditions, an increase in the reducing sugar concentration favoured ethanol-treated SGSW (Figure 5C) over ethanol-treated SJSW by a difference of 82.18%. In addition to 120 minutes incubation of 2N ethanol-treated OJSW (Figure 5B) at 50 ºC (2.444 mg/ml ± 0.017), an increasing release of reducing sugars was observed from 10 minutes to 120 minutes with a fold rise of 3.86%. The highest reducing sugar released was observed for at 1.25N ethanol-treated OGSW (3.554 mg/ml ± 0.025) and 28 ºC for 120 minutes. At a higher temperature of 50 ºC, reducing sugar concentration decreased by a difference of 37.51%. Compared to 2N ethanol-treated OGSW (Figure 5D) at 50 ºC for 60 minutes of incubation, ethanol-treated OJSW declined by a difference of 31.7%.

Like the NaOH solution, the ethanol solution pretreatment will require additional processing methods to make the resource of interest extractable and usable. Unfortunately, this may not be desirable for achieving cost-effectiveness in fermentation-based biochemical production in developing nations. Nevertheless, the possibility of generating a by-product might not be as high as when NaOH was employed.

Effect of ethanol, time and temperature on reducing sugars released

Using the Box-Behnken design, the response value for the analysis of reducing sugar concentration for ethanol-treated SJSW and ethanol-treated OGSW (Table S5 and Table S7), reveals each significant p-value with a value less than 0.05(Table S5.2 and Table S7.2), the insignificant lack of fit, and the adequate precision (greater than 4) indicates the model is suitable. The 3D response plot (Figure 6) shows the interaction effect of ethanol, time and temperature on the release of reducing sugars obtained from each ethanol-treated stick. Table S5.1 and Table S7.1 show the model predicted the outcome.

Analysis of reducing sugar concentration for ethanol-treated SGSW (Table S6), reveals the insignificant p-value (0.076) with a value greater than 0.05 (Table S6.1) and low R^2 (0.399) makes the model unsuitable to navigate the design.

Analysis of reducing sugar concentration for ethanol-treated OGSW (Table S8) reveals the data obtained was only suitable for the mean model. Hence, the design could not be navigated.

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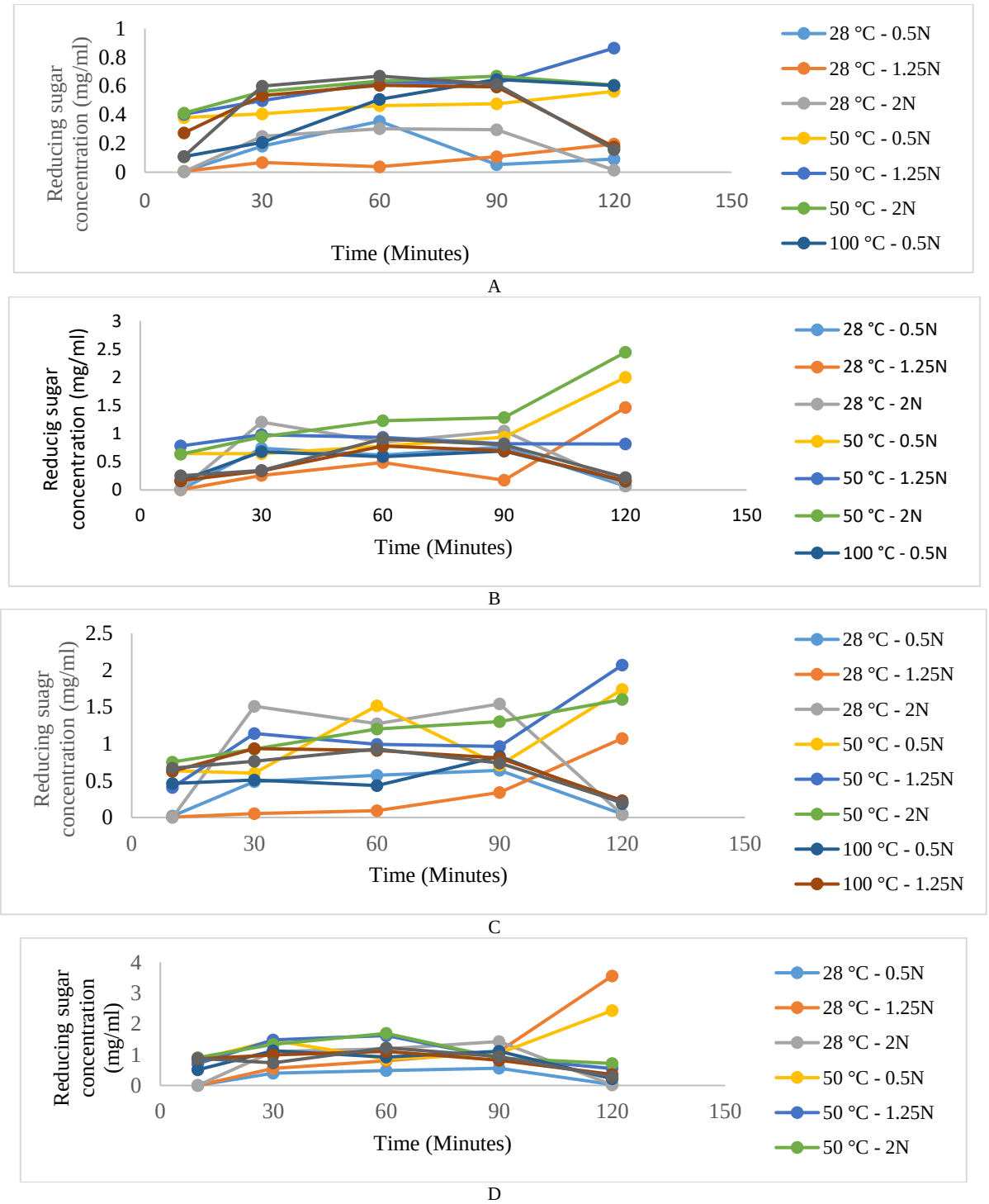

Figure 5: Effect of time and temperature on reducing sugars released from ethanol -treated SJSW (A), ethanol -treated OJSW (B), ethanol -treated SGSW (C) and ethanol -treated OGSW (D) at different concentrations.

Although the data fit only the mean model, the reducing sugar concentration of ethanol-treated OGSW (1.78 mg/ml ± 0.008) was higher than other ethanol-treated sticks.
Based on numerical optimization from analysis, the model predicted the actual outcomes for ethanol-treated SJSW (Table S5.1) and ethanol-treated OGSW (Table S7.1) as the coefficient of determination for reducing sugar concentration was at 84.2% (SJSW), and 86%(OGSW).

The equation below describes the interaction between the factors and the outcome for ethanol-treated SJSW (equation 5) and ethanol-treated OJSW (equation 6):

Reducing sugar concentration =1.490 - 0.004*Temperature – 1.533*Ethanol – 0.015* Time + 0.002*Temperature * Ethanol + 2.617E-06 Temperature * Time + 0.001*Ethanol * Time + 0.00005*Temperature² + 0.574* Ethanol² + 0.0001*Time…… (5)
[p-value: 0.037, F-value: 4.150, R^2:0.842, Adjusted R^2: 0.639, Predicted R^2: -1.023Adeq Precision: 6.109]

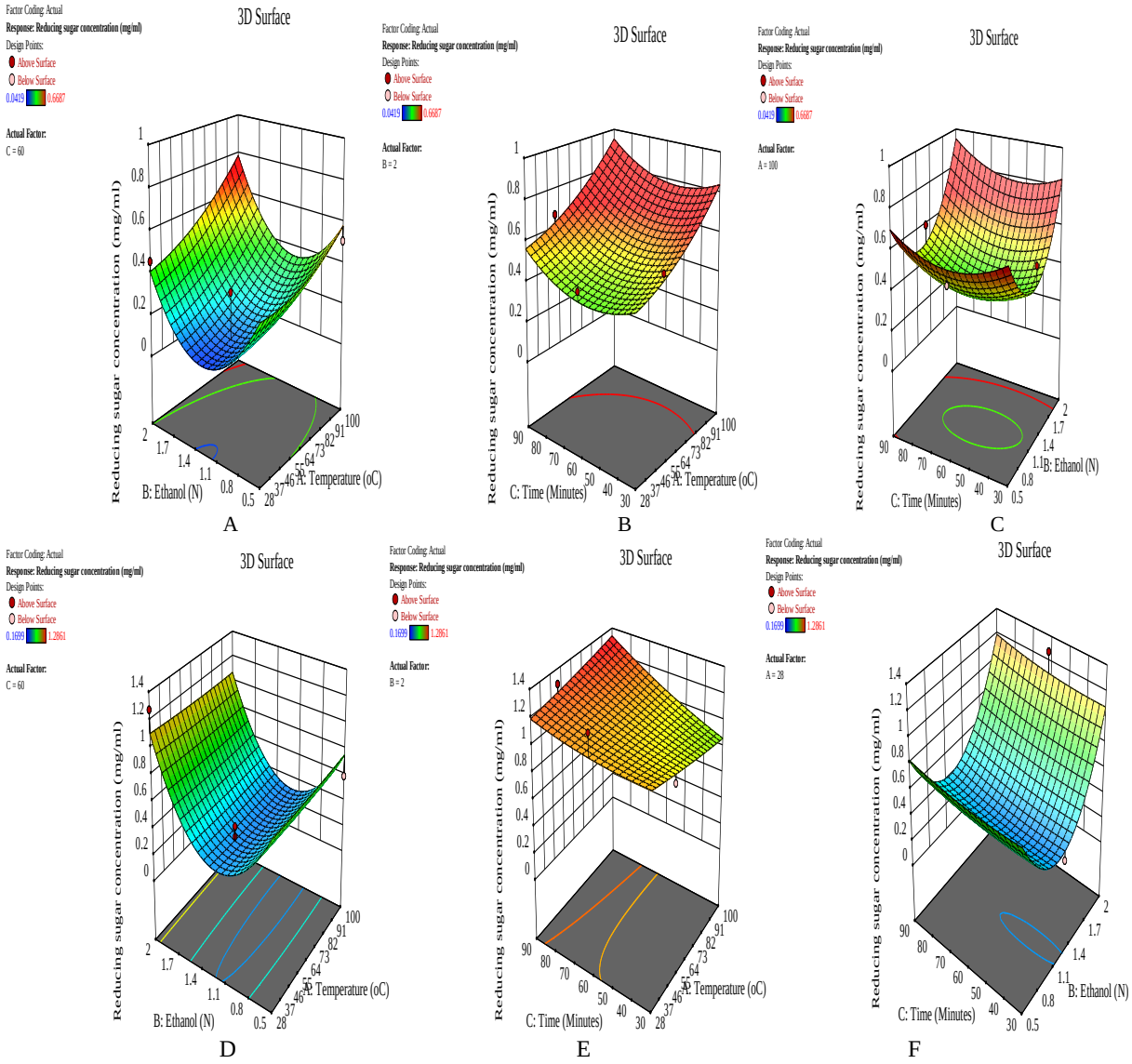

Figure 6: 3D surface plot showing the effect of variables on reducing sugar concentration from sun-dried jute stick [Variables: A) Temperature and ethanol; B) Temperature and time; C) Ethanol and time] and oven-dried jute stick at different concentration of variables [Variables: D) Ethanol and temperature; E) Temperature and time; F) Ethanol and time] at different concentrations.

The final equation for OJSW (actual factors):
Reducing sugar concentration =1.992 – 0.006*Temperature – 2.444*Ethanol – 0.006*Time – 0.0001*Temperature *Ethanol + 0.00008*Temperature*Time + 0.0007*Ethanol*Time + 8.119E – 6*Temperature² + 1.051*Ethanol² + 0.00004*Time²…… (6)
[p-value: 0.026, F-value: 4.780, R^2:0.860, Adjusted R^2: 0.680, Predicted R^2: -1.460, Adeq Precision: 6.889]

Analysis of the H_2O_2-Treated Extracts

With the discovery of lytic polysaccharide monoxygenases, oxidoreductase is gaining attention in lignocellulose degradation as an auxiliary enzyme to enhance hydrolase's function. [27,28] Its preference for H_2O_2 and the presence of H_2O_2 in the medium implies a potential interaction that could improve the process of cleaving and exposing end chains that could enhance the process of saccharification. [27,28] In circumstances where glucose oxidase functioned as an auxiliary enzyme, the release of aldonic acids and H_2O_2 is likely inevitable.[3] As an oxidizing agent, hydrogen peroxide's action on selected lignocellulose-based wastes could provide insight that would encourage the exploration of more oxidoreductase in enhancing saccharification in fermentation processes. SJSW (Figure 7A) incubated in the 0.5N H_2O_2 solution at 50 ºC for 120 minutes released a higher reducing sugar concentration of 0.934 mg/ml ± 0.04.

From 10 minutes of 0.5N H_2O_2 incubation of SGSW (Figure 7C) and OGSW (Figure 7D) at 28 ºC, an increase in the release of reducing sugars with subsequent colour change was observed compared to H_2O_2-treated jute stick samples. This colour change is indicative of the release of reducing sugars because, without the availability of the free anomeric carbon or free carbonyl group (C=O) on reducing sugars, the dinitrosalicylic acid solution will retain its orange colour after colour development. This increase favours the release of reducing sugars H_2O_2-treated SGSW over H_2O_2-treated OGSW by a difference of 12.48%. As the temperature rises to 50 ºC and the concentration level of H_2O_2 increases to 2N, maximal release that favours H_2O_2-treated OGSW was achieved at 120 minutes by a difference of 6.24% and 70.9% compared to H_2O_2-treated SGSW and H_2O_2-treated OJSW (Figure 7B). At an increased temperature of 100 ºC, the release of reducing sugars favoured the H_2O_2-treated OGSW by a difference of 31.45% at 90 minutes.

Effect of H_2O_2, temperature and time on reducing sugars released using Box-Behnken design

The response value for the analysis of reducing sugar concentration as presented in Table S9, Table S10 and Table S12 for H_2O_2-treated SJSW,

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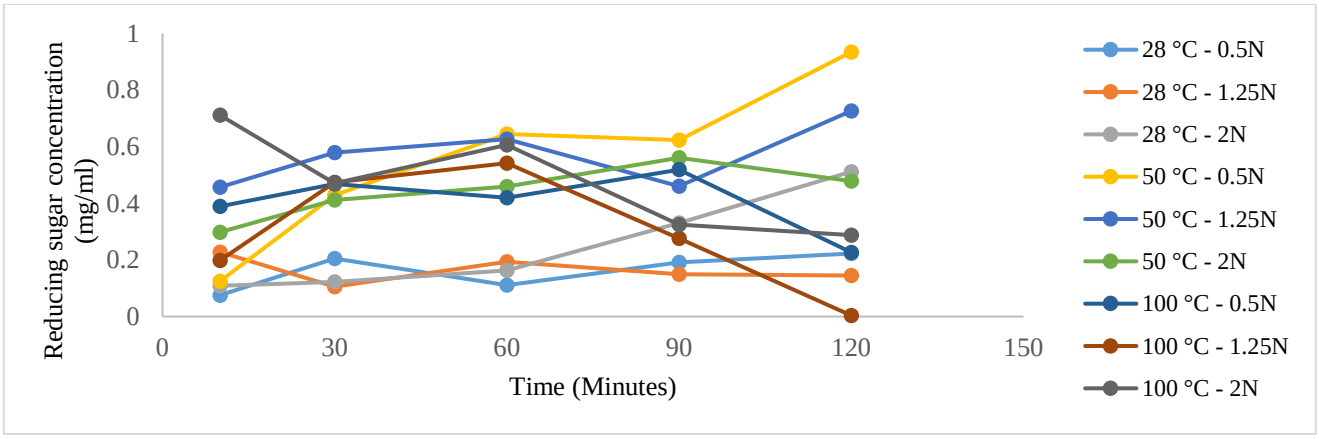
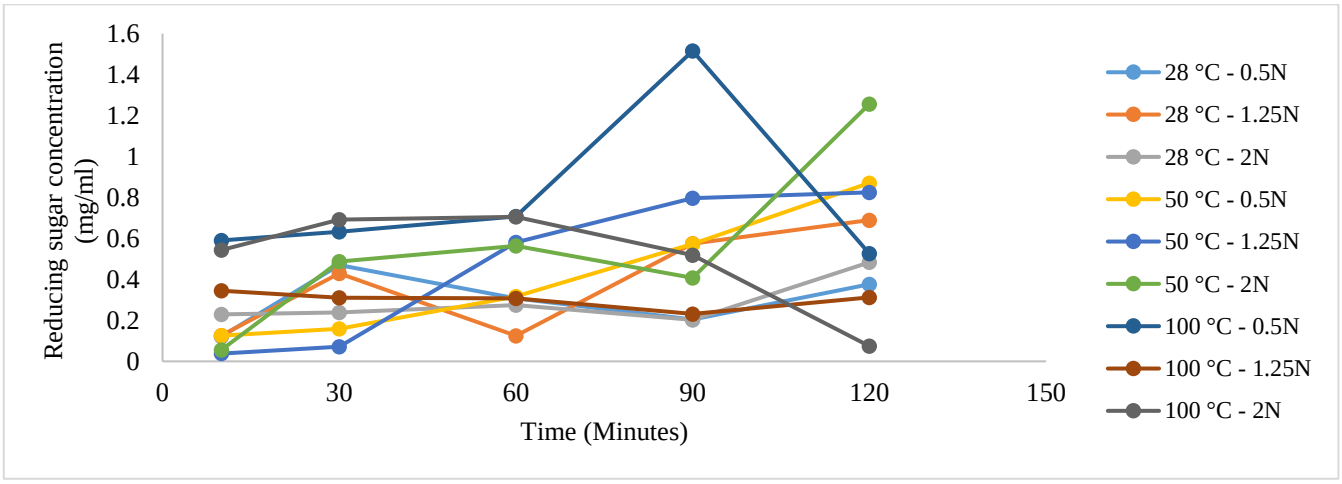
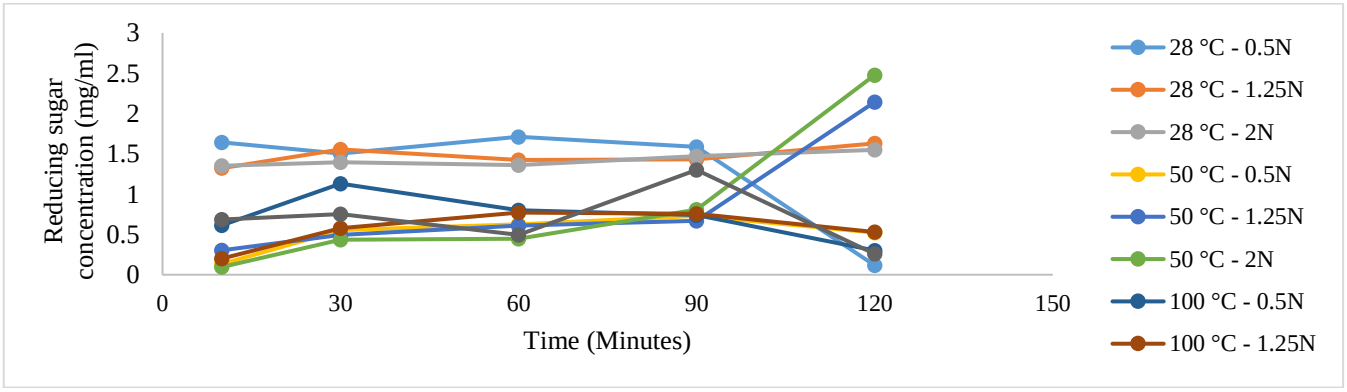


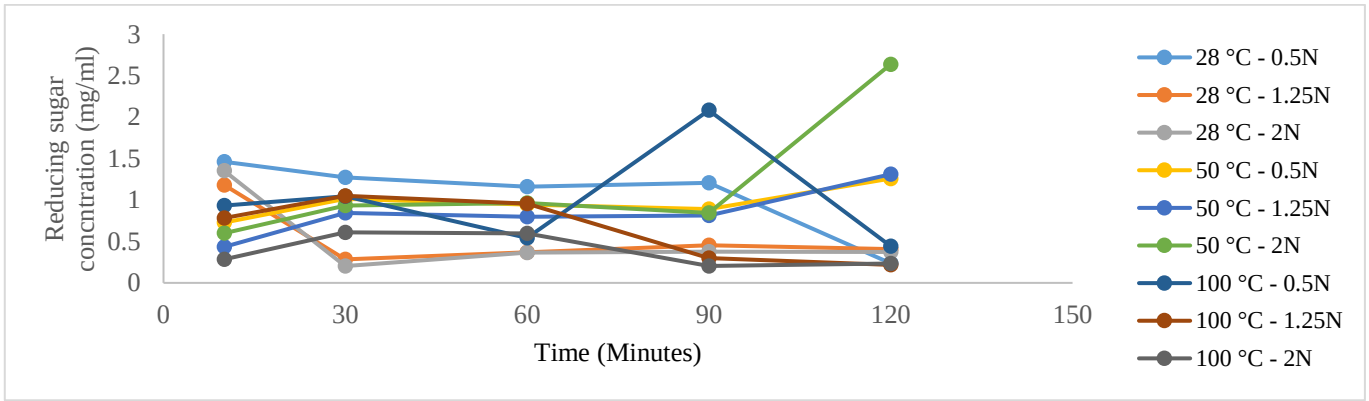
A



B



C



D

Figure 7: Effect of time and temperature on reducing sugars released from H_2O_2 -treated SJSW (A), H_2O_2 -treated OJSW (B), H_2O_2 -treated SGSW (C) and H_2O_2 -treated OGSW (D) at different concentrations.

Table 4: Summary of the regular two level factorial design for temperature, buffer(pH) and time

Factor	Name	Units	Minimum	Maximum	Coded Low	Coded High
A	Temperature	°C	28.00	50.00	-1 ↔ 28.00	+1 ↔ 50.00
B	Buffer (pH)		5.00	6.00	-1 ↔ 5.00	+1 ↔ 6.00
C	Time	Minutes	30.00	60.00	-1 ↔ 30.00	+1 ↔ 60.00

Table 5: Proximate analysis of wastes

Components	OJSW	SJSW	OGSW	SGSW
Moisture (%)	6.81±0.042	6.97±0.014	7.44±0.028	7.64±0.028
Fat (%)	0.55±0.014	0.63±0.014	0.58±0	0.67±0.014
Ash (%)	1.85±0.042	1.96±0.028	2.25±0.042	2.16±0.028
Protein (%)	0.57±0.014	0.535±0.007	0.48±0.014	0.45±0.014
CHO (%)	90.22±0.028	89.91±0.021	89.23±0.120	89.08±0.028

Mean ± Standard deviation; CHO: carbohydrates; OJSW: oven-dried jute stick waste; OGSW: oven-dried green stick waste; SJSW: sun-dried jute stick waste; SGSW: sun-dried green stick waste.

H_2O_2-treated SGSW, and H_2O_2-treated OGSW reveals each significant p-value with a value less than 0.05 (Table S9.2, Table S10.1 and Table S12.2), the insignificant lack of fit, and the adequate precision (greater than 4) makes the model suitable. The 3D response surface curves were plotted and expressed in Figure 8. The factor value for H_2O_2-treated SJSW predicted by the model was outside the design space hence, it was not achievable. The interaction effect between temperature and H_2O_2 significantly influenced the outcome (reducing sugar concentration) for H_2O_2-treated SGSW and H_2O_2-treated OGSW was observed.

Analysis of reducing sugar concentration (Table S11) for H_2O_2-treated OJSW revealed the data generated was only suitable for the mean model. Hence, the design could not be navigated.

Based on numerical optimization from analysis, it was revealed that the model predicted the actual outcomes for H_2O_2-treated SGSW (Table 9.1) and H_2O_2-treated OGSW (Table 12.1) as the model's coefficient of determination for reducing sugar concentration was at 87.9% (H_2O_2-treated SJSW), 71.6%(H_2O_2-treated SGSW) and 92.1% (H_2O_2-treated OGSW). For H_2O_2-treated SJSW, the factor value is outside the design space.

The equation below describes the interaction between the factors and the outcome for H_2O_2-treated SJSW (equation 7), H_2O_2-treated SGSW (equation 7) and H_2O_2-treated OGSW (equation 9):

Reducing sugar concentration = -1.591 + 0.050*Temperature + 0.342* H_2O_2 + 0.017* Time – 0.002*Temperature * H_2O_2 + 7.797E-06 Temperature * Time – 0.0009* H_2O_2 * Time – 0.0004*Temperature² - 0.082* $H_2O_2^2$ – 0.0001* Time²……. (7)
[p-value: 0.016, F-value: 5.66, R^2:0.879, Adjusted R^2: 0.724, Predicted R^2: 0.014, Adeq Precision: 5.787]

Reducing sugar concentration = 0.156 + 0.011*Temperature + 0.075* H_2O_2 – 0.003* Time – 0.007*Temperature * H_2O_2 + 0.00003*Temperature * Time + 0.002* H_2O_2*Time…….…… (8)
[p-value: 0.023, F-value: 4.20, R^2:0.716, Adjusted R^2: 0.546, Predicted R^2: -0.139, Adeq Precision: 8.247]

Reducing sugar concentration = 0.633 + 0.050*Temperature – 1.326* H_2O_2 + 0.0369*Time + 0.008*Temperature * H_2O_2 – 0.00007* Temperature * Time – 0.002* H_2O_2 * Time – 0.00043*Temperature² + 0.331* $H_2O_2^2$ - 0.0002*Time²…… (9)
[p-value: 0.004, F-value: 9.000, R^2:0.921, Adjusted R^2: 0.818, Predicted R^2: 0.652, Adeq Precision: 9.158]

Compared to other H_2O_2-treated samples, OGSW (1.24 mg/ml ± 0.022) significantly released reducing sugars in the presence of 1.25N H_2O_2 with higher saccharification of 2.24% at 50 °C for 60 minutes. Unlike other solvents, hydrogen peroxide might require enzymes such as catalase to decompose into water and oxygen. Unfortunately, H_2O_2-based treatment may not be cost-effective for any industry trying to conserve the cost of production as the value and required quantity of enzymes may not be affordable.

Interaction effect of citrate-phosphate buffer (pH), time and temperature on reducing sugars released

With the evidence of carbohydrate content, cellulose content and the maximal release of reducing sugars achieved with 2N NaOH-treated OJSW at 100 °C, 2N hydrogen peroxide-treated OGSW at lower temperature (50 °C) for 120 minutes and 1.25N ethanol-treated OGSW at 28 °C for 120 minutes, the regular two-level factorial design was employed to determine the interaction effect of citrate-phosphate buffer(pH), time and temperature on release of reducing sugars from buffer-treated oven-dried samples.

While the end products of other extraction solvents may affect fermentation organisms, the buffer has the potential to preserve the structural integrity of the cells of the organism by providing an environment that can support them as well as stabilize other components outside the cells. [10,29] In addition to their potential to display insignificant penetration through microbial membranes, mimicking the osmolyte's anti-denaturant properties with no reported toxicity and the capacity to maintain buffer capacity at a pH where the enzyme and protein stability is preserved make citrate-phosphate buffer preferable to other solvents. [29]

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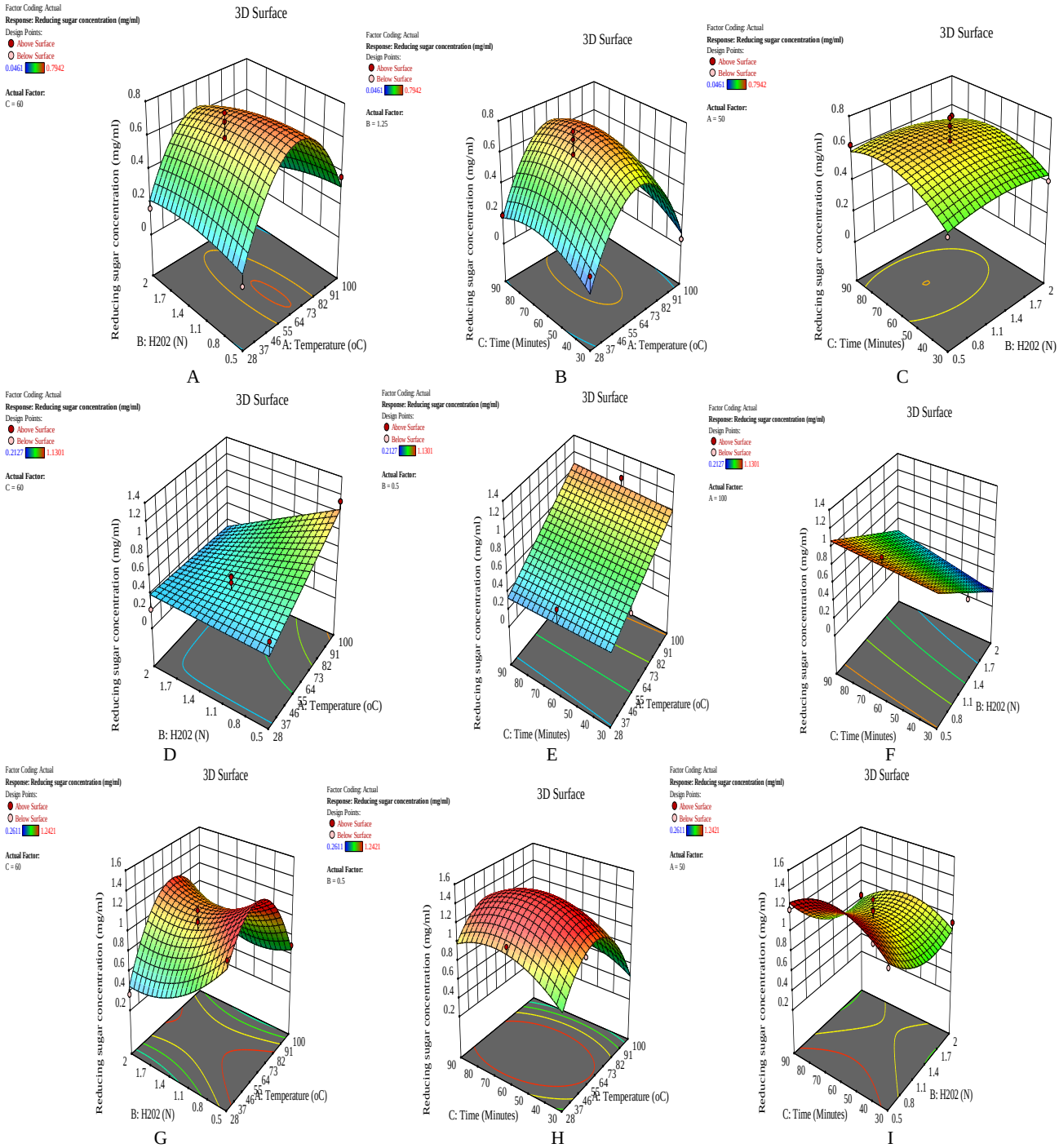

Figure 8: 3D surface plot showing the effect of variables on reducing sugar concentration from sun-dried jute stick waste [Variables: A) Temperature and H_2O_2; B) Temperature and time; C) H_2O_2 and Time], sun-dried green stick waste [Variables: D) Temperature and H_2O_2; E) Temperature and time; F) H_2O_2 and time] and oven-dried green stick [Variables: G) Temperature and H_2O_2; H) Temperature and time; 1) H_2O_2 and time] at different concentration of variables

Citrate-phosphate buffer (buffer) - treated OJSW (Table S13) and buffer- treated OGSW (Table S14) reveal each significant p-value with a value less than 0.05 (Table S13.2 and Table S14.2), the insignificant lack of fit, and the adequate precision greater than 4 makes the model suitable to navigate the design.

The half-normal plot and the Pareto plot (Figure 9) reveal the significant effects and express the percentage contribution of each factor. Temperature (40.6%) and time (22.29%) significantly contributed to the release of reducing sugars from the buffer-treated OJSW. Only temperature (19.8%) significantly contributed to the release of reducing sugars from the buffer-treated OGSW, followed by interaction between the three factors (17.45%), then the buffer (16.8%) and time (12.15%). The cube plot and 3D response surface curves/plots showing the interaction effects of variables for the buffer-treated OJSW (A and B) and the buffer-treated OGSW (C-F) were expressed in Figure 10.

Based on numerical optimization from analysis, it was revealed that the model predicted the actual outcomes for the buffer-treated OJSW (Table S13.1) and the buffer-treated OGSW (Table S14.1) as the coefficient of determination (model) for reducing sugar concentration was at 89.10% (buffer-treated OJSW) and 95.8% (buffer-treated OGSW).

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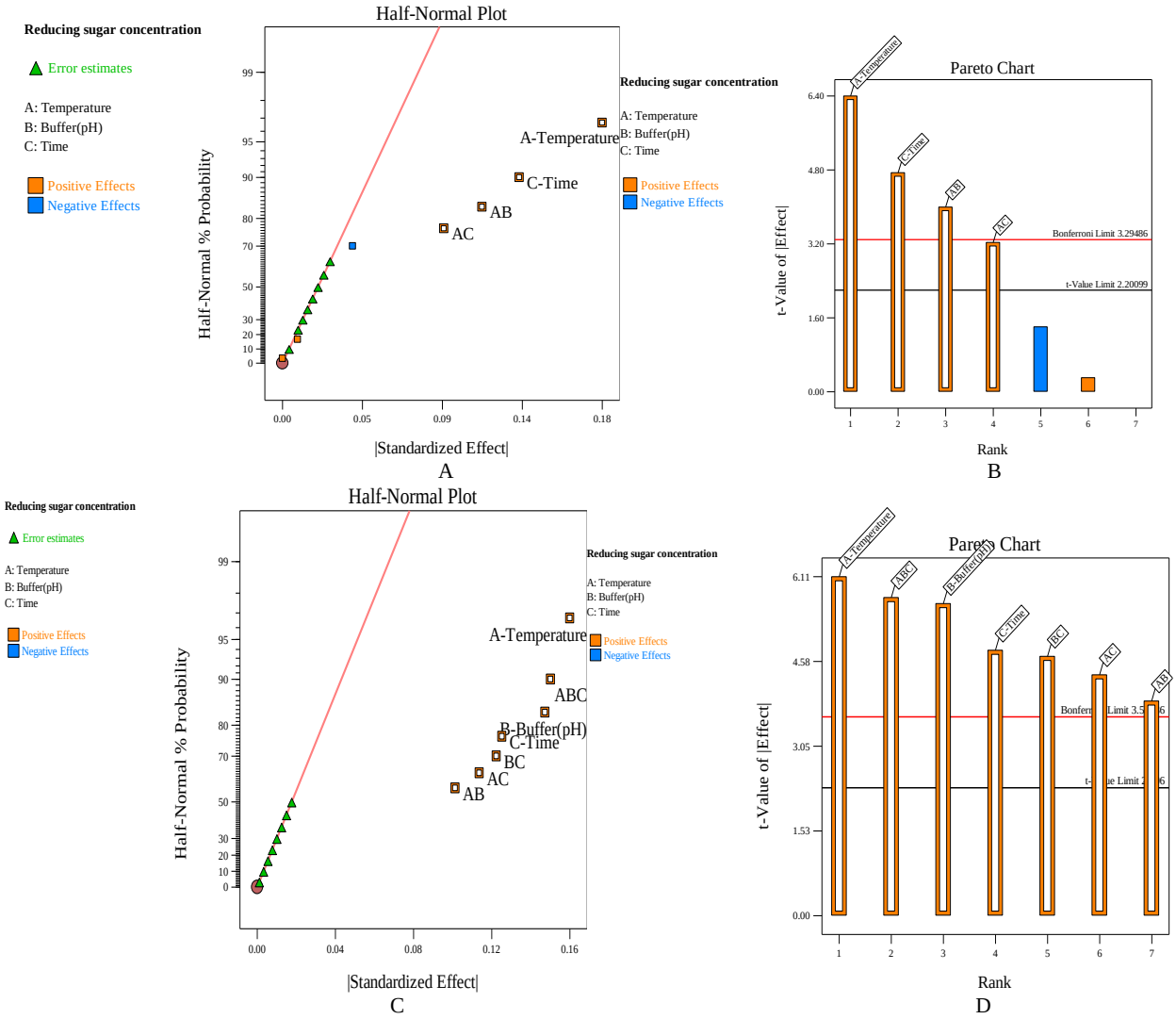
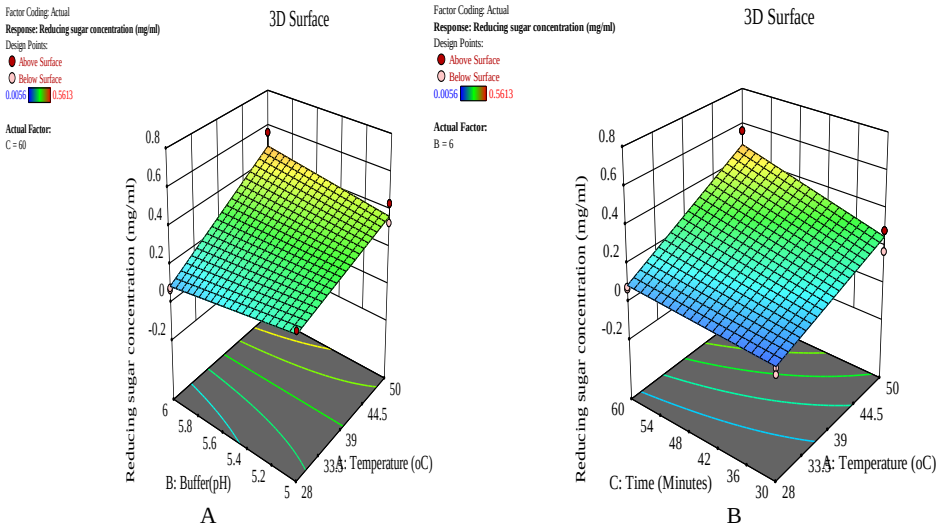


Figure 9: Half plot and Pareto plot showing the effect of variables (Temperature, buffer (pH) and time) on reducing sugar concentration from buffer-treated oven- dried jute stick [A and B] and buffer-treated oven-dried green amaranth stick [C and D].



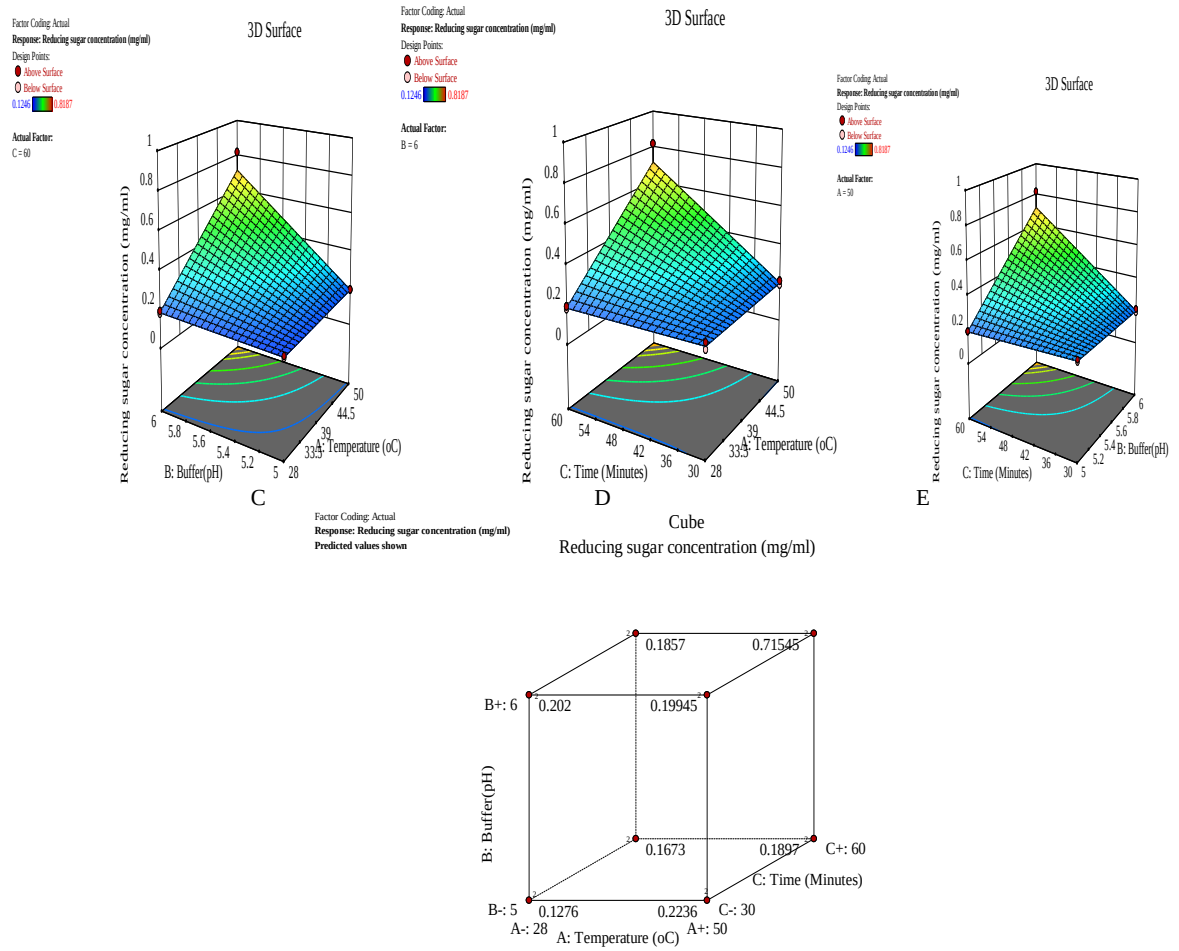


Figure 10: 3D surface plot showing the effect of variables on reducing sugar concentration from buffer-treated oven-dried jute samples [Variables: A) Temperature and buffer; B Temperature and time)] and buffer-treated oven-dried green samples [Variables: C) Temperature and buffer; D) Temperature and time; E) Buffer and time; F) Cube plot of buffer, temperature and time] at different concentrations.

The equation below describes the interaction between the factors and the outcome for buffer-treated OJSW (Coded factors: equation 10) and buffer-treated OGSW (equation 11):

Reducing sugar concentration = 0.219 + 0.092*Temperature + 0.069*Time + 0.058*Temperature* Buffer(pH) + 0.047 *Temperature* Time…… (10)
[p-value: < 0.0001, F-value: 22.48, R^2:0.8910, Adjusted R^2:0.8514, Predicted R^2: 0.7694, Adeq Precision: 13.5451]

Reducing sugar concentration = -5.263 + 0.168 Temperature + 1.027 Buffer(pH) + 0.142 Time – 0.032 Temperature * Buffer(pH) – 0.005 *Temperature* Time – 0.028 * Buffer(pH) * Time + 0.0009 Temperature * Buffer(pH)* Time…… (11)
[p-value: < 0.0001, F-value:25.77, R^2:0.958, Adjusted R^2: 0.920, Predicted R^2: 0.830, Adeq Precision: 15.73]

SEM of Oven-Dried Samples

As the accessibility of the resources present in oven-dried wastes is important for their valorization, their morphology is expected to provide more insight to achieve adequate depolymerization. Based on their surface morphology, OJSW (Figure 11A) displayed a mass of rough, compact, and spongy-like aggregation of lignocellulose structures. In contrast, OGSW (Figure 11B) showed a mass of coarse and irregular morphological patterns. This implies the need for tools that allow accessibility to resources of interest and their subsequent valorization.

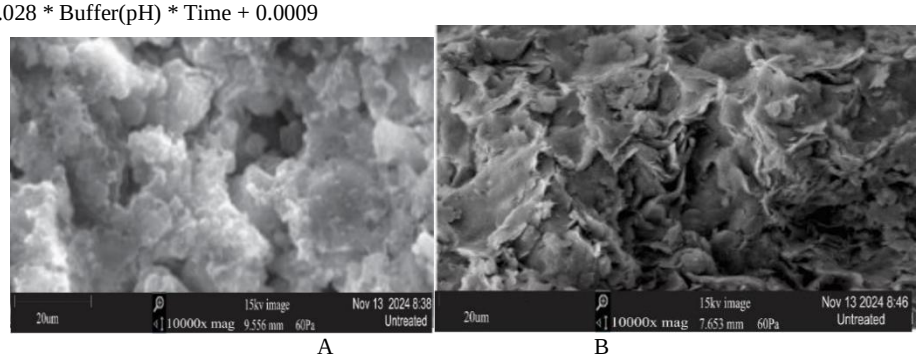


Figure 11: SEM processed OJSW (A) and OGSW (B

Conclusion

The solvent extraction was employed to release sugar resources trapped within the OGSW, OJSW, SGSW and SJSW. The Box-Behnken design revealed the released reducing sugars using 1.25N NaOH (2.393 mg/ml ± 0.494), 1.25N H_2O_2 (1.240 mg/ml ± 0.093) at 50 ºC and 2N ethanol (1.780 mg/ml ± 0.008) at 28 ºC for 60 minutes favour OGSW over OJSW by a difference of 61.92%, 73.28% and 31.92%. The ability of citrate-phosphate buffer to release reducing sugars favours OGSW over OJSW with a difference of 37.3% at the factor level of pH 6, 50 ºC, and 60 minutes. With the significant interaction effect of variables and the possibility of the absence of by-product formation, the citrate-phosphate buffer was considered a greener and more suitable option compared to ethanol and NaOH solution in extracting reducing sugars from the processed green amaranth and jute sticks.

As finding an economically feasible method with no impact on the environment is essential, the advantage of using an alkaline solution in extracting sugar-based resources from lignocellulose-based wastes is significant. Yet the drawback associated with its use has not been significantly explored from a biochemical perspective. Separating alkaline solutions from sugar-based resources within lignocellulose-based wastes, to make the sugars available for further processing should be given consideration that would subsequently impact the product yield as well as ensure the cost-effectiveness of the production process. Hence, attention focused on ascertaining the end products generated from the solvent-treated lignocellulose wastes, the requirement to separate reducing sugars from solutions that have the potential to be hazardous and effort channelled toward developing a cost-effective method that can separate reducing sugars from acidic or alkaline solutions should be considered a priority in lignocellulose-based fermentation processes.

Conflicts of interest

The authors declare no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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