

EFFECT OF VARIATION IN UREA CONCENTRATION USED IN RETTING, ON THE CHEMICAL AND MECHANICAL PROPERTIES OF KENAF FIBRES.

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Abstract

AIMS: (i) To determine the effect of 1% and 2% urea concentration on the proximate, chemical compositions and mechanical properties of Kenaf fibre

(ii). To determine the effect of variation in urea concentration on the enzyme and microbial activities during retting.

STUDY DESIGN: Data obtained from each treatment were subjected to analysis of variance (ANOVA). Means were separated using Duncan multiple range test. Significant difference was accepted at $P < 0.05$.

PLACE AND DURATION: The retting experiment was carried out in the Institute of Agricultural Research and Training, Obafemi Awolowo University. From April, 2015 to January, 2016.

METHODOLOGY: Two Kenaf stems each of the four varieties were tied (bundled) together as a replicate and three replicates were soaked in an improvised retting tank containing urea at 1% and 2% concentrations in 1500 litres of water and control (without urea). Fibres retted in 1%, 2% urea and control were subjected to proximate, microbiological, chemical and mechanical analyses.

RESULTS: The results showed that urea treatments slightly increased in α -cellulose. The lignin content of Kenaf fibres was 2% urea > 1% urea > control across the four varieties. The two indigenous varieties: Ifeken DI 400 and Ifeken 400 had least level of lignin. Hemi-cellulose content of Kenaf fibres retted from 1% urea was significantly lower than others. The highest protein and iron content were recorded in 2% urea followed by 1% urea and least in control. The tensile strength of 2% urea retted fibres was significantly higher than others.

CONCLUSION: This study observed that the synergistic interaction of the enzymes in urea solution had produced fibres with promising chemical and mechanical properties. Kenaf fibres retted in 2% urea had better tensile strength and higher α -cellulose content. Urea retting lasted for seven days irrespective of the Kenaf variety while control lasted for fourteen days. It can be inferred that urea treatments produced fibres with satisfactory levels (<30%) of lignin and (> 40%) of α -

1. INTRODUCTION:

Presently, natural fibres form an interesting option for the most composite technology, pulp, paper and other ligno-cellulose conversion industries [1-2].

Traditionally, kenaf bast fibres were usually retted in streams, rivers and ponds, and this practice was environmentally unfriendly as it deteriorates the water body

and predisposes the users to pathogenic micro-organisms. Recent research had shown that chemical retting using mild reagents like sodium hydroxide, potassium hydroxide and /or urea is a good substitute for whole stalk stream retting [3]. However, the World paper consumption has geometrically increased, although Hurter and Riccio[14] did report that the World paper consumption was 300 million tons in 1996 and was expected to rise to the tune of 400 million tons in 1997. In view of the shortage of raw materials for pulping and increasing demand for paper products, plaster of paris (POP), fibres glass, packaging bags for agricultural products among others worldwide, non-wood plants and agricultural residues attracted renewed interest, especially in countries like Spain, Italy and Greece with insufficient forest resources[14]. Non-wood plants offer several advantages including short growth cycles, biodegradability, moderate irrigation and fertilization requirements and low lignin content resulted in reduced energy and chemical uses during pulping [10]. Developing countries like Nigeria has also focused attention on the diversification of its economy by promoting programmes and activities that increase agricultural production amidst the dwindling oil revenue. In the light of this, the Federal government of Nigeria has renewed her interest in kenaf as a potential industrial crop with multipurpose uses including bioremediation of oil polluted areas.

Kenaf (*Hibiscus cannabinus* L.) is a natural fibre crop used for rope, twine and coarse sacking materials. Kenaf and other natural fibre composites have advantages of being renewable, environmental friendly, low cost, low density, flexibility of usage and biodegradability[4]. It is an understatement to say that Kenaf is a multi-purpose crop, as it has been found to be an important source of raw materials for other industrial applications [1].

The importance of treatments in raw plant materials goes a long way to determine the quality of the finished products. It was observed that the majority of variation in burst and tensile strength in hardwood pulp sheets could be accounted for by fibre length and cell wall thickness [4-5]. Updegraff *et al* [6] has categorically stated the significance of fibre dimensions in predicting wood pulp mechanical properties. Saikia *et al* [16] and Ogbonnaya *et al* [15] reported that the derived values like fibre length/fibre diameter, flexibility coefficient as well as Runkel ratio as $(2 \times \text{fibre cell wall thickness})/\text{lumen diameter}$ of softwoods and

hardwoods were compared to assess the suitability of the plant raw materials for paper. Aderonke *et al.* [18] reported that chemical treatment of selected agro-fibres were suitable to minimise water absorption rate in composite materials and thereby making the selected agro-fibres to be suitable for reinforcement in polyester composite and can be used for the production of composite with improved performance. Research has shown that the use of chemical (urea) in kenaf retting is very effective in reducing the retting time [3]. But there is a paucity of documentation on the effect of variation in the amount of chemical reagents (precisely urea) used in retting on the properties of kenaf fibre. Hence, this modification was to ascertain its impact on the physical, chemical and mechanical properties of the kenaf fibre. The specific objectives of this study were to determine:

- i. The effect of 1% and 2% urea solution on the α -cellulose, lignin and hemicellulose content of kenaf fibre
- ii. The effect 1% and 2% urea solution on the tensile modulus and tensile strength of kenaf fibre.
- iii. The effect 1% and 2% urea solution on the proximate compositions of kenaf fibre
- iv. The effect of variation in urea concentration on the enzyme and microbial activities during retting

2.0: MATERIALS AND METHODS:

2.1: Kenaf samples preparation

The four varieties of Kenaf namely: Cuba-108, Tianung-2, Ifeken-Di-400 and Ifeken 400 were collected from the Kenaf and Jute Improvement Programme, Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria.

Traditional whole stalk retting was carried out using water and urea in plastic tanks. Two kenaf stems each of the four varieties were tied together as a replicate and three replicates were soaked in an improvised plastic tank containing urea at 1% and 2% concentrations in 1500 litres of water and control

(without urea). Textures of the fibres were tested with fingers on daily basis to ascertain exact time taken to attain complete retting. Each completed retted fibre was removed, washed thoroughly with water and sundried. And was subsequently subjected to other analyses

2.2: Measurement procedure

The fibre samples were analysed for α -cellulose, hemi-cellulose and acid insoluble lignin; α -cellulose was determined using a colorimetric method with the anthrone reagent. 0.3g (dry weight) ground (0.5mm) samples were treated and boiled (at 100°C) with a mixture of nitric/acetic acid (1:8, v/v) for 1h to remove lignin and hemicelluloses after successive centrifugations, and diluted with 67% H_2SO_4 (v/v). Cellulose was then determined at 620nm using cold anthrone reagent [7]. Protein, ash and iron content of the fibres were determined according to the procedure described by AOAC [8].

Mechanical properties of the fibres

Mechanical testing of the fibres was carried out using an Instron (model 3369) fibre testing machine. This was in accordance with the prescribed procedure by the standard of American Society for testing and materials, ASTM D [9]. Tensile strength and tensile (Young's) modulus were automatically calculated from this test.

Organic tests

The properties of retting liquor were assessed at two days interval. And subsequently the organic tests for biochemical oxygen demand (BOD), chemical oxygen demand (COD) and dissolved oxygen (DO) were conducted to determine of the concentration (in mg/L) of carbon-based compounds aimed at establishing the relative strength of retting water [2].

Microbiological Procedure

The powered nutrient agar of 28g was dissolved in 1 litre of deionized water, allowed to soak for 10 minutes and then sterilized by autoclaving for 15 minutes at

121°C. It was thereafter cooled and then poured into petri dishes. Potato dextrose agar of 39g was also dissolved in 1 litre of distilled water and boiled to dissolve the medium completely before sterilizing with autoclave at 121 °C for 15 minutes. The pH of the liquor samples was adjusted to 3.5, after adding 10ml of lactic acid solution, to facilitate microbial growth. The medium was thereafter cooled to 55 °C and poured into petri dishes. 2ml of liquor were taken from each treatment at day 1, 3, 5 and 8 using syringe. Aliquot of each liquor was serially diluted in 10 folds. The highest three dilutions were considered for microbial counts. Each liquor was inoculated on nutrient agar and potato dextrose agar, incubated at 37 °C and 25°C in the incubators respectively and observed for 24 hours for five days, after which the different isolates were characterized using the slide culture technique. Microscopic examination was carried out after gram staining the bacterial isolates, while Lactophenol blue staining was carried out on fungi isolates [8, 17].

2.3: Statistical analysis

Data obtained from each treatment were subjected to analysis of variance (ANOVA) using Duncan multiple range test. Significant difference was accepted at $P < 0.05$

3.0:RESULTS AND DISCUSSION

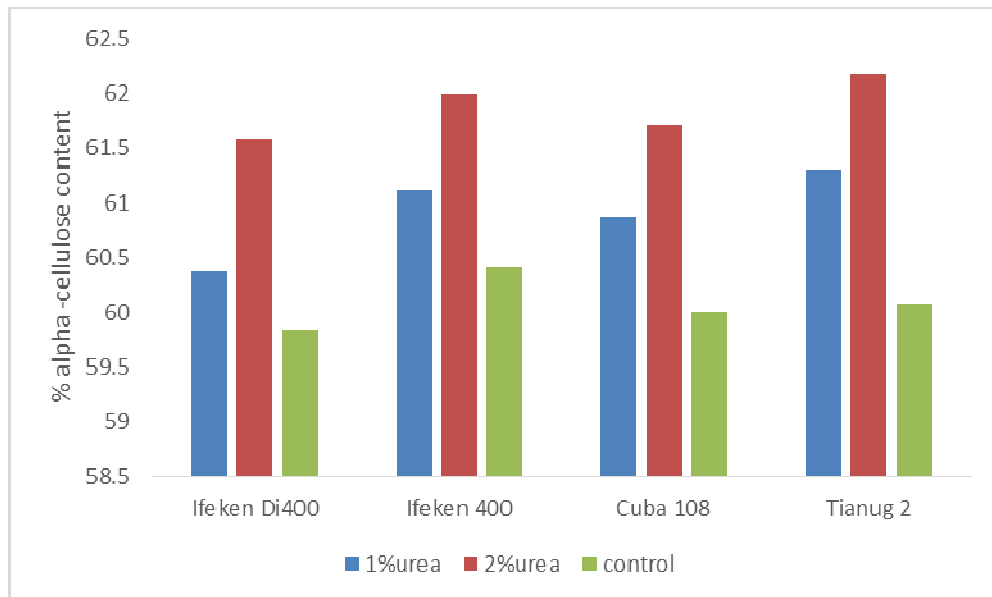


Figure 1: Alpha-cellulose content of retted fibre varieties.

3.1 Alpha –cellulose content

The α -cellulose content of the kenaf fibres from the four different varieties ranged from 59.91- 60.41% for Control; 60.38-61.30% for 1% urea and 61.58-62.17% for 2% urea (figure 1). Urea treatment significantly improved the α -cellulose content of the fibres across the four kenaf varieties (figure. 1). The α -cellulose content of the fibres increased gradually with an increase in concentration of the urea solution from 1% urea to 2% urea. (Fig 1). This result showed that kenaf has better α -cellulose content than most softwood fibres reported in Ververis et al[10]. In addition, the satisfactory level of α -cellulose in a fibre together with high tensile strength may produce composites with high specific properties due to their low densities. The urea treatment retains cellulose and removes hemi-cellulose from the kenaf fibres. The values for α -cellulose content in urea treatments were far above those (40.2- 43.8%) reported by Ververis et al[10]. Tianug-2 retted in 2% urea had the highest α -cellulose content followed by Ifeken 400 of the same treatment. The α -cellulose content in this range makes kenaf a suitable raw material for the paper and pulp industry. Plant materials with α -cellulose content of 40% and above have been characterized as promising for pulp and paper production [5,11]

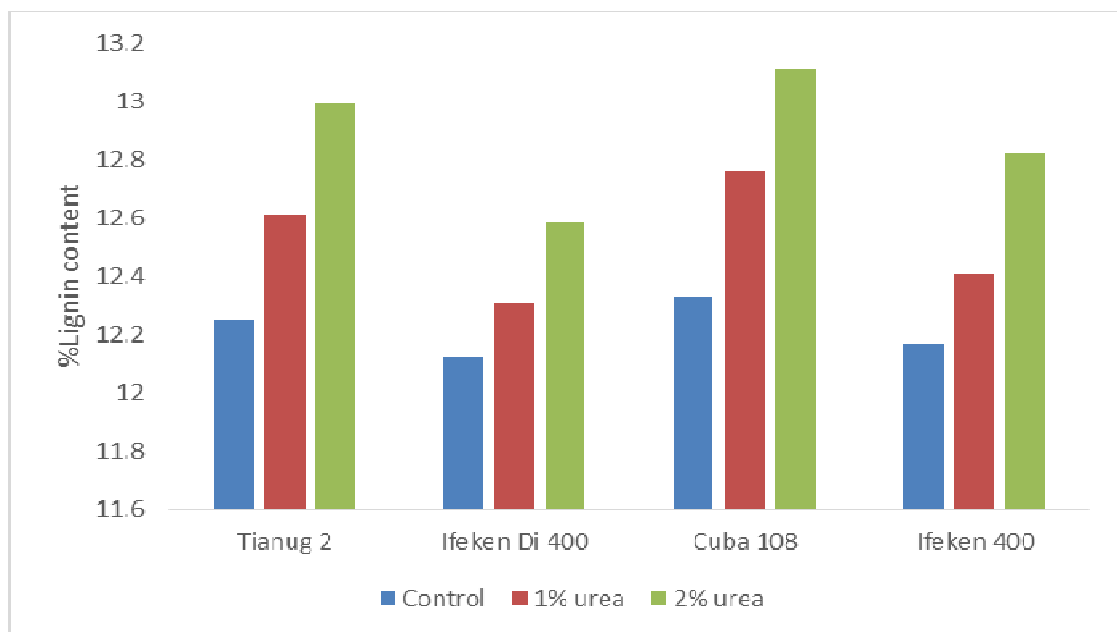


Figure 2: Percentage lignin content of retted fibre varieties.

3.2:Lignin content

Urea treatments had slightly increased the lignin content in the retted kenaf fibres when compared with control but the range of lignin in all the treatments was significantly lower than the data reported for softwoods and hardwoods [10].Cuba-108 fibre retted in 2% urea had the highest lignin content followed by Tianug-2 of the same treatment. However, this result was slightly at variance with MohdYuhazri et al [4] which stated that sodium hydroxide (NaOH) solution was effective to remove the impurities of the fibre surface. But increasing the soaking time in NaOH showed the damage of fibre surface.

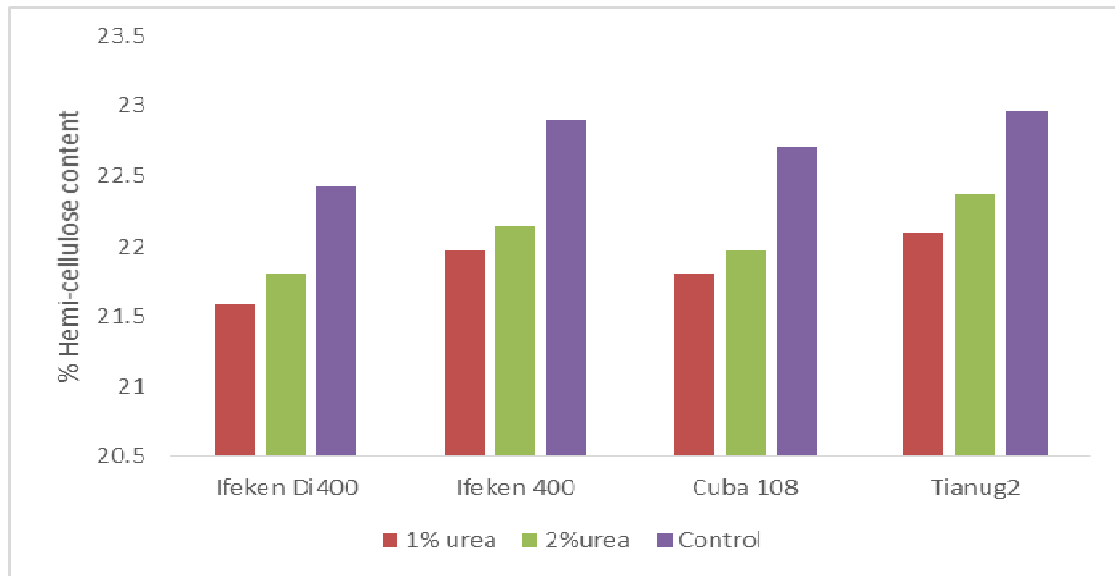


Figure 3: Hemi-cellulose content of retted fibre varieties.

3.3:Hemi-cellulose content

The highest level of hemi-cellulose was recorded in control followed 2% urea while 1% urea fibres had the least. This result implied that a significant reduction of hemi-cellulose in kenaffibres retted from 1% urea as shown in Figure 3. This finding was in consonance with the reported data by Cao et al [20] that alkali treatment caused the elimination of hemi-cellulose and some impurities. Ifeken DI-400 fibre retted in 1% urea had the least hemi-cellulose content. The reduced amount of hemicellulose will minimize the undesirable water absorption capacity of natural fibre when used for reinforced composites [11]. The findings of this study strongly agreed with El-Shekil et al [19] that alkali treatment of natural fibres has several advantages for the removal of undesired substances and this also affects the properties of the fibres and fibre-matrix interlocking

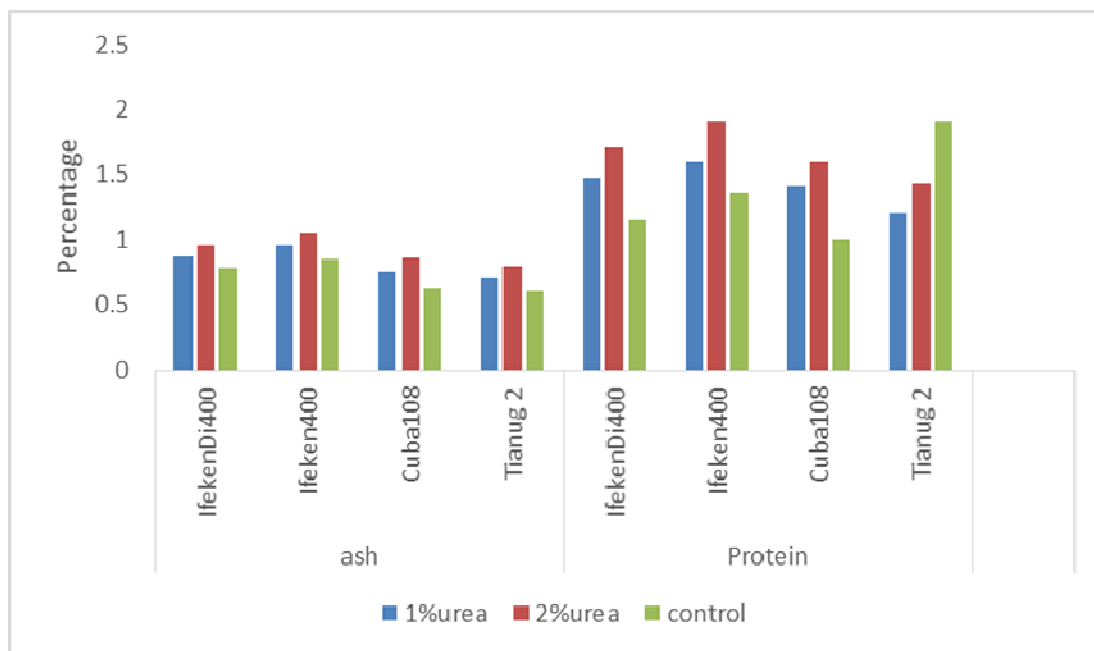


Figure 4: Percentage ash and protein content of retted fibres.

3.4:Proximate composition of the retted fibres

The ash contents of all the treatments was generally low, this quality is good because higher ash content is undesirable and it can affect the pulp mechanical properties. 2% urea retted fibres had the highest ash content (0.8- 1.05%) followed by 1% urea (0.71-0.96%) and least in control (0.61-0.85%), this may be due to the presence of some metallic salts like iron, calcium, etc. (figure 4). Ifeken 400 fibre retted in 2% urea had the highest (1.05%) ash content while Tianug-2 fibre of control had the least (0.61%).The values recorded in this work were significantly lower than 3.6-4.1% ash content of the part of kenaf stalk reported by Ververis et al[10].Urea treatments had slightly increased the percentage protein content of all the fibre varieties except the Tianug-2 fibre with 1.91% protein for control, 1.43% protein for 2%urea and 1.2% protein for 1%urea.This was in agreement the report by Neto et al[11-12].The protein content of the kenaf fibres was highest in 2% urea followed by 1% urea and least in control (figure 4).

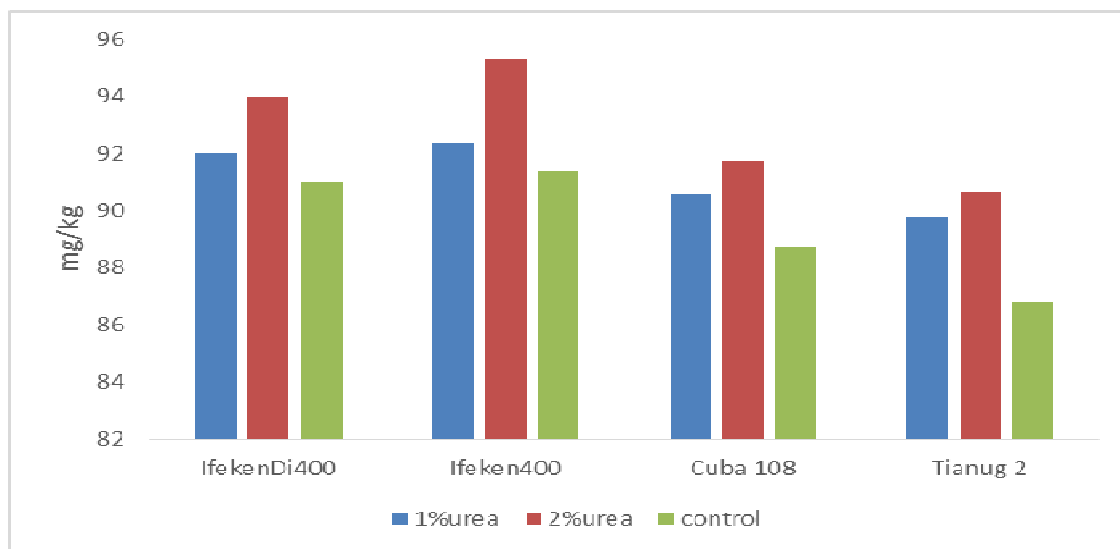


Figure 5: Iron content of retted fibres.

3.5:Mineral contents of retted fibres

The presence of inorganic substance like iron, carbonates, calcium, and potassium had significant effect on the ash content of kenaf fibres. Urea treatments had significantly increased the level of iron in all the varieties of kenaf fibres. Ifeken 400 fibre retted in 2% urea had the highest (95.35mg/kg) iron content followed by Ifeken Di-400 (94.00mg/kg) of 2% urea and least in Tianug-2 (86.80mg/kg) of control as shown in figure 5.

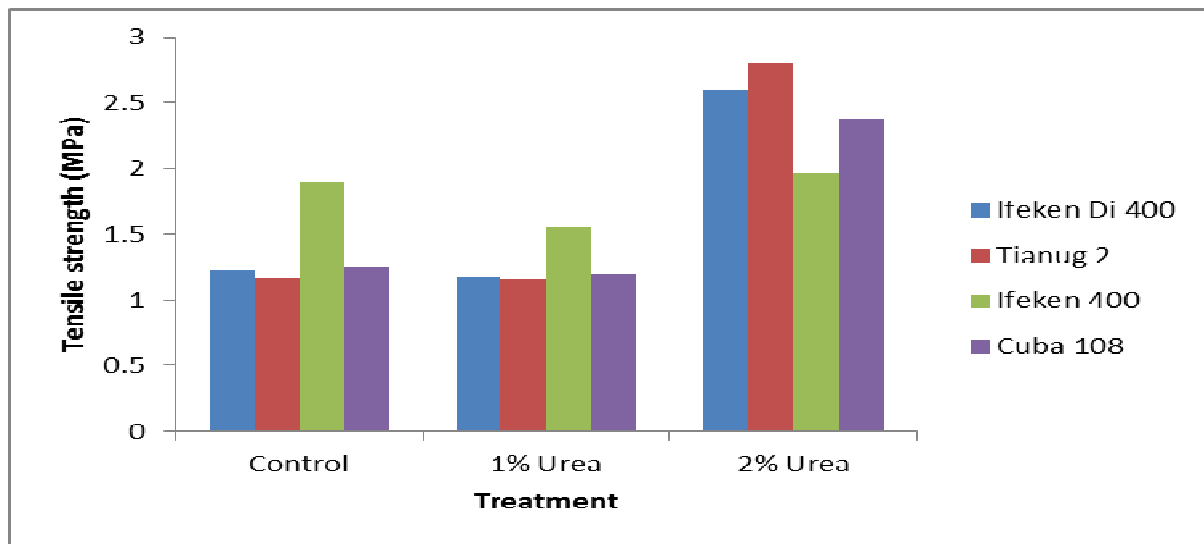


Figure 6: Tensile strength of the retted fibre varieties.

3.6: Tensile strength of retted fibres

Figure 6 showed that the tensile strength of 2% urea retted fibres was significantly higher than that of 1% urea and control for all the tested varieties of kenaf fibres. The strength and durability of kenaf composites are a function of the physical and mechanical properties of its fibres. The increased tensile strength of 2% urea retted fibres may be linked with the proportionate increase in its α -cellulose content. The indigenous variety, Ifeken 400 fibre had a remarkable tensile strengths in control and 1% urea except for 2% urea treatment where it was significantly lower than others. The data obtained in this findings strongly agreed with the report by MohdYuhazri *et al.* [4] that the tensile strength and modulus of kenaf composites increase when the concentration of sodium hydroxide solution (NaOH) was increased. In addition, the data obtained from this study in parts agreed with El-Shekeil *et al.* [19] who stated that the treatment of kenaf fibers with NaOH followed by PMDI had a significant effect on the composite, which was evident in the 30% increase in the tensile strength and approximately 42% increase in the tensile modulus. Moreover, this result followed the same trend with the data reported by Cao *et al.* [20] on the effect of heat and alkali treatment on the mechanical properties of kenaf fibre. Also, this study supported the publication by Cao *et al.* [21] that 10 and 15% NaOH solution, the tensile strength decreased, Young's modulus changed slightly and fracture strain increased drastically, which were two to three times higher than that of the untreated kenaf fibre.

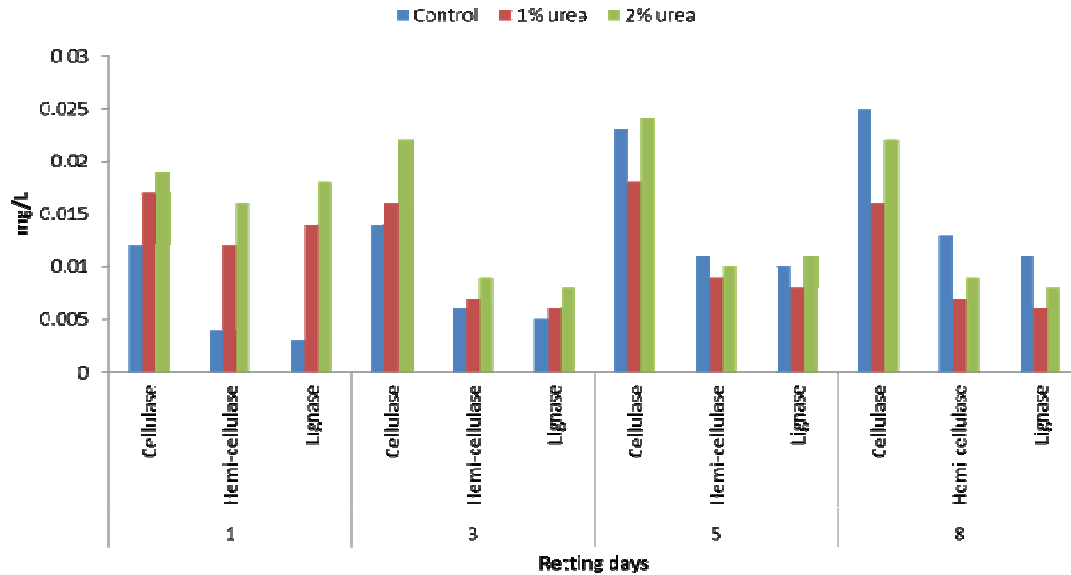


Figure 7: Enzyme activities at different retting days.

3.7: Enzyme activities in the liquor during fibre retting

There was partial removal of non-fibrous materials like hemi-cellulose and lignin which would make kenaf fibre considerably softer for **carding or spinning**. Lignase and hemi-cellulase enzymes allowed the selective removal of lignin and hemi-cellulose respectively without affecting the strength of the cellulosic fibre itself. The cellulase enzyme was significantly more active in 2% urea at day 1, 3 and 5 compared with 1% urea and control. The activities of the lignase and hemi-cellulase enzymes during retting were significantly higher in urea treatments than control for the first five days after which there was a decline as shown in figure 7. However, this result strongly agreed with the publication report by Biswapriya *et al.* [22] which stated that the action of cellulase enzymes during fibre retting does not show any damage on the tenacity of the fibres.

Table 1: The pH of retting water (liquor) at different days.

Trt	Day 1	Day 3	Day 5	Day 8
Control	6.80a	6.65a	6.50a	6.50a
1% urea	4.80b	4.3b	4.53b	4.05b
2% urea	4.51c	4.43c	4.25c	3.80c

Means with the same letter in a column are not significantly different at $P < 0.05$,

Trt = treatment.

3.8: Physico-chemical parameters of water before, during and after retting

Acidity /alkalinity of the retting media

In all the treatments, the pre-retting pH of the liquor (6.80 for control, 4.80 for 1% urea and 4.51 for 2% urea) was significantly higher than those recorded during and after retting (from 4.8 - 4.05 for 1% urea; 4.51- 3.80 for 2% urea and 6.80 - 6.50 for control). The decrease in pH value led to more acidity of retting water (table 1).The pH values of all the urea treated liquors were significantly lower than that of control.

The lowering of pH values during retting may be due to the release of organic acids and other gummy materials into the retting liquors.

Table 2: The total hardness of retting water at different days (mg/L).

Trt	Day 1	Day 3	Day 5	Day 8
Control	36.65c	36.50c	35.50c	35.25c
1% urea	38.41b	43.65b	44.65b	46.55b
2% urea	45.60a	45.60a	46.80a	47.75a

Means with the same letter in a column are not significantly different at $P < 0.05$

3.9: The degree of hardness (carbonate salts) of the retting water

Total hardness of the retting water increased significantly in all the urea treatments (38.41-46.65mg/L for 1% urea and 45.60 - 47.75mg/L for 2% urea) with the corresponding increase in retting period. Conversely, control had a decreasing trend of total hardness with the increase in retting time. Hardness is caused by the bicarbonate salts of calcium and magnesium.

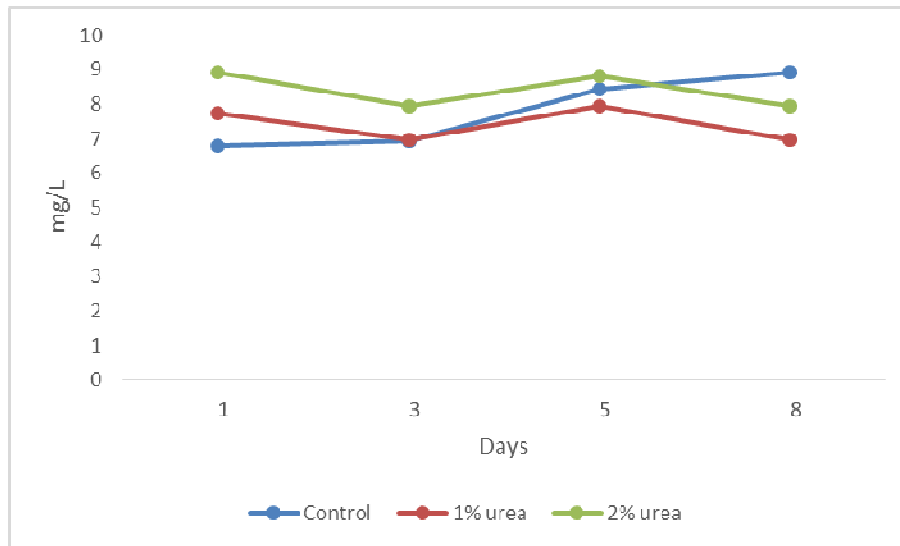


Figure 8a: Dissolved oxygen (DO) at different retting days.

3.9.2: Degradation of water retting

The level of water deterioration was revealed by the amount of dissolved oxygen (DO) in each liquor.

The liquor from 1% and 2% urea had significantly higher DO than control at day 1, 3 and 5 but at day 8, control had higher DO than the treated liquors (figure 8a). Biochemical oxygen demand of the treated liquors were significantly higher than that of control at day 1,3 and 5(figure 8b). A high BOD indicates a high content of easily degradable, organic material in the liquor, while a low BOD indicates a low volume of organic materials, substances which are difficult to break down

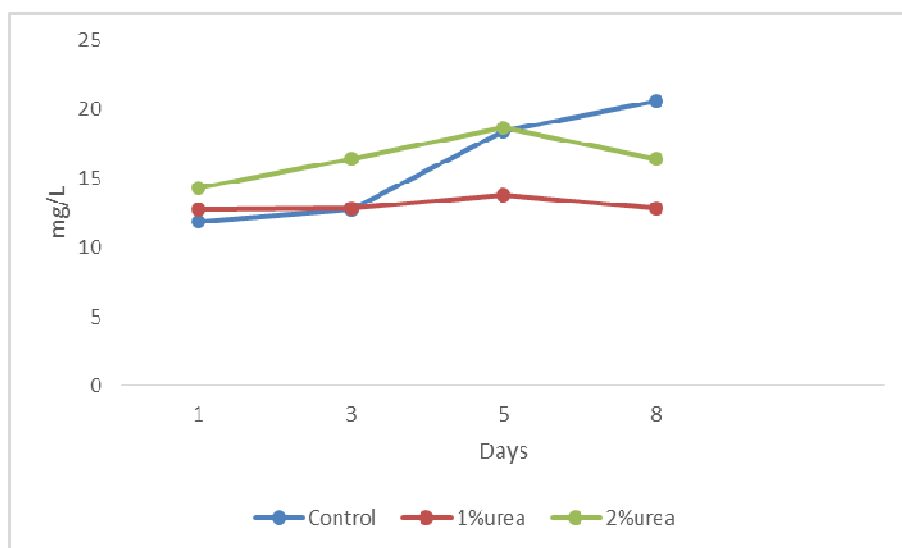


Figure 8b: Biochemical oxygen demand at different retting days

The chemical oxygen demand (COD) of the pre-retting liquors were 37.95mg/L for 1% urea, 41.33mg/L for 2% urea and 32.62mg/L for control. These values increased progressively with the retting in all the treatments (figure 8c). Urea treatments had higher amount of COD than control at the first three days of retting. There was rapid increase in COD of control at day 5 and 8. Chemical oxygen demand (COD) shows the oxygen equivalent of the organic matter content of each liquor that is prone to oxidation which can lead to water deterioration.

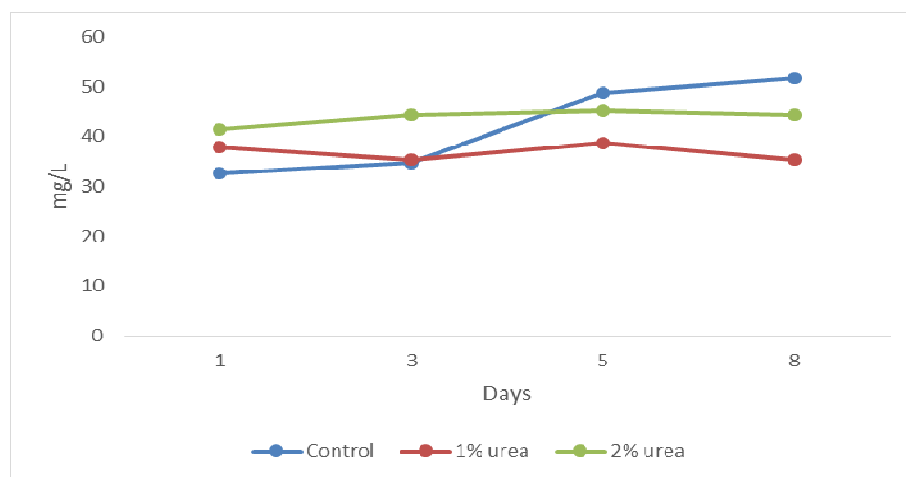


Figure 8c: Chemical oxygen demand (COD) at different retting days.

Table 3: Microbial counts at different interval (Cfuml¹).

Day 1	Control	1% urea	2% urea
Total bacteria count	3.1x10^{6c}	3.85x10^{6b}	4.25x10^{6a}
Total coliform count	0.3x10^{6c}	0.55x10^{6b}	0.8x10^{6a}
<i>E.coli</i>	0.2x10^{6b}	0.4x10^{6a}	0.4x10^{6a}
<i>S. aureus</i> count	0.45x10^{6c}	0.6x10^{6b}	1.2x10^{6a}
Total fungi count	0.2x10^{6b}	0.2x10^{6b}	0.25x10^{6a}
Day 3			
Total bacteria count	3.55x10^{6c}	4.35x10^{6b}	4.6x10^{6a}
Total coliform count	0.5x10^{6c}	0.7x10^{6b}	1.05x10^{6a}
<i>E.coli</i>	0.35x10^{6c}	0.5x10^{6b}	0.65 x 10^{6a}
<i>S. aureus</i>	0.65x10^{6c}	0.75x10^{6b}	1.6x10^{6a}
Total fungi count	0.35x10^{6c}	0.4x10^{6b}	0.5x10^{6a}
Day 5			
Total bacteria count	3.75x10^{6c}	5.0x10^{6b}	5.1x10^{6a}
Total coliform	0.7x10^{6c}	0.95x10^{6b}	1.0x10^{6a}
<i>E. coli</i>	0.5x10^{6c}	0.7x10^{6b}	0.75x10^{6a}
<i>S. aureus</i>	0.8x10^{6c}	1.0x10^{6b}	1.15x10^{6a}
Total fungi count	0.6x10^{6c}	0.7x10^{6b}	0.8x10^{6a}
Day 8			
Total bacteria	3.55x10^{6c}	4.6x10^{6b}	6.65x10^{6a}
Total coliform	0.5x10^{6c}	0.7x10^{6b}	1.7x10^{6a}
<i>E. coli</i>	0.35x10^{6c}	0.5x10^{6b}	1.1x10^{6a}
<i>S. aureus</i>	0.65x10^{6c}	1.35x10^{6a}	

			0.75x10 ^{6b}	
Total	fungi	0.4x10 ^{6c}		
count			0.5x10 ^{6b}	0.95x10 ^{6a}

Means with the same superscripts in a row are not significantly different (P< 0.05).Cfuml⁻¹ represents colony forming unit per millilitre.

3.9.2:Microbial activities

The activities of some microbes during retting were on the increase and their presence had hasten the retting process thereby reducing its duration. The result in table 3 showed that the highest microbial load was recorded at day 5.This range of values were not significantly different from those obtained at day 8 for both urea treatments. It implies therefore that 1% and 2% urea treatments induced the similar microbial load. It is pertinent to note that pathogenic microbes (*Aspergillus* spp, *Staphylococcus* spp and *Escherichia* spp) emanated during retting and this calls for high level of environmental sanitation and proper sewage (effluent) disposal or management. In line with Bello *et al* [13], washing of Kenaf fibre with clean water of potable quality could reduce the microbial load. Proper sensitization on the involvement of pathogenic bacteria in Kenaf retting should be carried out and adequate precaution must be taken while carrying out this farm-gate processing to prevent spread of the pathogenic micro-organisms to Kenaf farmers.

4.0: Conclusion

The synergistic interaction of enzymes in urea solutions had produced fibres with promising chemical and mechanical properties. Kenaf fibres retted in 2% urea had better tensile strength and higher α -cellulose content.Urea retting lasted for seven days irrespective of the Kenaf variety whilecontrol lasted for fourteen days. It can also be inferred that urea treatments produced fibres with satisfactory levels (< 30%) of lignin and (> 40%) of alpha-cellulose content.

Competing interest

This study is free from all forms of conflict of interest.

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Authors'contributions:

The first author (Omenna E.C) designed the experiment, supervised the retting exercise, biochemical and physico-mechanical analyses, while the second author(Oduwaye,O.F) carried out the microbiological analysis.

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