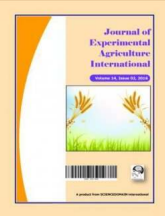

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Effect of Variation in Urea Concentration Used in Retting, on the Chemical and Mechanical Properties of Kenaf Fibres

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Authors' contributions

This work was carried out in collaboration between both authors. Author ECO designed the experiment, supervised the retting exercise, biochemical and physico-mechanical analyses while author OFO carried out the microbiological analysis. Both authors read and approved the final manuscript.

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Method Article

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ABSTRACT

The overall aims of this study were to determine:

i. The effect of 1% and 2% urea solution on the α-cellulose, lignin and hemi cellulose 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.

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* =.05.

Place and Duration: The retting experiment and laboratory analyses were carried out at the Institute of Agricultural Research and Training, Obafemi Awolowo University Ibadan, Nigeria. From April, 2015 to January, 2016.

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Methodology: Two Kenaf stems were tied together as a replicate (with three replicates per treatment) and were soaked in improvised retting tanks containing 1% and 2% urea in 150litres of water and control (without urea). Fibres retted in 1%, 2% urea and control were subjected to proximate, chemical and mechanical assays. Microbiological and physico-chemical properties of the retting liquors were determined at two days interval.
Results: The urea treatment increased α-cellulose in kenaf fibres while the lignin content of Kenaf fibres was in the order: 2% urea > 1% urea > control. Two local varieties Ifeken DI 400 and Ifeken 400 recorded the least lignin. Hemi-cellulose content of Kenaf fibres 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 the control.
Conclusion: High enzyme activities in urea liquors ease the retting process and this had produced fibres with promising chemical and mechanical properties. Kenaf fibres retted in 2% **u**rea had better tensile strength and higher α-cellulose content.

Keywords: Alpha-cellulose; hemi-cellulose; Kenaf fibre; lignin; tensile strength.

1. INTRODUCTION

Presently, natural fibres form an interesting option for the most composite technology, pulp, paper as well as other ligno-cellulose conversion industries [1,2]. Traditionally, harvested kenaf stems were usually immersed in natural water (streams, rivers, and ponds) in which indigenous bacteria attack the gum in an anaerobic process yielding much pollution and low quality of fibre [3]. However, this practice is environmentally unfriendly as it deteriorates the water body and predisposes the users to pathogenic micro-organisms. There are various types of retting methods namely: water, biological, microbe, ribbon, dew, chemical, enzymatic, and microbe-chemical, yet researchers have undertaken different studies to seek for pollution-free retting methods with faster rate [3-5]. In view of this, recent research had suggested that chemical retting using mild reagent like sodium hydroxide, potassium hydroxide and /or urea is a good substitute for whole stalk stream retting [4].

Globally, the World paper consumption has geometrically increased, although Hurter and Riccio [6] reported that the World paper consumption was 300 million tons in 1996 and was expected to have risen 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 [6]. 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 [7]. 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 and economic downturn. In the light of this, the Federal government of Nigeria has renewed her interest in kenaf as a potential industrial crop with multipurpose uses.

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 [8]. 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 [8,9]. Updegraff et al. [10] has categorically stated the significance of fibre dimensions in predicting wood pulp mechanical properties. Saikia et al. [11] and Ogbonnaya et al. [12] reported that the derived values like fibre length/fibre diameter, flexibility coefficient as well as Runkel ratio as (2 ×fibre cell wall thickness)/lumen diameter of softwoods and hardwoods were compared to assess the suitability of the plant raw materials for paper. Aderonke et al. [13] 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 [4]. But there is 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 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 hemi cellulose 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. 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 from the four varieties were tied together as a replicate (with three replicates for each treatment) and soaked in improvised plastic tanks containing 1% and 2% urea in 150 litres of water and the control (without urea). Textures of the fibres were checked daily with hands to determine when the fibres were completely retting. Each retted fibre was extracted, washed thoroughly with water and sundried. And it was subsequently subjected to other assays.

2.2 Measurement Procedure

The fibre samples were analysed for α-cellulose, hemi-cellulose and acid insoluble lignin. The α-cellulose was determined using a colorimetric method with the anthrone reagent. 0.3 g (dry weight) ground (0.5 mm) samples were treated and boiled (at 100℃) with a mixture of nitric/acetic acid (1:8, v/v) for 1 h as to remove lignin and hemicelluloses after successive centrifugations, and diluted with 67% H_2SO_4 (v/v). The α- Cellulose was then determined at 620nm using cold anthrone reagent [14]. Protein, ash and iron content of the fibres were determined according to the procedure described by AOAC [15].

2.2.1 Mechanical properties of the fibres

Mechanical testing of the fibres was carried out using an Instron fibre testing machine (Model 3369, Norwood NJ, USA). Tensile test was performed in an ambient temperature and then quasi-statically stressed at a constant cross-head displacement of 2 mm/min. The fibre strands response in terms of force and displacement was recorded automatically and then converted into stress versus strain. The ultimate tensile strength and the Young's modulus are then determined from the stress-strain curves. This procedure was in accordance with the international standard prescribed by the ASTMD [16].

2.2.2 Organic tests

The properties of retting liquor were assessed at the interval of two days. 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 [17]. A DO meter was used to measure the initial dissolved oxygen concentration (mg/L) in each bottle. For BOD determination, each bottle is then placed into a dark incubator at 20℃ for five days. After five days (± 3 hours) the DO meter is used again to measure a final dissolved oxygen concentration in mg/L [17,18]. The difference between the initial DO and final DO is the BOD concentration in mg/L. The COD is determined using a colorimeter. The colorimeter was set and calibrated per the specific instructions for that unit (i.e., proper wavelength, blank and standards) and each vial was placed in the unit and the COD concentration was read [18].

2.2.3 Microbiological procedure

The powdered nutrient agar of 28 g was dissolved in 1 litre of deionized water, allowed to

soak for 10 minutes and then sterilized by autoclaving for 15 minutes at 121℃. It was thereafter cooled and then poured into petri dishes. Potato dextrose agar of 39 g was also dissolved in 1 litre of distilled water and boiled to dissolve the medium completely before sterilizing with autoclave at 121℃ for 15 minutes. The pH of the liquor samples was adjusted to 3.5, after adding 10 ml of lactic acid solution, to facilitate microbial growth. The medium was thereafter cooled to 55℃ and poured into petri dishes. 2 ml 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℃ and 25℃ 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 bacteria isolates, while Lactophenol blue staining was carried out on fungi isolates [15].

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*=.05.

3. RESULTS AND DISCUSSION

3.1 Alpha –cellulose Content

The α-cellulose content of the kenaf fibres from the four different varieties ranged from 59.91-60.410% for Control; 60.38 - 61.30% for 1% urea and 61.58 - 62.17% for 2% urea. Urea treatment significantly improved the α-cellulose content of the fibres across the four kenaf varieties as shown in Fig. 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. 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. [7]. 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 [9,19].

3.2 Lignin Content

The effect of the urea treatments had 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 [7]. 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 Mohd Yuhazri et al. [8] 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 [20].

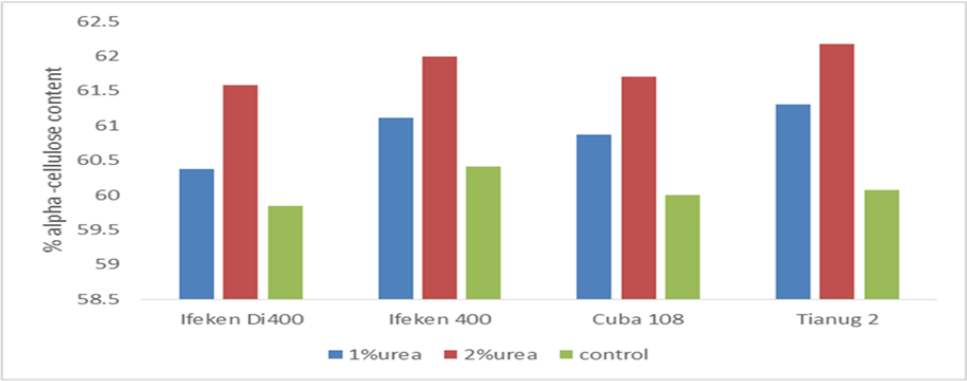

Fig. 1. Alpha-cellulose content of retted fibre varieties

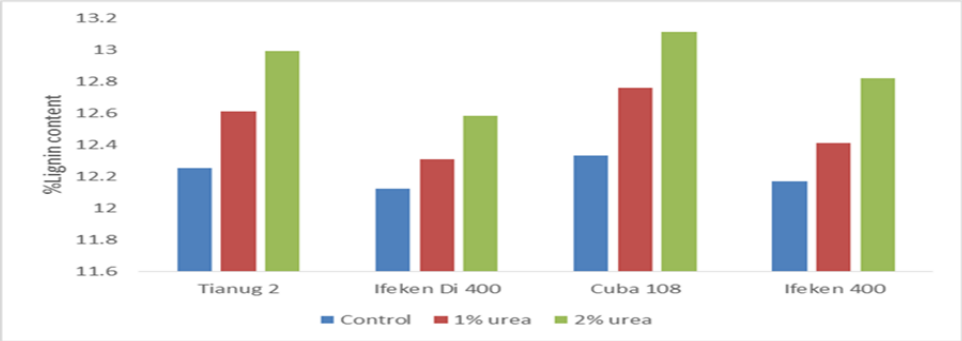

Fig. 2. Percentage lignin content of retted fibre varieties

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 kenaf fibres retted from 1% urea as shown in Fig. 3. This finding was in consonance with the reported data by Cao et al. [21] 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 [19]. The findings of this study strongly agreed with El-Shekil et al. [20] 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.

3.4 Proximate Composition of the Retted Fibres

The level of ash composition in the four varieties of kenaf fibres across 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. 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. [7]. 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 with the report by Neto et al. [22]. The protein content of the kenaf fibres was highest in 2% urea followed by 1% urea and least in control as shown in Fig. 4.

3.5 Mineral Contents of Retted Fibres

The mineral compositions of retted kenaf fibres were affected by the treatment applied. 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 (95.35 mg/kg) iron content followed by Ifeken Di-400 (94.00 mg/kg) of 2% urea and least in Tianug-2 (86.80 mg/kg) of control as shown in Fig. 5. The presence of inorganic substance like iron, carbonates, calcium, and potassium had significant effect on the ash content of kenaf fibres [17].

3.6 Tensile Strength of Retted Fibres

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 [8]. 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 Mohd Yuhazri et al. [8] which stated 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. [20] 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. [21] 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. [23] which stated that 10 and 15% NaOH solution, the tensile strength decreased, Young's modulus slightly changed and fracture strain increased drastically, which were two to three times higher than that of the untreated kenaf fibre.

3.7 Enzyme Activities in the Liquor during Fibre Retting

The observation of this study was in line with the literature which stated that the enzymes activities partially degrade the non-fibrous materials like hemi-cellulose and lignin which would make kenaf fibre considerably softer for spinning [14]. Lignase and hemi-cellulase enzymes allowed the selective removal of lignin and hemi-cellulose respectively without affecting the strength of the cellulosic fibre itself [24]. The cellulase enzyme was significantly more active in 2% urea at day 1, 3 and 5 compared with 1% urea and control. Th e 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 Fig. 7. However, this result strongly agreed with the publication report by Biswapriya et al. [25] which stated that the action of cellulase enzymes during fibre retting does not show any damage on the tenacity of the fibres. Also Marek et al. [26] stated that pectinase, xylanase and cellulase are the main enzymes acting in retting process. Marek et al. [26] added that combination of enzymes with different substrate specific action would broaden its application such as the use of pectinases in textile industry for retting and degumming fibre crops.

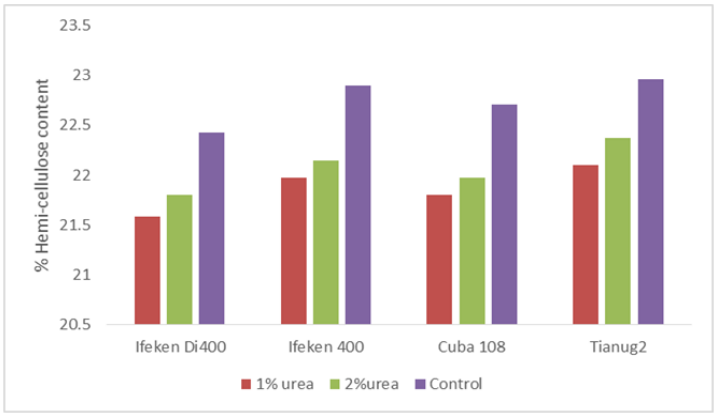

Fig. 3. Hemi-cellulose content of retted fibre varieties

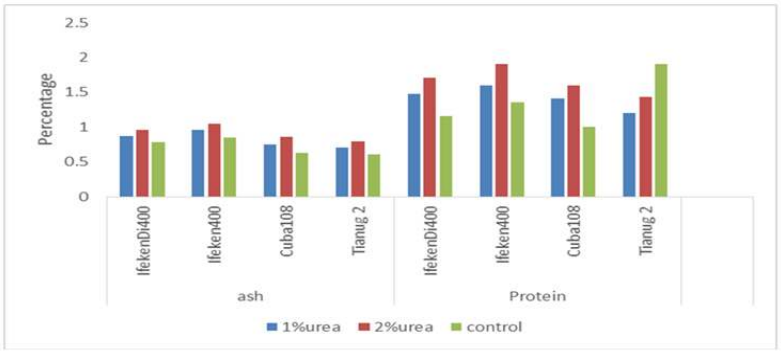

Fig. 4. Percentage ash and protein content of retted fibres

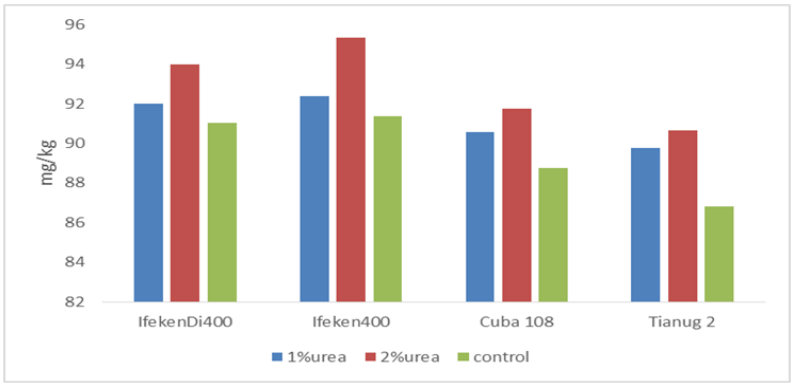

Fig. 5. Iron content of retted fibres

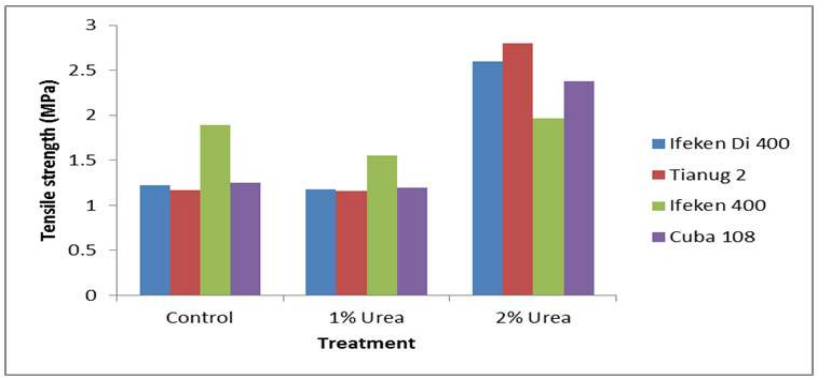

Fig. 6. Tensile strength of the retted fibre varieties

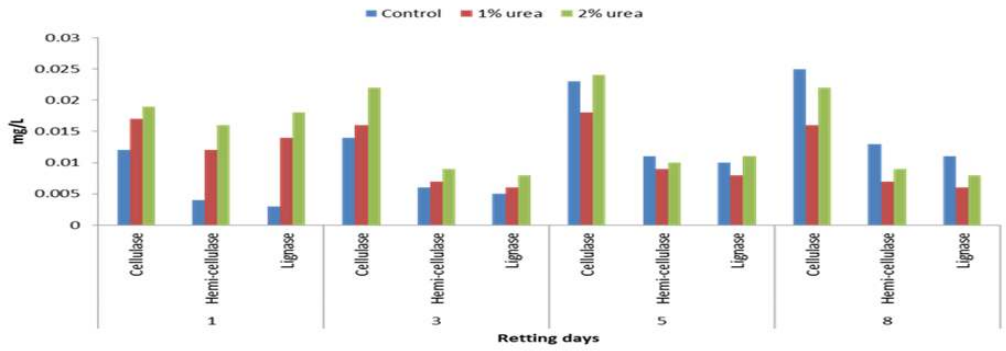

Fig. 7. Enzyme activities at different retting days

3.8 Physico-chemical Parameters of Water Before, during and After Retting

The measure of acidity or alkalinity of the retting media reflected the concentration of hydrogen ion [H^+] or hydroxide ion [OH^-] of the effluents. 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 [27].

3.9 The Degree of Hardness (Carbonate Salts) of the Retting Water

The total hardness of the retting water increased significantly in all the urea treatments (38.41-46.65 mg/L for 1% urea and 45.60 - 47.75 mg/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 [18]. Dhanalaxmi and Jyoti [3] reported that Ca and Mg cations bind pectin to kenaf stalk.

3.9.1 Degradation of water retting

The level of water deterioration was revealed by the amount of its physico–chemical parameters such as dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) 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 as presented in Fig. 8a. Biochemical oxygen demand of the treated liquors were significantly higher than that of control at day 1,3 and 5 as shown in Fig. 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 [17]. The chemical oxygen demand (COD) of the pre-retting liquors were 37.95 mg/L for 1% urea, 41.33 mg/L for 2% urea and 32.62 mg/L for control. These values increased progressively with the retting in all the treatments as shown in Fig. 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 [18].

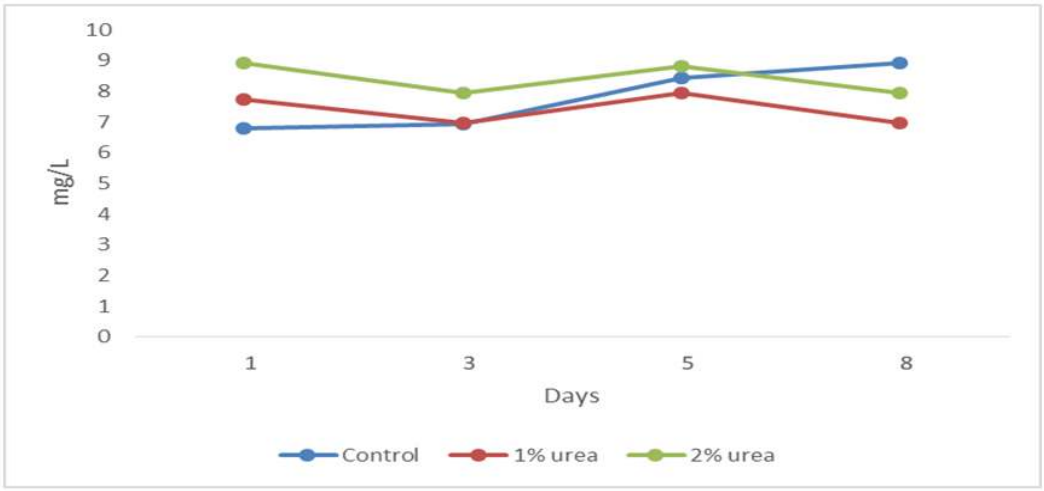

Fig. 8a. Dissolved oxygen (DO) at different retting days

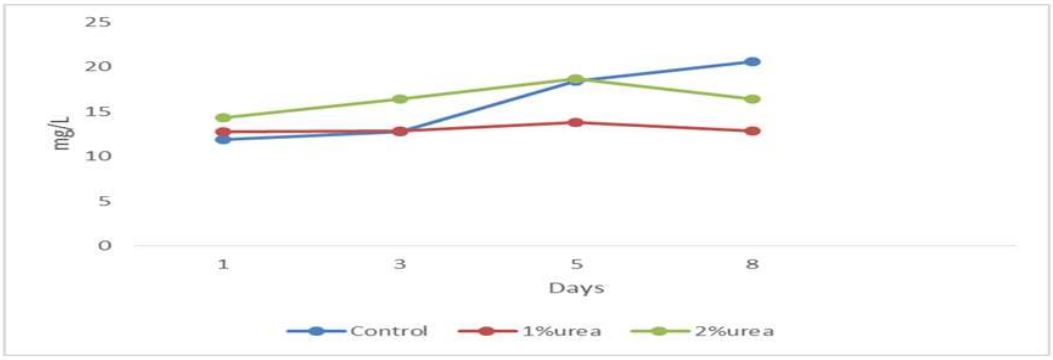

Fig. 8b. Biochemical oxygen demand at different retting days

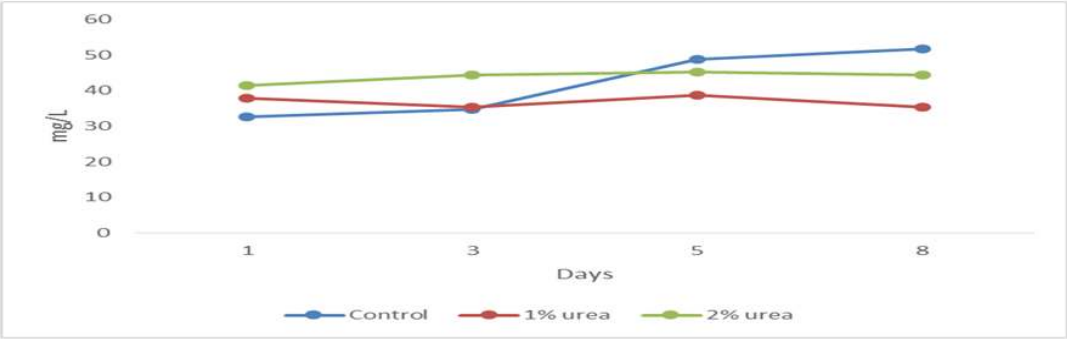

Fig. 8c. Chemical oxygen demand (COD) at different retting days

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 =.05, Trt = treatment

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=.05

Table 3. Microbial counts at different interval ($Cfuml^{-1}$)

Day 1	Control	1% urea	2% urea
Total bacteria count	$3.1x10^{6c}$	$3.85x10^{6b}$	$4.25x10^{6a}$
Total coliform count	$0.3x10^{6c}$	$0.55x10^{6b}$	$0.8x10^{6a}$
E. coli	$0.2x10^{6b}$	$0.4x10^{6a}$	$0.4x10^{6a}$
S. aureus 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	$0.5x10^{6c}$	$0.7x10^{6b}$	$1.05x10^{6a}$
E. coli	$0.35x10^{6c}$	$0.5x10^{6b}$	0.65×10^{6a}
S. aureus	$0.65x10^{6c}$	$0.75x10^{6b}$	$1.6x10^{6a}$
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	$0.7x10^{6c}$	$0.95x10^{6b}$	$1.0x10^{6a}$
E. coli	$0.5x10^{6c}$	$0.7x10^{6b}$	$0.75x10^{6a}$
S. aureus	$0.8x10^{6c}$	$1.0x10^{6b}$	$1.15x10^{6a}$
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	$0.5x10^{6c}$	$0.7x10^{6b}$	$1.7x10^{6a}$
E. coli	$0.35x10^{6c}$	$0.5x10^{6b}$	$1.1x10^{6a}$
S. aureus	$0.65x10^{6c}$	$0.75x10^{6b}$	$1.35x10^{6a}$
Total fungi count	$0.4x10^{6c}$	$0.5x10^{6b}$	$0.95x10^{6a}$

Means with the same superscripts in a row are not significantly different (P =.05). $Cfuml^{-1}$ represents colony forming unit per millilitre

3.9.2 Microbial activities

The result of the microbial load for the retting days and the activities of these microbes during retting were on the increase and their presence had hasten the retting process thereby reducing its duration [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 same level of microbial load. This finding strongly supports the publication report by Dhanalaxmi and Jyoti [5] which stated that the microbial growth help in loosening the fibre at faster rate. From the report by Dhanalaxmi and Jyoti [5] which stated that urea aids the growth of bacteria in soil and water, It is therefore, pertinent to note that pathogenic microbes (*Aspergillus* spp*, Staphylococcus* spp *and Escherichia* spp) emanated during the kenaf retting process and this calls for high level of environmental sanitation and proper sewage (effluent) disposal or management. In line with Bello et al. [28], 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. CONCLUSION

There was high enzyme activities in urea liquors and this had produced fibres with promising chemical and mechanical properties. Kenaf fibres retted in 2% urea had better tensile strength and higher α-cellulose content. 1 and 2% urea treatments were comparably higher in microbial load. Urea retted fibres had satisfactory levels (< 30%) of lignin and (> 40%) of alpha-cellulose content.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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