



ASHESI UNIVERSITY

DEVELOPING A MICROPATTERNED SURFACE TO PREVENT BACTERIAL ADHESION

CAPSTONE PROJECT

B.Sc. Mechanical Engineering

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2021

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BACTERIAL ADHESION**

CAPSTONE PROJECT

Capstone Project submitted to the Department of Engineering, Ashesi
University in partial fulfilment of the requirements for the award of Bachelor
of Science
degree in Mechanical Engineering.

Oluwatobiloba Omole

2021

DECLARATION

I hereby declare that this capstone is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: Omole

.....

Candidate's Name:

.....

Date:

I hereby declare that preparation and presentation of this capstone were supervised in accordance with the guidelines on supervision of capstone laid down by Ashesi University.

Supervisor's Signature:

.....

Supervisor's Name:

.....

Date:

Acknowledgements

Firstly, I would like to thank God for the grace and protection to complete this capstone in a global pandemic. Secondly, I would like to thank my supervisor, Dr. Elena Rosca, for her dedication and time in providing me with relevant pointers and suggestions that ultimately helped to steer the course of the capstone. Furthermore, I appreciate the guidance of other Ashesi engineering lecturers on their input on how to improve this project and possible flaws that should be avoided.

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Finally, I would like to thank my parents for their general support thus far.

Abstract

Hospital Acquired Infections (HAIs), also commonly referred to as Nosocomial infections, present a significant problem to hospitals around the world. The problem is more pronounced in African hospitals due to the lack of thorough and efficient sanitation and hygiene practices. They present a substantial danger to infants in neo-natal care who do not have well developed immune systems. Hospital acquired infections also pose a significant economic and social problem in urban areas. Although a wide range of pathogens can cause nosocomial infections, bacteria is responsible for a larger number of infections. With this in mind, a safe, effective and cost-effective solution is essential to minimize the occurrence of nosocomial infections. The strategy employed will be to try to reduce bacterial colonies in hospitals to prevent infection by bacteria. Natural fibres have been documented to exhibit anti-microbial action and they are also very cheap. The creation of a mat using such fibres that can be used in hospitals to reduce bacterial growth will form the basis of the solution.

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1. Chapter 1: Introduction

1.1. Introduction

Hospital associated infections are infections contracted by patients or health personnel within the hospital. These infections account for 7% of total infections in developed countries and 10% in developing countries [1]. Common pathogens of these types of infections are bacteria, viruses and fungi. In a study conducted in ten Ghanaian government hospitals, there was an overall hospital acquired infection (HAI) rate of 8.2% [2]. The prevalence ranged from 3.5% to 14.4% in the individual hospitals [2]. In countries such as Nigeria and Ethiopia, the prevalence of HAIs in hospital wards varied between 5.7%-45.8% with Ethiopia having an incident density of '26. 7 infections per 1000 patient days' [3]. Although reliable data is scarce in developing countries, this study gives a good benchmark for the (HAI) prevalence. HAIs can prove to be fatal and adversely affect an individual's supposed recovery. The mortality rates of HAI's are estimated to be over 10% in developing countries [3]. These infections have an economic and social cost as they increase the patient's hospital stay increasing medical expenses for the individual and hospital while also rendering the individual unproductive. Prolonged hospital stays also take away spaces for individuals.

Nosocomial infections are dependent on the size of the microbe, the route of transmission and well as the present state of the patient's immune system [3]. Microorganisms can enter the skin through wounds, moist mucosal surfaces like respiratory and gastrointestinal tract [3]. Transmission could occur from contact with droplets, food, medication and hospital equipment [3]. Patients with compromised immunity such as those with medical devices imbued in them such as catheters and respirators are at increased risk [3]. Nosocomial infections include a myriad of bacteria with common examples such as

Streptococcus agalactiae, Klebsiella spp., Candida spp., clostridium tetani and Esheria coli.

Preventing nosocomial infections from occurring is a difficult task as it involves several key considerations. Protecting possible infection sites and interrupting transmission routes are some of the best ways to do it [3]. However, it is only possible to achieve this if the entire management of a health care facility plays a role [3]. Practices such as hand washing, use of protective wear, aseptic practices in operating theatres, quarantine on highly contagious patients and the use of antiseptics will drastically curb accumulation of pathogens and reduce infection risk [3]. Other methods like the proper sanitation of food and water sources and the proper disposal of hospital waste including blood and sputum from the lab samples as they are considered a pathogen reservoir [1].

1.2. Problem Statement:

One particular area in healthcare in which HIA becomes of particular importance is neonatal care. Subjects in neonatal care have underdeveloped immune systems and are at a significant risk of contracting bacterial infections easily. A study in southern Brazil showed the prevalence of nosocomial infections in neonatal care was as high as 45.8% [4]. This comes with a corresponding mortality of 33.8% [4]. The most commonly identified agent in blood cultures of these infants was in fact *Staphylococcus* which is a common bacterium [4]. Therefore, there is a need to innovate and try and to identify better ways of reducing bacterial infections amongst infants.

1.3. Conceptual Framework

This project will harness the relationship between surface roughness and bacterial adhesion to develop a micropatterned surface that curbs bacterial growth [5]. Natural fibers with antimicrobial properties are materials that will be used as they have a low cost and are readily available materials. to create a mat like structure to be applied on a range of surfaces like tables, beds and incubators.

1.4. Project Scope

This project will investigate properties of natural fibers that will determine if they have relevant characteristics to be used as an antiadhesive surface that will prevent bacterial colonization. It will do this through experiments and data analysis of two chosen natural fibers.

2. Chapter 2: Literature review

2.1.Literature Review

Bacteria are ubiquitous and colonize almost every ecological niche on the planet [6]. They have been able to adapt, over their long evolutionary history to develop ways that, make them able to reproduce effectively on almost any surface [6]. Biological fouling, or commonly referred to as biofouling, refers to the accumulation of microorganisms on either natural or artificial surfaces [5]. These microorganisms include bacteria and pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* can accumulate on medical surfaces like implants and lead to infections [5]. The accumulation of these bacteria form biofilms. Bacterial adhesion is the first step of biofilm formation [6]. Bacteria attach to an available surface experiencing a local force normal to the surface known as the adhesive force [6]. Once attached, they proliferate with time forming an initial biofilm developing extracellular polymeric substances (EPSs) that reinforce attachment [7]. EPSs are simply the building blocks of a biofilm and include the flagella, pili and several other matrix components [6]. The initial formed biofilm will mature into a complex structure that provides protection from external factors and allows the bacteria to thrive on surfaces. The biofilms can be disrupted by physically removing them or removing dormant cells. However, the three-dimensional structure of the mature biofilm makes it well protected and thus difficult to remove. This is why it is important to stop the biofilms forming in the first place by trying to prevent the adhesion of these bacteria [7].

Currently, there are two main ways to curb bacterial adhesion onto surfaces. These include physical modification where surface topography is altered. Chemical

modifications in which the surface is coated with organisms that inhibit bacterial adhesion or even active agents that kill bacteria [7].

The relationship between surface roughness and bacterial adhesion has been extensively studied in numerous experiments. Some scholars argue that surface roughness reduced the adhesion of bacteria onto surfaces, others argued it did not reduce adhesion and other said it had no effect. Therefore, there is a lack of consensus on the issue [5]. However, microstructure surfaces of some animals (surfaces with tiny projections on them) were found to have hindered bacterial adhesion passively by virtue of their structure. The skins of these animals are patterned with microstructures that increase surface roughness and encourage anti-fouling abilities. It is believed the spacing between the structures is where the performance of their ability lies. They have wrinkled surfaces that remain free of biofouling [8].

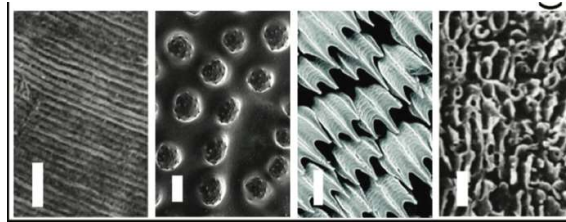


Fig 2.1. Showing the surface roughness on some naturally occurring animal skins [8,

Fig 4a.]

Another reason these surfaces have anti-fouling abilities might be their patterned natures. The influences of the shapes and heights of the microstructure patterns has an effect on bacterial adhesion. Patterned surfaces displayed a significant reduction to bacterial adhesion as compared to smoother and flat surfaces. Bacterial adhesion was inhibited in its early stages [5].

2.2.2 The Role of Natural Fibers

Fibers refer to long continuous or discrete pieces with filament hairs. Natural fibers can be extracted from either plants, animals or mineral sources. Animal fibers are extracted from animal furs and include wool and silk. Mineral fibers are obtained from minerals. Plant fibers consist of fibers that contain high levels of cellulose [9]. They can be categorized as primary plant fiber or secondary plant fibers. Primary fibers are grown for their inherently high fiber contents like cotton while secondary fibers are obtained as by-products. Fibers have extensive uses in numerous industries. The antibacterial properties of natural fibers have been studied extensively in recent times. The anti-bacterial properties could be applied to not only healthcare, but industries like packaging. Plant fibers also contain high levels of hemicellulose and lignin other than cellulose. Lignin has been identified to be the source of potentially potent antibacterial action in fibers such as bamboo and cotton stalks. This is due to the high phenolic contents in lignin [10]. It could be inferred that fibers containing high percentages of lignin will exhibit great antibacterial action. Numerous fibers such as sisal, hemp, kenaf etc. owe their antimicrobial action to their lignin concentrations. This project is inspired by the natural antimicrobial properties of natural plant fibers and the fact their natural surfaces exhibit a rough texture.

3. Chapter 3 – Requirements & Options Evaluation

3.1. Requirements

Several different structures could be manufactured with natural plant fibers due to modern technological advances and the versatility of these fibers. However, a mat like structure is the most readily obtainable structure from natural fibers as they exist as long or short bundles of fiber that can be easily layered to produce a mat of one or more layers of flat sheet fibers. Natural fibers are strong, eco-friendly, lightweight, renewable, low cost and biodegradable giving them an inherent advantage against other natural antibacterial materials. However, some fibers have better physical properties than others or are more readily available and easier to work with. These will serve as the principal criteria for selecting the best natural fibers for the mat structure. The mat should be:

- i. Lightweight
- ii. Cost Effective
- iii. Made from locally available resources (Natural fibres)
- iv. Exhibit Anti-microbial action
- v. Physically Durable

Natural fibers might be natural and abundant, but their availability differs in West Africa i.e., some are easier to obtain than others. Natural fibers might possess natural antimicrobial action but the strength of this varies from fiber to fiber. Fibers also demonstrate different physical strengths in terms of their tensile strengths. They also vary greatly in cost amongst themselves.

With these criteria, four different natural plant fibers were chosen and evaluated. These four fibers satisfy all requirements but outperform each other in each regard. The five chosen fibers are Musa fibers from Banana plants, Sisal fibers from the Sisal Plant, PAL fibers from Pineapple, Coir fibers from Coconut fibers.

		Option 1	Option 2	Option 3	Option 4
Criteria	Weight	PAL fibers (Datum)	Musa fibers	Sisal fibers	Coir fibers
Cost	2	0	0	0	0
Availability	3	0	+	+	+
Anti- Microbial action	3	0	-	0	0
Tensile Strength	2	0	0	+	0
+		0	2	3	2
0		4	2	2	3
-		0	1	0	0
Total		0	1	3	2

Fig 3.1. Pugh Chart showing the four fibers compared to each other using PAL fiber as the Datum

From the above Pugh chart, it can be seen that Sisal and Coir fibers generated higher tally scores than the Musa and datum PAL fiber. They represent the best two options for the mat.

3.2. Solidworks Modelling

A prototype mat was designed using Solidworks in order to visualize how the mat will appear in the real medical setting. Two designs were created using groups of fibers arranged in bulk rows and columns braided to mimic indigenous African braiding designs. The kind of braiding patterns that can be seen on objects like a basket.

3.2.1 Prototype A

This design was made with interlocking row and columns of fibers. Each row and column is made with several individual strands of fibers that constitute to a thicker bunch. This was done so that the mat would not light enough to be affected by wind or a strong breeze. However, it is still lightweight enough for easy transport. The problem with this design is it uses a lot of fiber to form the complete structure.

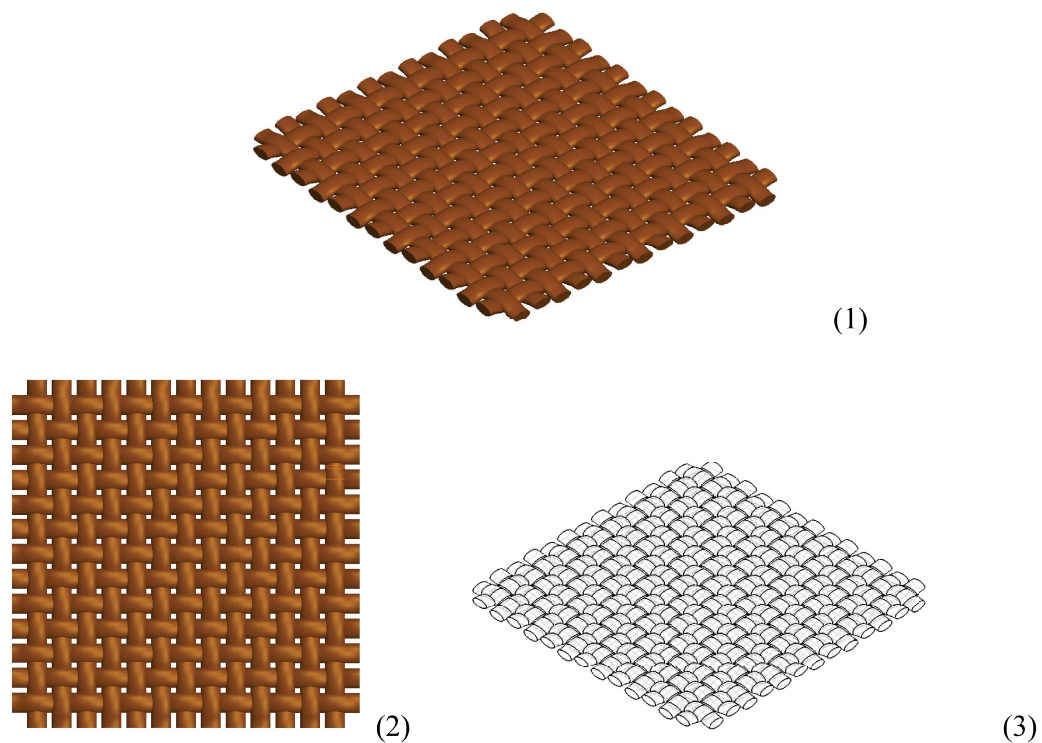


Fig 3.2.1a. Isometric view of Prototype A (1), Side view of Prototype A (2),
Structure of Prototype A (3)

3.2.2 Prototype B

This design was also made with interlocking row and columns of fibers. Each row and column is made with several individual strands of fibers that constitute to a thicker bunch. However, in order to save material, much less fiber is combines to form the intersecting rows and columns. This results in larger spaces forming. Therefore, this mat has to be placed on a thin plastic surface.

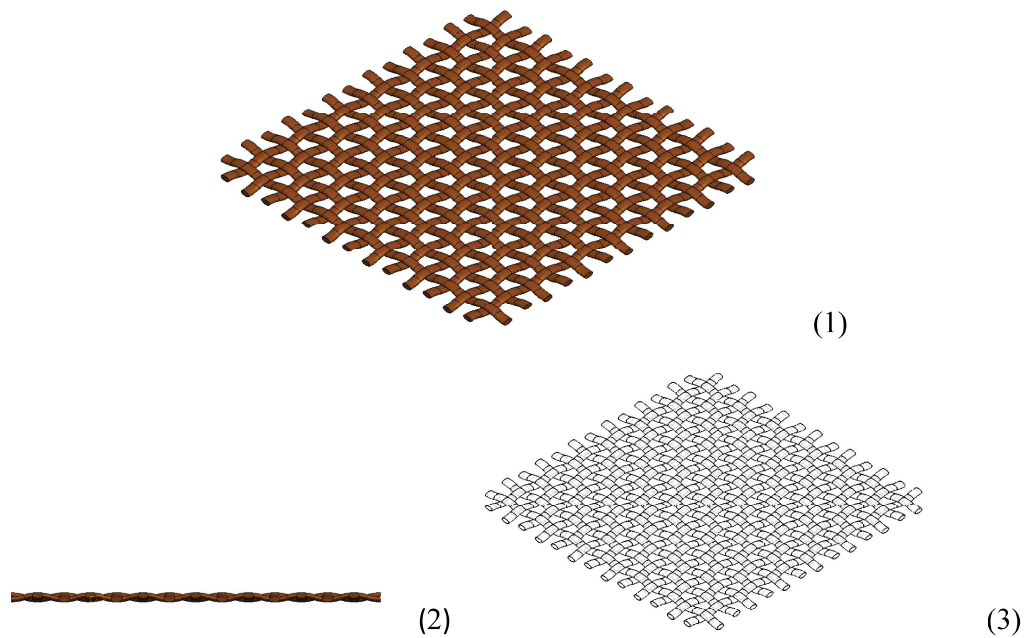


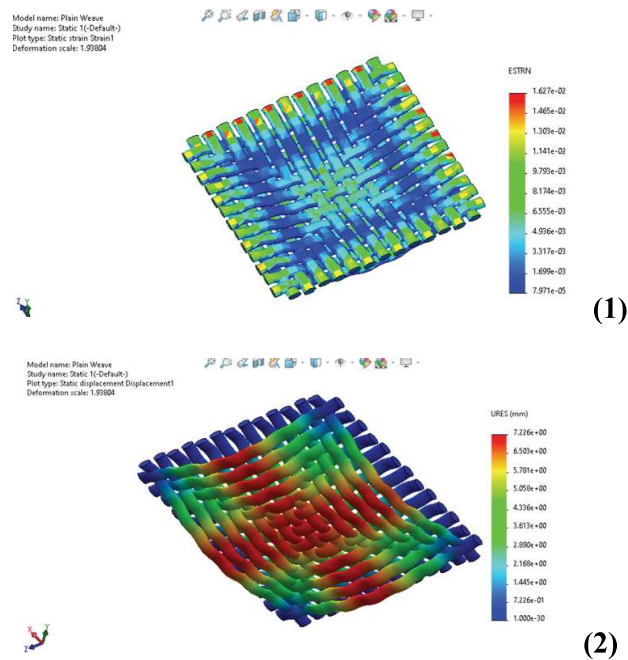
Fig 3.2.1b. Isometric view of Prototype B (1), Side view of Prototype B (2), Structure of Prototype B (3)

The design chosen was Prototype A. Although Prototype B saves a lot of material, the existence of larger spaces inhibits the microbial action of the fibers. This is too great a disadvantage to neglect.

3.3. Solidworks Simulations of Coconut and Sisal Mat

Solidworks was used to simulate the behavior of the mat under an applied load to mimic forces acting on it while in use. Custom materials were created in SolidWorks for the coir and sisal fiber. Properties of the fibers were derived from Cambridge Education Selector (CES) EduPack 2013 Granta design. Contact sets (bonded) was used to establish contact of the fibers in the SolidWorks assembly. Fixtures were applied to the mat. 200N load was applied to the top face of the mat. Stress, displacement, strain and factor of safety plots were graphed. This should simulate the physical properties of the mats if a mass of 20kg is placed on it.

3.3.1. Coconut Mat simulations



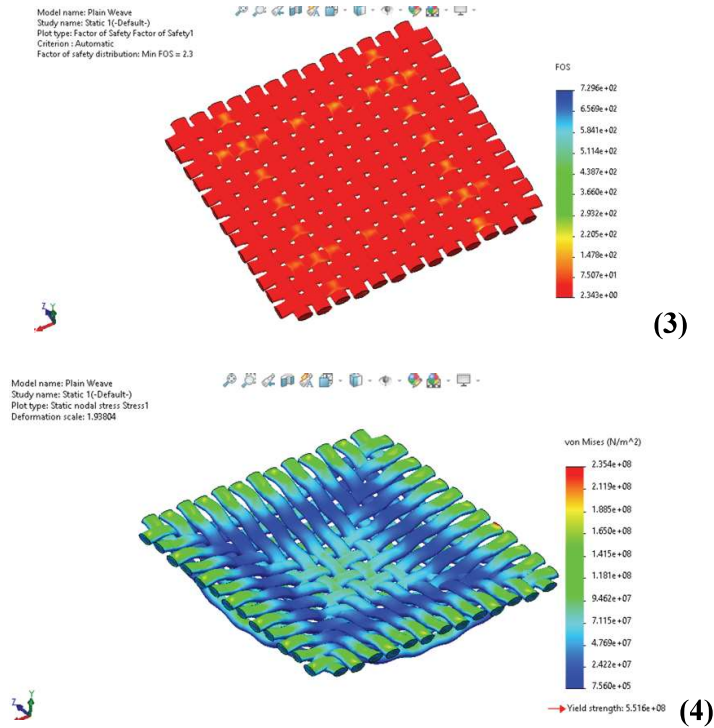


Fig. 3.3.1 Simulation results of Coconut Mat Strain (1), Displacement (2), Factor of Safety (3), Stress (4) under applied load

Under a load of 200N, the above results were obtained. The simulation of the Strain shows that there was little effect of the applied on the strain property of the mat on the outer side but is more pronounced on the inside. The simulation of the Displacement shows the load deformed the mat quite significantly towards the inner side meaning a load higher than this will very likely tear the mat. The highest deformation values were about 7mm while the lowest were about 1mm. The factor of safety is about 2.43 which fulfils the engineering criteria of the Factor of Safety being above 2. This means the design is very safe and can withstand forces much higher than the typical stress applied. The simulation of the Stress shows the mat didn't undergo significant stress as a result of the applied load. The highest stress recorded was about $1.145 \times 10^7 \frac{N}{m^2}$. Which is lower than the yield strength. This is a successful test at this applied load as the mat took it well without any failure even though there was significant deformation of some parts.

3.3.1. Sisal Mat simulations

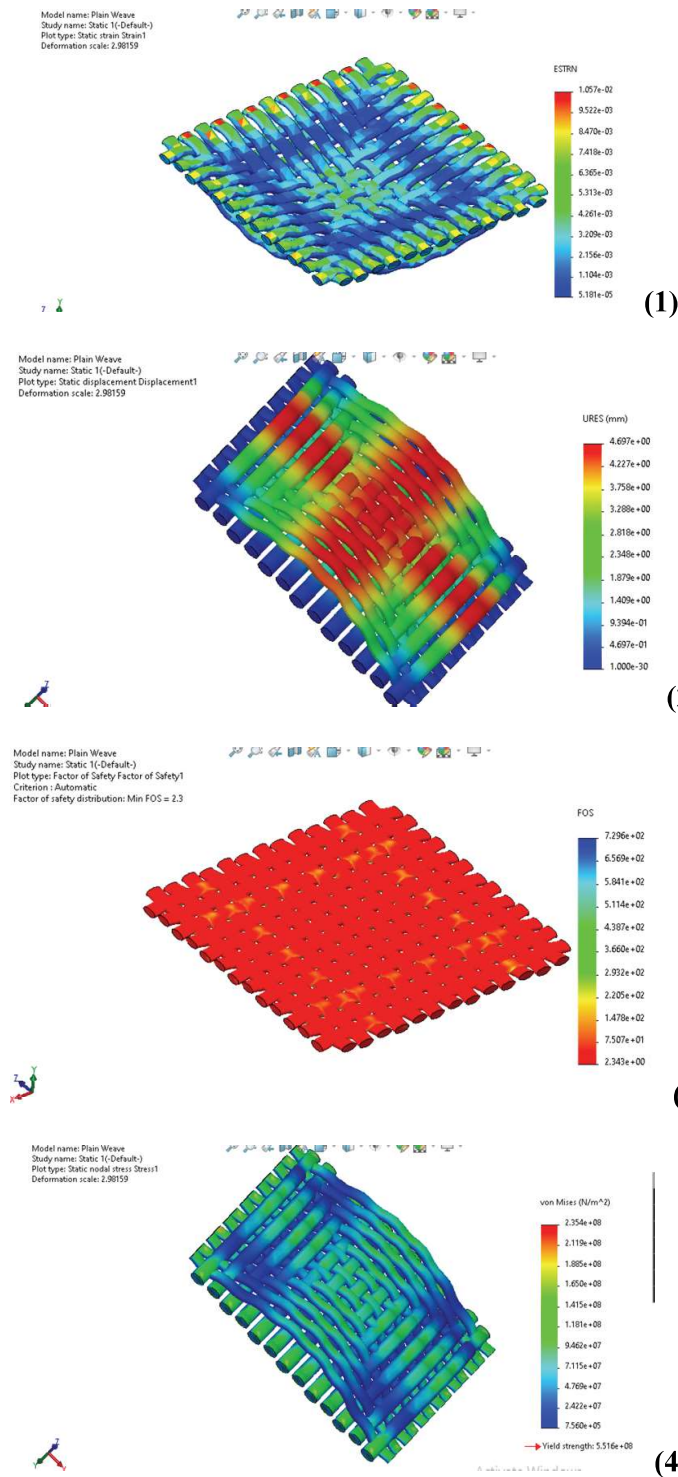


Fig. 3.3.2 Simulation results of Sisal Mat Strain (1), Displacement (2), Factor of Safety (3), Stress (4) under applied load

Under a load of 200N, the above results were obtained. The simulation of the Strain shows that there was little effect of the applied on the strain property of the mat on the outer side but is more pronounced on the inside. The simulation of the Displacement shows the load deformed the mat quite significantly towards the inner side meaning a load higher than this will very likely tear the mat. The highest deformation values were about 4mm while the lowest were about 1mm. The factor of safety is about 2.43 which fulfils the engineering criteria of the Factor of Safety being above 2. This means the design is very safe and can withstand forces much higher than the typical stress applied. The simulation of the Stress shows the mat didn't undergo significant stress as a result of the applied load. The highest stress recorded was about $9.462 * 10^7 \frac{N}{m^2}$. Which is lower than the yield strength. This is a successful test at this applied load as the mat took it well without any failure even though there was significant deformation of some parts.

3.4. Significance

Both fibers performed similarly based on the Simulation results. However, the sisal mat outperforms the Coconut slightly as less stress was generated in the Sisal mat. It also deformed less under the same load.

4. Methodology and Results

4.1.Introduction:

Several experiments were carried out on the fibre samples of coconut and sisal fibres to determine which of the fibres will exhibit the best properties as the base material for a micro surfaced mat that prevents bacterial growth. The experiments were carried out in a controlled setting with necessary precautions observed wherever the need may arise.

4.1.1 Materials

1. Microscope slides
2. Transparent cello tape
3. Paper cello tape
4. Fiber samples
5. Analytical weighing balance
6. Pyrex beakers 100ml
7. Steel tongue
8. Ocular micrometer by Erma
9. Stage micrometer by Erma
10. Binocular light microscope
11. Forceps
12. Tryptone Soya Agar
13. Petri dishes by Silver Health Diagnostics
14. Colony counter
15. Spectrophotometer
16. Biological safety cabinet by Esco Laboratories
17. Micro pipettor

18. Micropipette tips.
19. Autoclave by Express Laboratories
20. Water bath by Uniscope Laboratories
21. Hot air oven by Uniscope Laboratories

4.1. Measurement of Diameter of Fiber Samples using Micrometry

4.1.1. Aims

The aim of this experiment was to obtain measurements for the average random girth distribution for both fibers. These random samples should approximate the size of the larger population of the fibers since all fibers had an equal chance of random selection.

4.2.2. Procedure 1: Sample preparation

Samples were first subjected to length reduction by cutting each individual fiber into lengths of 1.5cm. They were loaded on the transparent nylon tape cut into the width of the microscope slide by aligning on the tape. The loaded cello tape was quickly inverted on the microscope slide with the adhesive side faced down. The paper cello tape was used for slide numbering with the sample name. Ten randomly selected fibers were studied, and average girth sizes were recorded.

4.2.3. Procedure 2: Ocular slide calibration

The ocular slide was calibrated using the stage micrometer. To calibrate the ocular micrometer at a particular objective lens, place the stage micrometer on the ocular micrometer. The number of stage micrometer divisions that coincided with the ocular division were recorded and used for the ocular micrometer calibration. According to the x4 objective used, 39 ocular divisions equal 100 stage division. Therefore, one stage division equals 0.39

ocular division. The inscribed lines on the stage micrometer are 0.01mm apart.

Therefore one 1 ocular division equals $\frac{100}{39}$ divisions of the stage micrometer. Hence 1

ocular division equals $\frac{100}{(39)(0.1)} = 0.0256\text{mm} = 25.6\mu\text{m}$

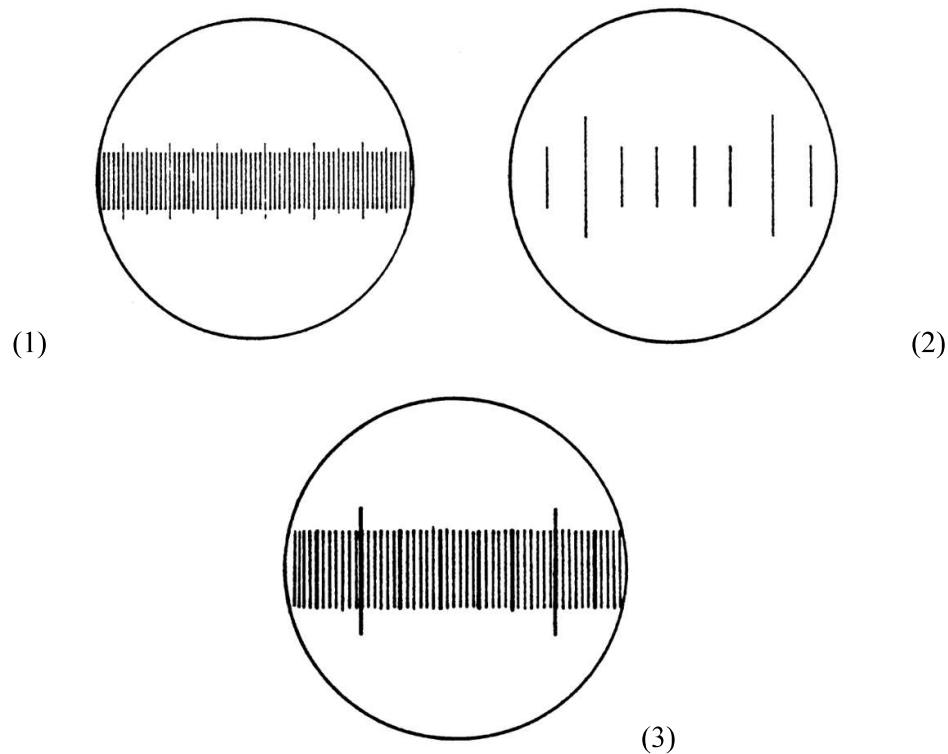
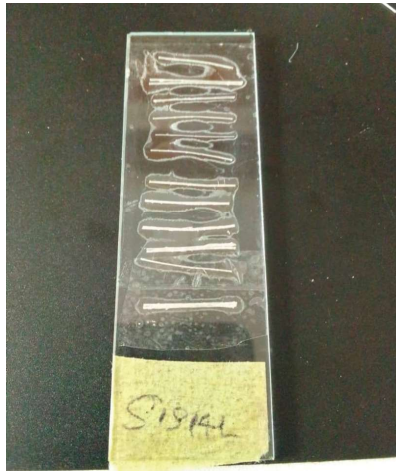


Fig. 4.2.3 Showing an Ocular micrometer (1), Showing a Stage micrometer (2)

Showing a stage micrometer imposed on an ocular micrometer (3)

4.2.4. Slide reading

All slides were read using the low power dry lens x4 objective lens. The reading was taken starting from the zero mark of the ocular divisions. The number of ocular divisions a fiber measured was taken and tabulated. The readings were converted to the actual readings using the values obtained from ocular calibration as the multiplier. The comprehensive raw data is included in the appendix.



(1)



(2)

Fig 4.2.4a Showing prepared samples of sisal fibers
used in ocular micrometry (1), Showing prepared samples of coconut fibers
used in ocular micrometry (2)

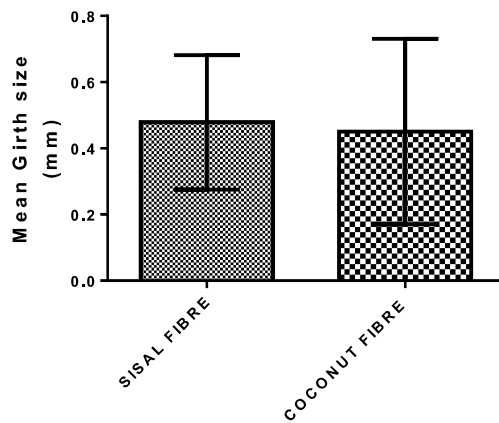
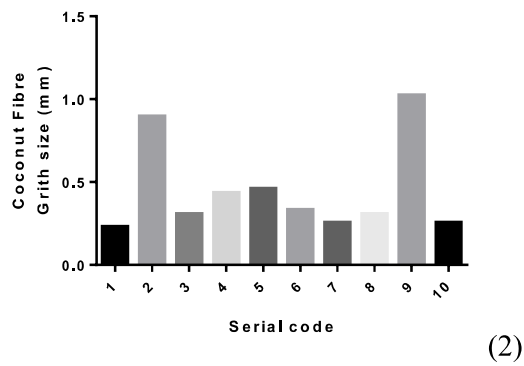
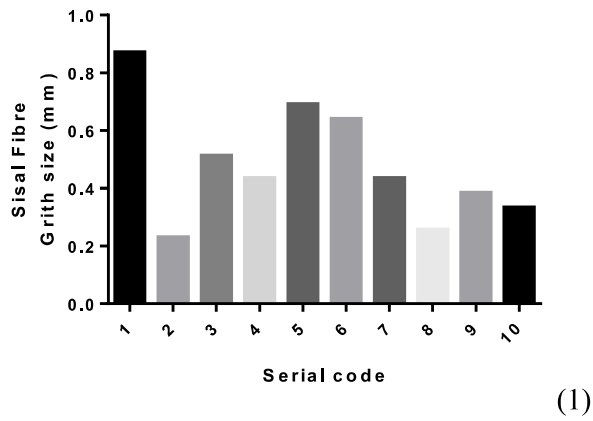


Fig 4.2.4b. Bar Chart showing the distribution of sizes of Sisal fibers (1), Bar Chart showing the distribution of sizes of Coconut fibers (2), Plot showing the comparison between the mean of the distribution of sizes of Sisal fibers and Coconut fibers (3)

4.2.5. Significance of Measurements

There was no statistical significance between the two groups as a t test yielded a p value of 0.7998 greater than the criteria of $p < 0.05$ for the results to have statistical significance. Therefore, there we conclude there is no difference between both groups. Although, the Sisal fibers appeared to have higher average diameters.

4.3. Determining the Anti- Bacterial Qualities of Fiber Samples

4.3.1. Aims

The aim of this experiment is to quantify the number of bacteria that a certain amount of uncontaminated fiber holds. This will help determine of the roughness of the fibers has an effect on its anti-bacterial qualities.

4.3.2 Procedure 1: Media Preparation

Media were all weighed, and their weights recorded. The agar gels were first prepared by heating to melt in the water bath and then mixed to avoid segregation before autoclaving at 121°C for 15 minutes. Tryptone soya agar was prepared by suspending 40g in a liter of distilled water. It was heated to melt in water bath at 100°C . After thorough mixing, it was autoclaved at 121°C for 15 minutes. This agar was used for Total Aerobic Microbial count.

4.3.3 Procedure 2: Sterilization of Glass wares

All the glassware were washed and rinsed with tap water. They were then rinsed with distilled water and dried in a hot air oven at 100°C until they were dry. They were then wrapped with aluminum foil and sterilized at 160°C for one hour in the hot air oven and allowed to equilibrate with the Laboratory ambient temperature before use.

4.3.4. Procedure 3: Diluents Preparation

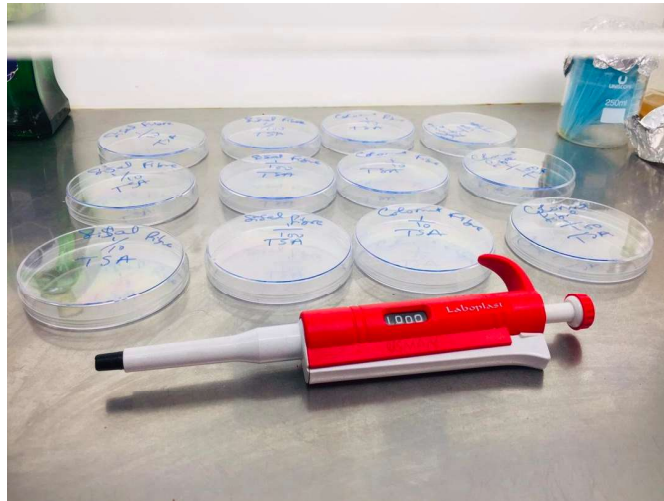
The diluent was 4% tween 20. Tween is a polysorbate for the dilution of samples that might contain preservatives and natural antimicrobials. It is also a good emulsifier and therefore enhance mixing of samples that might contain trace oils. It is prepared by making 40ml of tween 20 to 1000ml with distilled water. 1ml of sodium thiosulphate was incorporated to remove residual chlorine from the samples.

4.3.5. Procedure 4: Sampling

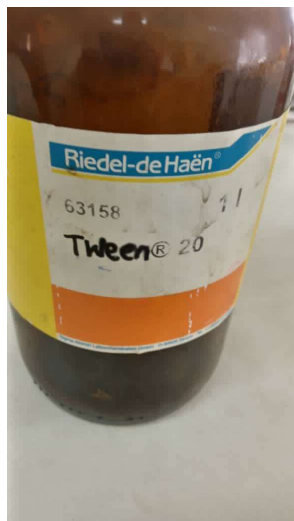
There were two working dilutions, one - in - ten (1 in 10) and one- in- hundred (1 in 100) dilutions. 10g portion of the sample was weighed aseptically and transferred to 90ml of diluent in a bottle 1 in 10 dilution concentration. It was shaken and then 10ml portion was transferred to another 90ml of diluent in a bottle of 1 in 100 diluent concentration. 1ml of each of the dilutions was aseptically transferred to appropriately labeled Petri dishes. They were overlayed with 19ml of sterile agar and allowed to set. The plates for bacterial load were incubated at 37°C and observed daily for 72 hours.

4.3.6. Procedure 5: Quantifying Bacterial growth

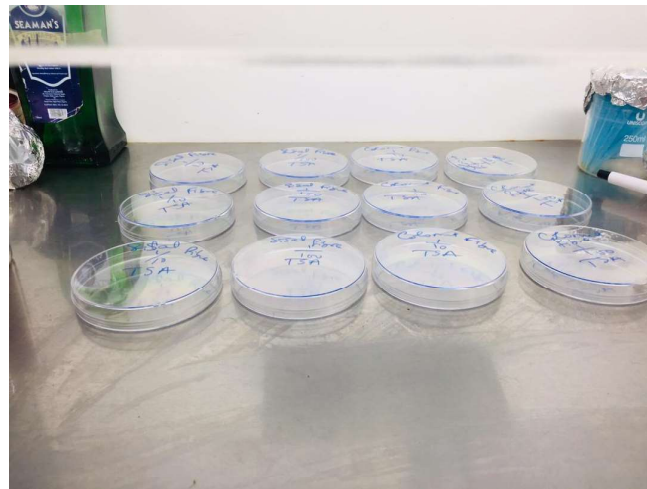
The bacteria in the petri dishes were quantified into the number of Colony forming units per gram. The counting of the colonies was done manually using a pen and a click counter to record the number of dots on each plate. The comprehensive raw data is included in the appendix



(1)



(2)



(3)

Fig 4.3.6a Pen and counter used to record CFU/G quantity (1), 4% Tween 20 used to dissolve samples (2), Petri dish layout before bacteria colonialization (3)



(1)



(2)

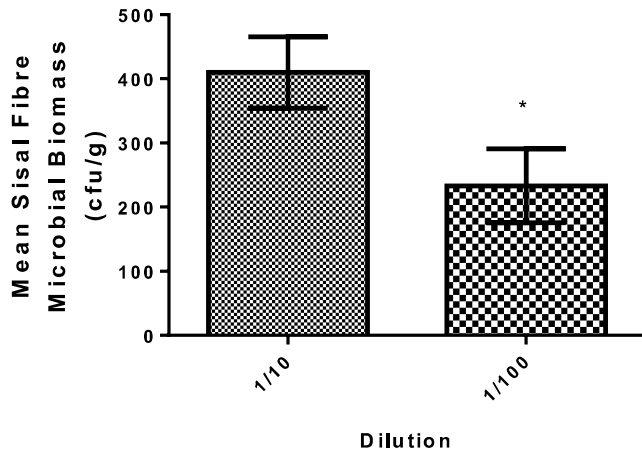


(3)

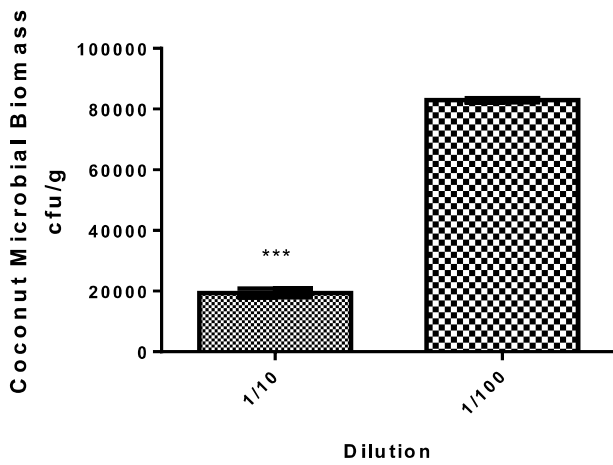


(4)

Fig 4.3.6b. Sisal Ccolony Fforming Units (CFU) at 1/10 dilution (1), Sisal CFU at 1/10 dilution (2), Cocunut CFU at 1/10 dillution (3), Coconut CFU at 1/10 dilution



(1)



(2)

Fig 4.3.6c: Graph Showing Mean Sisal Fibre Microbial Biomass (1), Graph Showing Mean Coconut Fibre Microbial Biomass (2)

4.3.7. Significance of experiment:

There was statistical significance between the two groups as a t test yielded a p value of 0.0001 greater than the criteria of $p < 0.05$ for the results to have statistical significance. Therefore, there we conclude that there is a difference between both groups. The Coconut fibers have a higher average Colony Forming Unit per gram count. The surface roughness is known to reduce bacterial adhesion and thus bacteria population, as observed under a microscope in the figures below, the coconut fibres exhibit greater surface roughness than sisal fibres so one would expect the population of the coconut fibres to be less than sisal. However, this wasn't the case as sisal fibres, even though less rough, exhibited less bacterial growth. This could be attributed to the fact that 4 % Tween 20 could have dissolved essential anti-microbial oils in the coconut fibres. As is it an emulsifier and solvent, it could very well have hampered the microbial action of the fibre. Autoclaving the fibres could also alter anti-microbial structures and chemicals in the fibre component. The larger surface area of coconut fibres compared the sisal fibres of the same mass could have allowed more microorganisms to settle in the fibre's spaces and inner cavities. These are just some of the proposed explanations as to why the results may have differed from the reviewed literature.

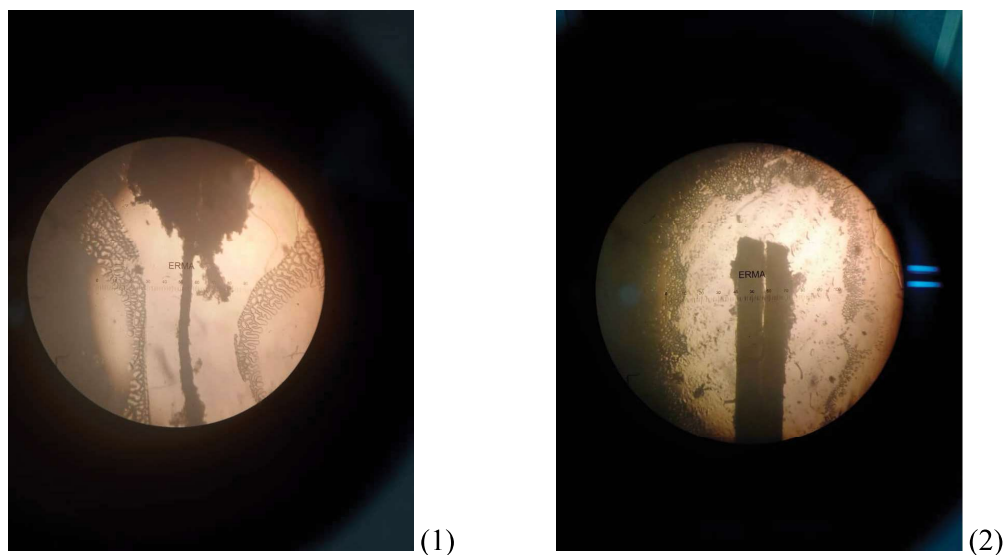


Fig 4.3.7a Showing surface roughness of Coconut fibre (1), surface roughness of Sisal fiber (2)

4.4 Water Absorbance Test

4.4.1. Aims

The aim of this test is to determine the degree to which a fiber has affinity for water. This is important as bacteria are influenced by the hydrophobicity of a surface during attachment.

4.4.2. Procedure

1g of both samples were weighed and placed into different beakers with labels for the respective time durations the fibres will be submerged in water i.e., 6hrs, 12hrs, 24hrs, 48hrs and 72hrs respectively. 20ml of deionised water was added to the samples in the beaker. After each respective duration, the samples were removed and blotted with blotting paper and weighed. The differences in the final weight from the initial dry weight of the fibres represent the quantity of water absorbed. The rate of water absorbed is compared and the absorbance is determined. The comprehensive raw data is included in the appendix

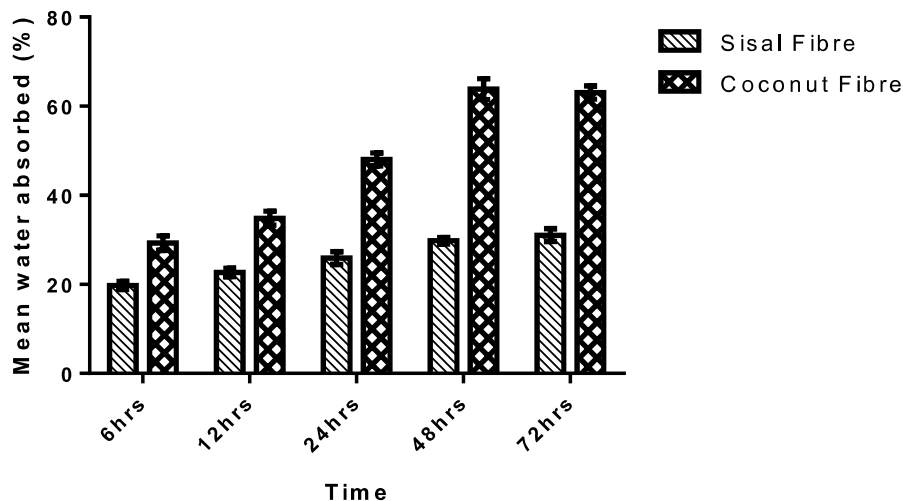


Fig 4.4.2 Graph Showing the comparison of means of the water absorbed between Sisal and Coconut fibres

4.4.3. Significance of Results

An unpaired t -test was done for each test duration time, and for each of them it yielded a p value of less than 0.005 ($p < 0.005$), therefore each test was statistically significant. Coconut exhibits greater water absorbance properties than sisal as it absorbed more water than sisal. Thus, it can be inferred that Sisal exhibits greater hydrophobic properties than coconut as it absorbed less water than coconut. Highest biofilm production is observed on more hydrophobic biomaterials [11]. This means coconut fibers would be resistant to bio film pollution from more prevalent hydrophobic bacteria strains.

4.5 Ease of stain removal from fibers

4.5.1 Aims

The aim of this experiment is to determine the ease of cleaning stains from fiber samples using ethanol. This experiment aims to mimic the cleaning of surfaces in medical settings as these surfaces are typically sterilized with disinfectants like methylated spirit [3]. The easier a surface is to clean, the better it is as it means less of stain remains.

4.5.2. Stainability of fibers

Spectrometry involves the separating a beam of visible light into its different wavelengths. The monochromatic wavelengths are in turn split into two with one being the sample beam that will pass through the solution of the compound being studied in a transparent solvent. The other beam, known as the reference beam, will pass through only the solvent. The sample beam and reference beam are analyzed in a logarithmic scale in the spectrometer to yield absorbance values. The absorbance values therefore are a logarithmic comparison between the sample beam of the solution containing the compound being studied and reference beam containing just the solvent. Absorbance is rated from 0 to 2 with 0 being a transparent liquid and 2 being a darker liquid. There were six holding times for the staining of the fiber in order to study the stain absorbability of the fiber. A quantity of 0.1g of the fiber was stained with 0.005% solution of crystal violet. The stained fiber was also decolorized at the same holding times. The holding times were 10s, 20s, 30s, 40s, 50s and 60s. The absorbance values of the dye released into the decolorizing agent at the different timings were also taken at 620nm λ_{max} using a spectrometer. Control standard

solutions of the dye were also prepared for quantitative studies of the dye released into the decolorizing medium. The decolorizer was 70% solution of ethanol.

4.5.3. Preparation of dyes for staining

The stock solution of 0.005% was prepared by weighing 0.01g of crystal violet powder in 200ml of preparation in a 200ml capacity volumetric flask. This stock was used for staining and preparation of working standard concentrations of the dye.

A. 0.1g of crystal violet in a 200ml volumetric flask. Distilled water was added to 200ml mark. Concentration = 0.005%

B. 5ml of content of bottle A + 5ml of distilled water, concentration= 0.0025%

C. 5ml of content of bottle B + 5ml of distilled water, concentration= 0.00125%

D. 5ml of content of bottle C + 5ml of distilled water, concentration= 0.000625%

E. 5ml of content of bottle D + 5ml of distilled water, concentration= 0.0003125%

F. 5ml of content of bottle E + 5ml of distilled water, concentration= 0.00015625%

Contents of bottles B, C, D, E and F were used for standard concentrations to control the experiment by using the values for standard plot and determination of the concentrations of the unknown dye released into the decolorizing agent.

4.4.4. Preparation of 70% Alcohol

A. Is a proprietary solution of 96% ethanol

B. Is 145.83ml portion of A

C. Is a carefully measured 54.17ml portion of distilled water.

D. Mix solution B and C, concentration = 70%

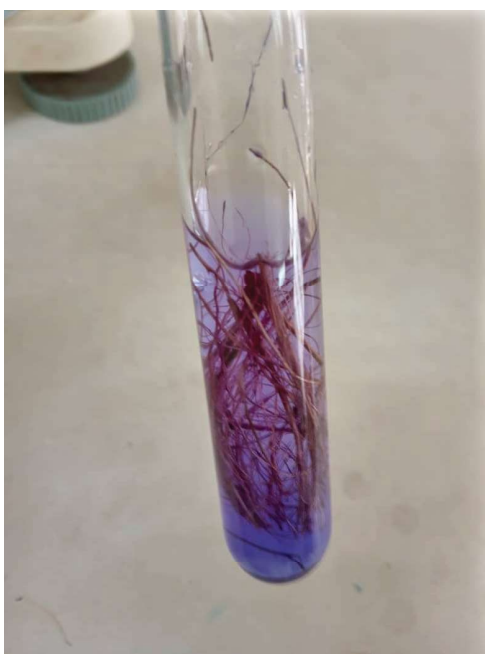
Solution D was used as decolorizing agent throughout the experiment. Fractional volumes were taken with micro pipettor. The comprehensive raw data is included in the appendix



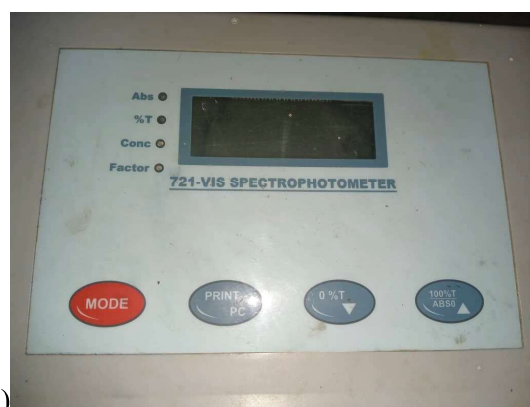
(1)



(2)

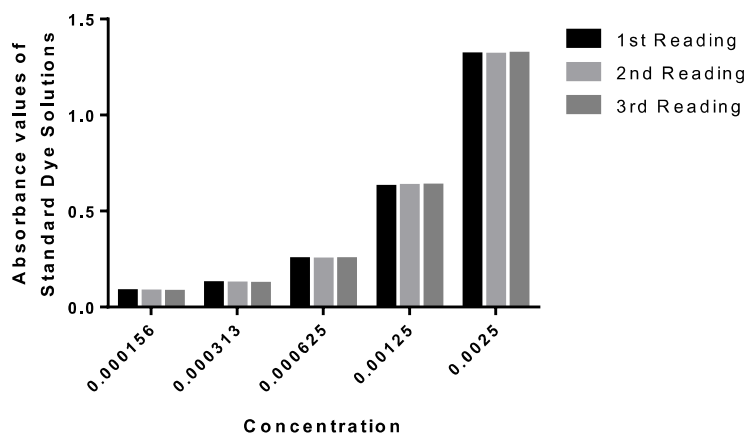


(3)

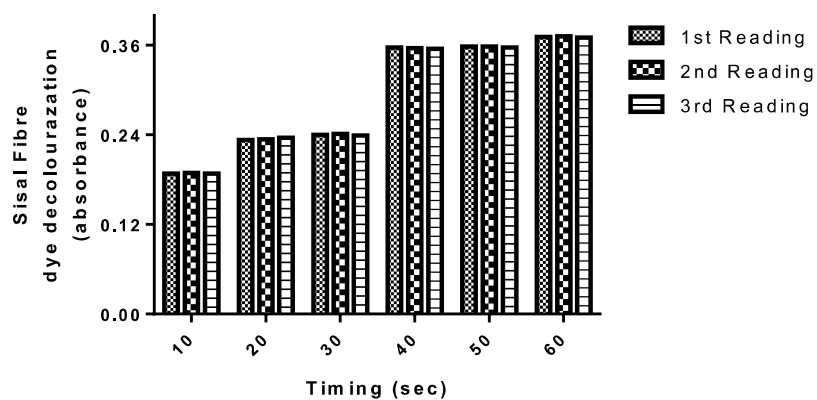


(4)

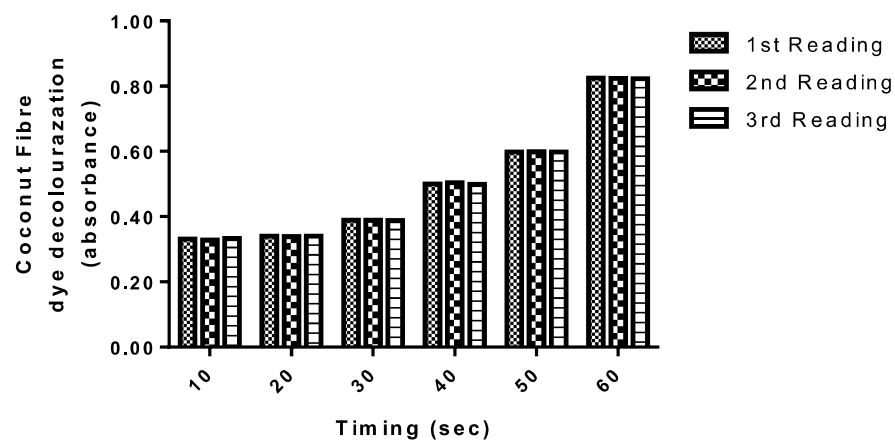
Fig 4.5.4a Showing stock standard solutions which had their absorbance values recorded as reference (1), Showing rack of fibre samples before decolorization (2), Showing the process of decolourization of a coconut fibre sample (3), Spectrometer (4).



(1)



(2)



(3)

Fig 4.5.4b Graph Showing Absorbance Values of Standard Dye Solutions (1), Sisal dye deculturalization (2), Coconut dye decolorization (3)

4.5.5 Significance of experiment:

An unpaired t -test was done for each test duration time, and for each of them it yielded a p value of less than 0.005 ($p < 0.005$), therefore each test was statistically significant. Coconut exhibits greater absorbance than sisal as it released more dye into the solvent than sisal. Thus, it can be inferred that Coconut will be an easier fibre to clean than sisal. This is of particular importance as mats in a medical setting are subjected to bodily fluids such as blood, sputum, sweat, urine and faeces. Commonly used cleaning agents contain typically contain certain percentages of ethanol. This experiment tried to mimic these stains using crystal violet. Thus, it gives a good idea as to how easy it will be to remove body fluids from each fibre.

4.6 Determination of Mechanical Properties of Fiber Samples

4.6.1 Aims:

This experiment aims to determine key physical properties of the fibers that will influence their strength and durability.

4.6.2 Procedures:

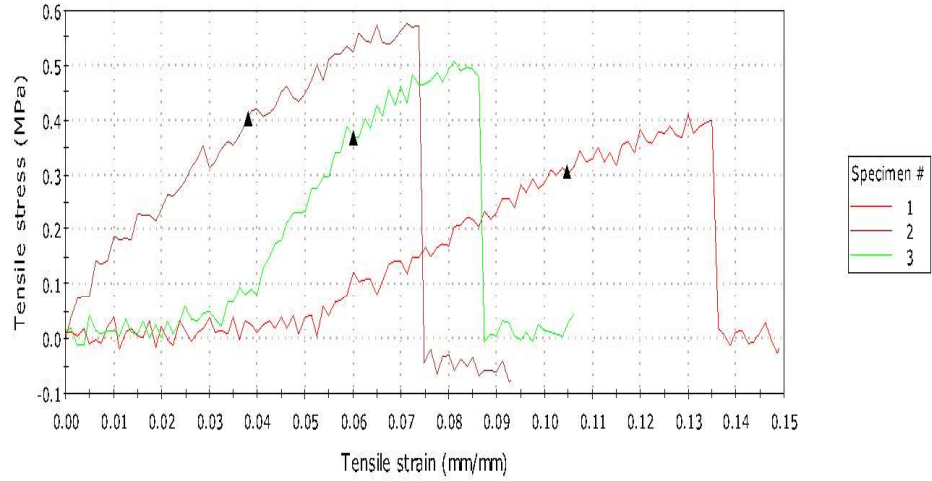
To evaluate the fibers, tensile tests were done on the fibers using a universal testing machine. The bottom and top of the fibers were then clipped to the machine for testing. Load extension curves were obtained and the stress-strain behavior on each fiber was subsequently calculated using the following equations:

$$\sigma = \frac{F}{A} \quad (4.1)$$

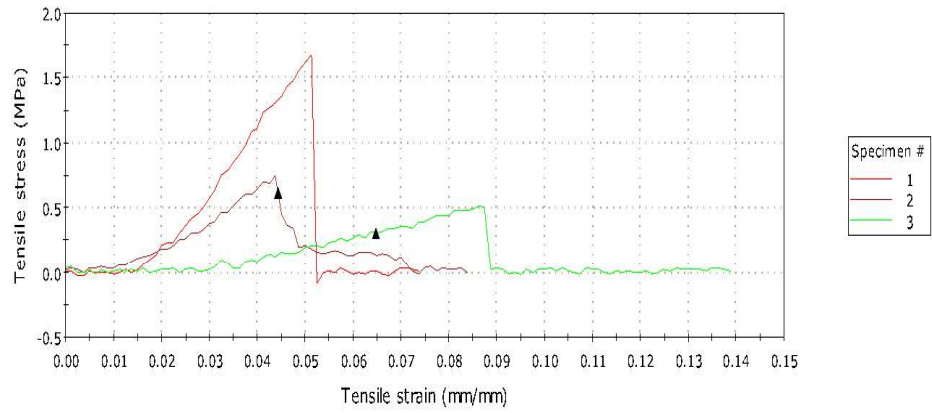
Where σ represents the generated stress when a certain force (F) is applied over a fiber sample with an Area (A).

$$\varepsilon = \frac{\Delta L}{L_0} \quad (4.2)$$

Where ε represents the generated strain obtained from the change in length of the fiber (ΔL) divided by the initial length of the fiber (L_0).



(1)



(2)

Fig 4.5.2. Graph Showing the Tensile Stress against Strain of Coconut fiber (1), Tensile Stress against Strain of Sisal fiber (2).

4.6.3 Ultimate Tensile Strength and Strain at Necking

The ultimate tensile strength is the stress at the maximum applied load on the fiber sample. From the stress-strain curve obtained from the tensile test, it was extrapolated, and the point with highest stress was determined. The strain at necking was also inferred.

4.6.4 Yield Strengths and Strain at Yielding

The yield strength was found at the yield point extrapolated parallel to the elastic region of the stress-strain curve, at 0.2% offset strain value. The strain at the yield point is inferred at the point of the stress-strain curve in which the material transitioned from elastic deformation to plastic deformation i.e., yielded.

4.6.5 Stiffness

The stiffness (E) of a fiber sample denotes its resistance to plastic deformation. It was obtained from the slope of the Tensile stress versus strain curves of the fiber samples

$$E = \frac{\Delta\sigma}{\Delta\epsilon} \quad (4.3)$$

4.6.6 Modulus of Resilience

The observed area under the elastic region of the stress-strain curves gave the modulus of resilience for both fiber samples. For linear elastic behavior, the modulus of resilience, E_r is

$$E_r = \frac{1}{2} \cdot \sigma_y \cdot \varepsilon_y \quad (4.4)$$

Where σ_y represents the yield strength of the fiber and ε_y represents the strain at yielding.

4.6.7 Determination of Critical Fiber Length

The critical length (l_c) is the minimum length at which the fiber will perform on average in terms of both strength and stiffness. It was obtained from the equation:

$$l_c = \frac{\sigma \cdot d}{2 \cdot \tau} \quad (4.5)$$

Where d is diameter of the fiber, τ is shear strength of the fiber and σ is the ultimate tensile strength of the fiber.

The properties of these fibers were compared against one another and tabulated below and summarized. The comprehensive raw data from the experiment is included in the appendix.

Physical Property	Value
1. Ultimate Tensile Strength	0.49609 MPa
2. Strain at Necking	0.11607 mm/mm
3. Yield Strength	0.03955 MPa
4. Strain at Yielding	0.09417 mm/mm
5. Modulus of Resilience	$1.86 * 10^{-3}$ MPa
6. Critical Length	5.65 mm

Table 4.5.5. Summary of Mechanical properties for Coconut fiber

Physical Property	Value
1. Ultimate Tensile Strength	0.81935 MPa
2. Strain at Necking	0.08636 mm/mm
3. Yield Strength	0.52066 MPa
4. Strain at Yielding	0.05125 mm/mm
5. Modulus of Resilience	$1.33 * 10^{-2}$ MPa
6. Critical Length	0.75 mm

Table 4.5.5. Summary of Mechanical properties for Sisal fiber

5. Conclusion, Limitations and Recommended future work

5.1 Conclusion

The two fiber mats performed similarly under load based on the Solidworks simulation results. Notwithstanding, the sisal fiber mat beats the Coconut marginally as less deformation was produced in the Sisal fiber mat under same loading. From the mechanical tests, Sisal exhibited greater resistance to stress and greater strength overall. The Sisal fibers appeared to have higher average diameters, although slightly. The Coconut fibers have a higher average Colony Forming Unit per gram count. Coconut exhibits greater water absorbance properties than sisal as it absorbed more water than sisal. This means coconut fibers should resist bio film pollution from more prevalent hydrophobic bacteria strains. Coconut exhibits greater absorbance than sisal as it released more dye into the solvent than sisal. Thus, it can be inferred that Coconut will be an easier fibre to clean than sisal.

In conclusion, physical tests favour sisal. However, in terms of determining anti-microbial activity, the tests proved inconclusive in proving is superior. More testing and sophisticated measurements are necessary in finalizing this.

5.2. Limitations

The main challenge experienced with this project is the lack of certain equipment including things like a Scanning Electron Microscope (SEM), equipment capable of mapping out the detailed topographical surface structure of each fiber and equipment capable of developing advanced computer simulations. The lack of these kinds of equipment will have thoroughly increased available information into the behavior of the nanostructure of the fibers. The scope of this project was limited to the micro- structure which is not sufficient to fully understand bacterial behavior and its adhesion principles in detail. The nanoscale is where most of the important bacterial behavior and studying it at this level will develop more sophisticated

models and inferences. It is also impossible to model adhesion principles on the micro- scale as it only depicts the immediate surface structures of the fibers. The more hidden structures like the grooves, crevices, pores and lines in the fiber are where bacteria actually begin adhesion, and this requires work in the nanoscale. The lack of an accurate description of the full surface characteristics of the fiber hindered work on a detailed model. Elaborate simulation packages that can mimic bacteria behavior on certain surfaces were not available. A culmination of these contributed to the short comings in the project.

5.3 Recommended future work

The surface topography and structures of these fibers need to be mapped out in immense detail to fully understand how bacteria will behave on these fibers more accurately. This is the only way accurate models of bacterial adhesion can be developed. The treatment of the fibers with certain chemicals could be investigated to determine their effect on its microbial properties, physical properties and surface topography. Evaluating other fibers could give another interesting area of study.

Reference

[1] H. A. Khan, F. K. Baig, and R. Mehboob, “Nosocomial infections: Epidemiology, prevention, control and surveillance,” 07-Jan-2017. [Online]. Available: <https://www.sciencedirect.com/science/article/pii/S2221169116309509>. [Accessed: 20-Mar-2021].

The article gives a general overview of the occurrence of nosocomial infections on the global scale. It discusses the types of nosocomial infections and their relative prevalence and severity. It also discusses the key pathogens responsible for the diseases associated with the infecting citing modes of transmission and possible preventive measures to curb the transmission levels. It hints on the problems associated with tackling the infections as well as stipulating clear approaches to curb the spread of nosocomial infections.

[2] A.-K. Labi, N. Obeng-Nkrumah, E. Owusu, S. Bjerrum, A. Bediako-Bowan, G. Sunkwa-Mills, C. Akufo, A. P. Fenny, J. A. Opintan, C. Enweronu-Laryea, S. Debrah, N. Damale, C. Bannerman, and M. J. Newman, “Multi-centre point-prevalence survey of hospital-acquired infections in Ghana,” 03-May-2018. [Online]. Available: <https://www.sciencedirect.com/science/article/pii/S0195670118302573>. [Accessed: 20-Mar-2020].

The article cites the prevalence of nosocomial infections in acute care hospitals across ten hospitals. It emphasizes on the types of nosocomial infections occurring and the dominating pathogen responsible as well. It mentions the features some of these hospitals possess that make infections widespread. A study carrying out point prevalent surveys according to guidelines stipulated by European Centre for Disease Prevention and Control. It was discovered there was an overall nosocomial infection rate of 8.2%

[3] E. Mbim, C. Mboto, and B. Agbo, “A Review of Nosocomial Infections in Sub-Saharan Africa,” *British Microbiology Research Journal*, vol. 15, no. 1, pp. 1–11, 2016.

[4] K. Dal-Bó, R. M. da Silva, and T. M. Sakae, “Nosocomial infections in a neonatal intensive care unit in South Brazil,” Dec-2012. [Online]. Available: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4031819/>. [Accessed: 20-Mar-2021].

The article investigates the incidence of nosocomial infections and its gravity amongst infants in neonatal care in an ICU in Southern Brazil. It discusses the severity of these infections on such young patients whilst identifying key prevailing pathogens alongside the diseases they trigger. A study was conducted across a year with 239 infants in care for up to 48 hours. It was discovered the nosocomial infection rate was as high as 45.8% with Coagulase-negative Staphylococcus being the main causative agent and nosocomial mortality rate reaching 33.8% .

[5] . S. Wu, B. Zhang, Y. Liu, X. Suo, and H. Li, “Influence of surface topography on bacterial adhesion: A review (Review),” *AVS*, 27-Nov-2018. [Online]. Available: <https://avs.scitation.org/doi/10.1116/1.5054057>. [Accessed: 29-Mar-2021].

- [6]. A. Persat, C. D. Nadell, M. K. Kim, F. Ingremeau, A. Siryaporn, K. Drescher, N. S. Wingreen, B. L. Bassler, Z. Gitai, and H. A. Stone, “The Mechanical World of Bacteria,” *Cell*, vol. 161, no. 5, pp. 988–997, 2015.

The article highlights the effects of the mechanics of surfaces on single celled bacterium behavior and their respective development into multi celled biofilms (bacterial communities). It discusses the roles clear physical structures bacteria possess on generating certain forces, particularly adhesive forces, on the colonization of surfaces.

- [7] F. Ghilini, D. E. Pissinis, A. Miñán, P. L. Schilardi, and C. Diaz, “How Functionalized Surfaces Can Inhibit Bacterial Adhesion and Viability,” *ACS Biomaterials Science & Engineering*, vol. 5, no. 10, pp. 4920–4936, 2019

- [8] S. Rigo, C. Cai, G. Gunkel-Grabole, L. Maurizi, X. Zhang, J. Xu, and C. G. Palivan, “Nanoscience-Based Strategies to Engineer Antimicrobial Surfaces,” 08-Mar-2018. [Online]. Available: <https://onlinelibrary.wiley.com/doi/full/10.1002/advs.201700892>. [Accessed: 05-Oct-2020].

The article looks at nanostructured synthetic surfaces with functional coatings and antimicrobial compounds that reduce bacteria proliferation for use in medical devices. It investigates the antifouling abilities of naturally occurring organisms using that inspiration to propose novel surfaces and patterns to annihilate bacterial colonization. Micropatterned surfaces with varying motifs could reduce bacterial coverage to as little as 11% on a surface

- [9] M. K. Gupta, “Natural Fibre Reinforced Polymer Composites: A Review on Dynamic Mechanical Properties,” *Current Trends in Fashion Technology & Textile Engineering*, vol. 1, no. 3, 2017.
- [10] B. A. Khan, P. Warner, and H. Wang, “Antibacterial Properties of Hemp and Other Natural Fibre Plants: A Review,” *BioResources*, vol. 9, no. 2, 2014.
- [11] De-la-Pinta, I., Cobos, M., Ibarretxe, J. et al. Effect of biomaterials hydrophobicity and roughness on biofilm development. *J Mater Sci: Mater Med* 30, 77 (2019). <https://doi.org/10.1007/s10856-019-6281-3>

Appendix

Table Showing Diameter Size Determination of Sisal Fiber

Fiber Serial Code	No. Of Ocular Divisions	Actual Girth Size Millimeter (Mm) (No. Of Ocular Divisions X 0.0256)
1	34.00	0.8704
2	9.00	0.2304
3	20.00	0.5120
4	17.00	0.4352
5	27.00	0.6912
6	25.00	0.6400
7	17.00	0.4352
8	10.00	0.2560
9	15.00	0.3840
10	13.00	0.3328
Average Readings		0.47872

Table Showing Diameter Size Determination of Coconut Fiber

Fibre Serial Code	No. Of Ocular Divisions	Actual Girth Size Millimetre (Mm) (No. Of Ocular Divisions X 0.0256)
1	9.00	0.2304
2	35.00	0.8960
3	12.00	0.3072
4	17.00	0.4352
5	18.00	0.4608
6	13.00	0.3328
7	10.00	0.2560
8	12.00	0.3072
9	40.00	1.024
10	10.00	0.2560
Average Readings		0.45056

Statistics Table Showing Unpaired T Test Of Girth Size: Tabular Results

Table Analysed	GIRTH SIZE
Column B vs. Column A	COCONUT FIBRE vs. SISAL FIBRE
Unpaired t test	
P value	0.7998
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.2573 df=18
How big is the difference?	0.4787 ± 0.06420 N=10
Mean ± SEM of column A	0.4506 ± 0.08862 N=10
Mean ± SEM of column B	-0.02816 ± 0.1094
Difference between means	-0.2581 to 0.2017

Table Showing Microbial Biomass Of Sisal And Coconut Fibers (Cfu/G)

Dilutions	1/10	1/10	1/10	1/100	1/100	1/100
Sisal Fibre	420	350	460	300	200	200
Coconut Fibre	18000	19320	20800	83600	82400	82800

Table Showing Average Microbial Biomass Of Sisal And Coconut Fibers (Cfu/G)

Microbial Parameter	Sisal Fiber	Coconut Fiber	Environmental Control Plates
Average Aerobic Total Biomass	251.67	51153.33	0.00
Unit	Cfu/G	Cfu/G	Cfu/G

Table Showing the Percentage of water absorbed from the 20ml in beaker by Sisal fibers

Duration of test (Hours)	Percentage of water absorbed (%), Sample 1	Percentage of water absorbed (%), Sample 2	Percentage of water absorbed (%), Sample 3
6	20.21	18.75	20.43
12	22.33	23.76	22.00
24	27.55	24.75	25.49
48	29.81	30.48	29.13
72	31.68	29.41	32.04

Table Showing the Percentage of water absorbed from the 20ml in beaker by Coconut fibers

Duration of test (Hours)	Percentage of water absorbed (%), Sample 1	Percentage of water absorbed (%), Sample 2	Percentage of water absorbed (%), Sample 3
6	27.88	28.85	31.07
12	34.31	36.63	33.65
24	49.48	46.53	48.04
48	61.76	66.33	63.37
72	61.32	64.08	63.73

Unpaired T Test of Water Absorbed: Tabular Results

Duration of test (Hours)	Sisal		Coconut		P-value	df	t
	Mean	SD	Mean	SD			
6	19.667	0.9130899	29.26667	1.63530	0.0009	4	8.7576
12	22.6977	0.9355392	34.86333	1.565162	0.0003	4	11.558
24	25.93	1.45093	48.01667	1.475139	0.0001	4	18.4887
48	29.806	0.6750063	63.82	2.317997	0.0001	4	24.4023
72	31.043	1.425915	63.04333	1.502676	0.0001	4	26.7561

Absorbance Values Of Standard Dye Solutions

Concentration	0.00015625	0.0003125	0.000625	0.00125	0.0025
Absorbance 1	0.082	0.123	0.249	0.625	1.315
Absorbance 2	0.081	0.122	0.247	0.631	1.313
Absorbance 3	0.078	0.121	0.248	0.632	1.318
Mean Value	0.080	0.122	0.248	0.629	1.315

Absorbance Values Of Dye Released Into The Decolorizer By Sisal Fiber

Timing (Seconds)	10	20	30	40	50	60
Absorbance 1	0.188	0.233	0.240	0.357	0.358	0.371
Absorbance 2	0.189	0.234	0.241	0.356	0.358	0.372
Absorbance 3	0.188	0.236	0.239	0.355	0.357	0.370
Mean Value	0.188	0.234	0.240	0.356	0.357	0.371

Table showing Absorbance Values Of Dye Released Into The Decolorizer By Coconut Fiber

Timing (Seconds)	10	20	30	40	50	60
Absorbance 1	0.331	0.340	0.390	0.500	0.598	0.825
Absorbance 2	0.328	0.339	0.389	0.504	0.599	0.824
Absorbance 3	0.333	0.340	0.388	0.499	0.598	0.823
Mean Value	0.331	0.340	0.389	0.501	0.598	0.824
Mass Of Fibre (G)	0.1	0.1	0.1	0.1	0.1	0.1

	Sisal		Coconut				
Timing (Seconds)	Mean	SD	Mean	SD	P- value	df	t
10	0.1883333	0.0005773514	0.3306667	0.002516609	0.0001	4	95.47
20	0.2343333	0.001527528	0.3396667	0.00057736	0.0001	4	111.72
30	0.24	0.001000002	0.389	0.0009999871	0.0001	4	182.4
40	0.356	0.001000002	0.501	0.002645751	0.0001	4	88.79
50	0.3576667	0.00057736	0.5983333	0.0005773428	0.0001	4	510.3
60	0.371	0.001000002	0.824	0.0009999871	0.0001	4	554.3

Coconut Fibre Universal Testing Machine Data

	Length (mm)	Maximum Tensile stress (MPa)
1	40.00000	0.40844
2	40.00000	0.57512
3	40.00000	0.50472
Mean	40.00000	0.49609
Standard Deviation	0.00000	0.08367

	Load at Maximum Tensile stress (N)	Tensile strain at Maximum Tensile stress (mm/mm)	Tensile extension at Maximum Tensile stress (mm)	Energy at Maximum Tensile stress (J)	Tensile stress at Break (Standard) (MPa)
1	4.08444	0.13000	5.20000	0.00761	-0.01788
2	5.75117	0.07125	2.84994	0.01008	-0.07434
3	5.04724	0.08125	3.25000	0.00625	0.04515

	Load at Maximum Tensile stress (N)	Tensile strain at Maximum Tensile stress (mm/mm)	Tensile extension at Maximum Tensile stress (mm)	Energy at Maximum Tensile stress (J)	Tensile stress at Break (Standard) (MPa)
Mean	4.96095	0.09417	3.76665	0.5500798	-0.01569
Standard Deviation	0.83671	0.03143	1.25733	0.00194	0.05977

	Load at Break (Standard) (N)	Tensile strain at Break (Standard) (mm/mm)	Tensile extension at Break (Standard) (mm)	Energy at Break (Standard) (J)	Tensile stress at Yield (Zero Slope) (MPa)
1	-0.17881	0.14900	5.96000	0.00851	0.03955
2	-0.74335	0.09295	3.71794	0.01043	-----
3	0.45146	0.10625	4.25006	0.00744	-----
Mean	-0.15690	0.11607	4.64267	0.00879	0.03955
Standard Deviation	0.59771	0.02929	1.17146	0.00151	-----

	Modulus (E-modulus) (gf/tex)
1	217974.53695
2	410558.18629
3	574733.46322
Mean	401088.72882
Standard Deviation	178567.87459

Sisal Fiber Universal Testing Machine results

	Length (mm)	Maximum Tensile stress (MPa)
1	40.00000	1.67264
2	40.00000	0.74485
3	40.00000	0.52066
4	40.00000	0.33927
Mean	40.00000	0.81935
Standard Deviation	0.00000	0.59255

	Load at Maximum Tensile stress (N)	Tensile strain at Maximum Tensile stress (mm/mm)	Tensile extension at Maximum Tensile stress (mm)	Energy at Maximum Tensile stress (J)	Tensile stress at Break (Standard) (MPa)
1	16.72636	0.05125	2.04994	0.01136	0.00457
2	7.44850	0.04375	1.75000	0.00466	-0.00896
3	5.20657	0.08625	3.45006	0.00591	0.00715
4	3.39268	0.02375	0.95000	0.00092	0.03971
Mean	8.19353	0.05125	2.05000	0.00571	0.01062
Standard Deviation	5.92549	0.02606	1.04246	0.00432	0.02064

	Load at Break (Standard) (N)	Tensile strain at Break (Standard) (mm/mm)	Tensile extension at Break (Standard) (mm)	Energy at Break (Standard) (J)	Tensile stress at Yield (Zero Slope) (MPa)
1	0.04566	0.07375	2.94994	0.01175	-----
2	-0.08959	0.08417	3.36700	0.00688	-----

	Load at Break (Standard) (N)	Tensile strain at Break (Standard) (mm/mm)	Tensile extension at Break (Standard) (mm)	Energy at Break (Standard) (J)	Tensile stress at Yield (Zero Slope) (MPa)
3	0.07153	0.13875	5.55000	0.00649	0.52066
4	0.39709	0.04875	1.95000	0.00138	-----
Mean	0.10617	0.08636	3.45423	0.00662	0.52066
Standard Deviation	0.20641	0.03796	1.51843	0.00424	-----

	Modulus (E-modulus) (gf/tex)
1	-----
2	887461.12771
3	379348.59828
4	-----
Mean	633404.86299
Standard Deviation	359289.81517