



# Microbial Assessment of Grey Water Samples Treated with Activated Carbon Forms of Selected Agro-wastes

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors NCA, OJN and PC designed the study & performed the statistical analysis. Author OJN wrote the protocol. Author NCA wrote the first draft of the manuscript. Authors NCA and PC managed the analyses of the study. All authors read and approved the final manuscript.

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## ABSTRACT

This study evaluated the efficacy of activated carbon from rice husk, corn cob and coconut husk wastes in the reduction of microbial properties of grey water samples harvested from students' hostels. Microscopic characterization, enumerations and identification of microbial isolates were carried out to determine the microbial community before and after the treatment with activated carbon. *Staphylococcus* sp, *Micrococcus* sp, *Bacillus* sp, *Salmonella* sp, *Saccharomyces* sp and *Penicillium* sp were observed to be present in the grey water. Before treatment, Total Heterotrophic Count (THC) was  $1.2 \times 10^{11}$  cfu/ml, Total Coliform Count (TCC),  $6.4 \times 10^6$  cfu/ml and Total Fungi Count (TFC)  $2.2 \times 10^{10}$  cfu/ml. THC after the treatment ranged from  $1.69 \times 10^9$  -  $7.6 \times 10^{10}$  cfu/ml; TCC,  $2.2 \times 10^5$  -  $7.3 \times 10^9$  cfu/ml and TFC  $1.0 \times 10^8$  -  $1.2 \times 10^9$  cfu/ml. Reduction in the microbial load after treatment revealed that activated carbons from rice husk, corn cob and coconut husk can be used singly or in combined states for the treatment of wastewater.

Keywords: Activated carbon; agrowastes; grey water.

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## 1. INTRODUCTION

Grey water/sullage is domestic wastewater that is collected from dwelling units, commercial building, and institutions of the community [1,2]. At global level, about 80% all domestic wastewater (grey water) is discharged into the environment untreated causing widespread water pollution [3]. These result in the contamination of water supplies and ground waters which are used for numerous activities like irrigation, drinking and recreation [4,5]. Studies done by Alens [6] and Environmental Protection Agency, EPA [7], revealed that pollution alone contributes to significant cases of aquatic live poisoning, gastroenteritis, hepatitis and infections in the eyes or nose during dermal exposure.

Like other developing countries, the management of waste and wastewater has been one of the major problems in Nigeria [8,9,5]. Major problems leading to wastewater pollution in Nigeria result from population, industrialization and urbanization [10]. Effective monitoring of physico-chemical and microbiological parameters, proper education and enlightenment of local people on the importance of water sanitation and good waste disposal method and reduction or prevention at the source are keys for proper management and prevention of water pollution [11,12]. The microbiological examination of water features a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health [13].

Generally, the physicochemical and microbiological parameter of wastewater varies [2], it is dependent on the factors such as the age and number of occupants, differences in hygiene conditions, variation in water economy (how they use water) [2,14,15]. The microbiological composition of greywater often contains  $10^5 - 10^8$  colony forming unit (CFU)/mL of coliform organisms,  $10^3 - 10^4$  CFU/mL fecal streptococci,  $10^1 - 10^3$  protozoan cysts, and  $10^1 - 10^2$  virus particles [16]. The microorganisms usually associated with grey water are *Enterobacter aerogenes*, *Proteus* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* species, *Staphylococcus aureus*, *Rhizopus* species, *Penicillium* species, *Aspergillus* species and *Trichoderma* species [17,2,18].

Activated carbon from agrowastes has lately been of study interest for their ability in the removal of pollutants from wastewater. Some

recent works include, coconut wastes and black oak bark [19]; corn cob, walnut shells [20]; corn cob, rice husk and coconut husk [21,22]; coconut husk [23]; banana peels [8]; corn cob [24]; oil bean (ugba or ukpaka) and snail shell [25]; Rice husk [26]; coconut shell [27]; corn cob [28]. Agrowastes have great potentials as inexpensive adsorbents [29,8]. Their abundance and availability in nature make them good sources of materials for activated carbon [30,31].

Activated carbon is defined as carbon that has been heated or otherwise treated to increase its adsorptive capacity [23]. The key property of activated carbon is adsorption, which allows gases and chemicals to adhere to millions of microscopic pores on the internal surface area of the material [25]. "Activating" carbon is the process of making the carbon high in surface area to facilitate adsorption. Adsorption is a process in which pollutants are adsorbed on the solid surface [32]. In adsorption technique, when a solution that contains adsorbable solutes is in contact with a solid with highly porous surface structure, liquid-solid intermolecular forces of attraction causes some of the solutes from the solution to be concentrated or deposited at the solid surface [33]. The solute retained (in the solid surface) in adsorption is called adsorbate, whereas the solid on which it is retained is called an adsorbent [34]. There are two basic methods by which activation can be produced: physical activation and chemical activation [32]. Physical activation comprises of two steps, carbonization of the precursor in an inert atmosphere, and subsequent activation of the resulting char in the presence of carbon gasification reactants (gaseous) such as carbon dioxide, steam or air at high temperature ranging between  $800^{\circ}\text{C}$  and  $1100^{\circ}\text{C}$  [35]. In this process, pore creation and development is not equal in all parts, it also takes longer time and consumes more energy for microporous activated carbon production [35]. Hence, the yield is very poor. Chemical activation involves heat treatment in an inert atmosphere at a temperature of  $400 - 600^{\circ}\text{C}$  and then impregnation [32]. The chemicals used as activators include transition metal salts like zinc chloride ( $\text{ZnCl}_2$ ), acidic group such as phosphoric acid ( $\text{H}_3\text{PO}_4$ ) and sulphuric acid ( $\text{H}_2\text{SO}_4$ ) and alkaline group such as potassium hydroxide (KOH) and potassium carbonate ( $\text{K}_2\text{CO}_3$ ). Compared to physical activation, chemical activation is more economical (uses lesser energy, short processing time and higher carbon yield) [36,37]. The efficiency or adsorption capacity depends on the nature of adsorbate

(easily ionized materials are adsorbed to a greater extent whereas low ionized materials are adsorbed to a lesser extent with pH as the controlling factor for ionization [38,39]. Other factors include: nature of adsorbent (each adsorbent has its own characteristics, and functional groups are the main metal binding factors); Specific area of the adsorbent (the more the surface area the more will be the biosorption [40,41]; pH (the concentration of OH<sup>-</sup> ion increases with the increase of pH, and number of exchangeable anions on the outer surface of the adsorbent will remain at higher pH values [42]; activation of the adsorbent (addition of activation agent results in the increase of spore, as a result of this the adsorption capability becomes quicker and increase adsorption capacity) [40]; and contact time (the removal is higher within the starting and step by step drops with time, that is most likely because of the larger surface area of the biosorbents being available at the start for the adsorption of metals) [25].

The current study is solely on the microscopic characterization, enumeration and identification of microbial isolates to evaluate the microbial community distribution before and after the treatment with activated carbon from selected agrowastes: rice husk, coconut husk and corn cob.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Samples

Agrowastes were sourced from Relief Market, Owerri, Imo State. Grey water samples were collected in triplicates from male and female hostels of Federal University of Technology, Owerri, using 500 ml sterile containers. All the analyses were carried out at Federal University of Technology, Owerri. Grey water samples from male hostel were labeled M; female, as F; while combined samples of the water at a ratio of 1:1 were labeled MF. Combined Sample (MF) was treated with the agrowastes.

### 2.2 Carbonization and Activation of Adsorbent [21,22,25,43].

Each agrowaste (750 g) was washed with deionized water, dried and grounded. They were carbonized in electric muffle furnace at 600°C for 45 min. The carbonized samples were sieved using 1.18 mm mesh size. To achieve activation, carbonized samples were mixed with 20% H<sub>3</sub>PO<sub>4</sub> solution at a ratio of 1:1 (Acid: Char) and stirred

for 30 minutes. The samples were then filtered, washed with deionized water until the washed off water gave a pH of 7.0. The washed activated carbon was later dried at 120°C in an oven and stored in airtight plastic container.

### 2.3 Adsorption Column Preparation

The adsorption column was constructed according to the work of Swraup & Umesh [44] as reported by Nduka et al., [21]. An apparatus (burette) of 50 cm length and 1cm diameter, and a collection flask were provided for this experiment. An outlet was provided at the bottom of the tank. Experiment was conducted by placing the individual adsorbents in column apparatus separately at first and then in combination. From the tank waste water was allowed to pass through the apparatus and collected water analyzed. Experiment was carried out at room temperature of 30 ± 2°C.

### 2.4 Microbial Analysis

#### 2.4.1 Preparation of samples and inoculation

One milliliter of the samples (wastewater) was transferred into 9 ml of physiological saline (diluent) and serially diluted until 10<sup>6</sup> were obtained. Aliquot portion (0.1 ml) of appropriate dilution was inoculated into the pre-sterilized and surface dried nutrient agar medium. Inocula were spread evenly to ensure uniform and countable colonies. Plates were incubated at 37°C for 24 - 48 hours [45].

#### 2.4.2 Determination of microbial population

Colony counts obtained on the media were expressed as colony forming units per ml (CFU/ml) by multiplying with dilution factor to obtain total population [46].

#### 2.4.3 Characterization and identification of microbial isolates

Microbial isolates were characterized based on cultural (colonial), microscopic and biochemical methods with reference to standard manuals for the identification of bacteria [46].

#### 2.4.4 Microscopic characterization

##### 2.4.4.1 Gram staining

An isolate smear was developed with a drop of water on grease free glass slide and permitted to

dry. The smear was mounted by delicate heating and flooded with crystal violet, then allowed to stand for 30 seconds and rinsed with water. Lugol's iodine was then added, allowed to stand for 30 seconds and rinsed with water and acid alcohol, till no colour change was observed. Safranin was used to counter stain; allowed to stand for 10 seconds, rinsed with water and air-dried. On the slide, oil immersion (1 drop) was added and viewed using the microscope's x100 objective lens [46].

#### 2.4.4.2 Spore staining test

This test was used to verify the presence of spores. Isolates were heat-fixed on a slide and flooded with 5% malachite green. It was steamed for 3 minutes (without boiling); allowed to dry, washed off and later stained with Safranin for 30 seconds. The slide was rinsed, dried and observed under the microscope using oil immersion lens. Green colour indicates spore while colour pink indicates negative spores.

#### 2.4.4.3 Motility test

This test was carried out to determine the motility of bacteria isolates. A semi-solid agar medium was used. The medium was poured into test tubes, autoclaved and allowed to set in an upright position. Isolates were inoculated using an inoculation needle by stabbing it into the medium in the test tube and then incubated at 37°C for 24 hours. Diffused growth from the straight line of inoculation indicates positive result [45].

#### 2.4.4.4 Biochemical characterization of bacteria isolates

Microorganisms were further subjected to few biochemical tests [45,46].

#### 2.4.4.5 Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. One drop of 3% hydrogen peroxide solution was placed on a clean slide. A loop full from 24 h culture was added. The release of bubbles (of oxygen) indicated the presence of catalase in the culture under test.

#### 2.4.4.6 Coagulase test

The coagulase test identifies whether an organism produces exoenzyme. This enzyme

clots the blood plasma by a mechanism that is similar to normal clotting. Samples were added to rabbit plasma in test tubes and incubated at 37°C for a specified period of time. Presence of clot (coagulation) within four hours indicates positive result and indicates a virulent *S. aureus* strain. After 24 hours, the absence of coagulation indicates a negative result.

#### 2.4.4.7 Oxidase test

This was used to check for the presence of terminal enzyme cytochrome c oxidase or cytochrome a. Members of enterobacteriaceae give negative oxidase test.

A piece of paper was placed in a clean petri dish and 2-3 drops of freshly prepared oxidase reagent (1% tetramethyl-p-phenylene diamine dihydrochloride) was added. A small portion of culture was placed in the filter paper with the help of a sterile glass rod and a smear was made. Colour change was examined within 10 seconds. Blue-purple colour indicated the presence of terminal enzyme cytochrome c oxidase or cytochrome a.

#### 2.4.4.8 Oxidation / sugar fermentation

This test was used to differentiate between bacteria groups that oxidize carbohydrate such as members of Enterobacteriaceae. One milliliter (1 ml) of 10% glucose, maltose, lactose, fructose, mannitol, and sucrose were separately transferred into duplicate tubes containing 9 ml of sterile Hugh and Leifson's medium to obtain a final concentration of 1% of each of sugar. The tubes were stab-inoculated in duplicates while two un-inoculated tubes served as control. One set of the duplicate tubes were covered with Vaseline and one control to discourage oxidative utilization of sugar. All tubes were incubated at 37°C for 48 h and observed for acid production in the culture. Yellow coloration in the open tubes indicated acid production, suggesting oxidative utilization of the sugar. Acid production in the sealed tubes indicated a fermentative reaction.

#### 2.4.4.9 Hydrogen sulphide production ( $H_2S$ ) test

Isolates were inoculated into a tube containing triple sugar iron agar by stabbing the agar to the bottom and streaking the surface of the slant. The inoculated tube was incubated at 37°C for 72 h and was examined daily. Black precipitate indicated  $H_2S$  production while yellow colouration indicated lactose, glucose and sucrose fermentation.

#### 2.4.4.10 Urease test

Isolates were inoculated in Urease Agar slant in McCartney bottle at 30°C for 4 hours then overnight. Pink colour in the medium indicated a positive result.

#### 2.4.4.11 IMViC test

This test determined the physiological properties of microorganisms. It consists of four different tests: indole test, methyl-red test, Voges Proskauer test and citrate utilization test. They are especially useful in the differentiation of Gram-negative intestinal bacilli, particularly *Escherichia coli* and the *Enterobacter-Klebsiella* group.

#### 2.4.4.12 Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid-Tryptophan to Indole. The bacteria isolates were inoculated into the medium and incubated at 37°C for 48 hours. At the end of incubation period, 3 drops of Kovac's reagents were added and then shaken. A red colour ring at the interface of the medium indicates a positive result.

#### 2.4.4.13 Methyl red test and Voges-Proskauer

Methyl red and Voges-Proskauer tests are considered together since they are physiologically related. Opposite test is usually obtained from the MR and VP test, that is, MR+, VP-, or MR-, VP+.

Methyl red test demonstrates the capacity of different organisms to produce acid from the fermentation of sugar (dextrose).

Inoculated glucose phosphate medium was incubated at 37°C for 2 days. Two drops of methyl red solution were added, shaken well and examined. Red colour indicated positive reaction while yellow indicated negative reaction.

The Voges-Proskauer test demonstrates the ability of organisms to produce acetoin from glucose metabolism. One milliliter (1 ml) of six percent alcoholic solution of alpha-naphthol and 1 ml of 16% KOH was added into 1 ml of the culture and stood for 15-20 minutes. Red to pink colour indicates a positive test.

#### 2.4.4.14 Citrate utilization test

This test demonstrates the ability of an organism to use citrate as its only source of carbon. It is

effective and used to assist in the identification of Enterobacteria. It was carried out using Simmon's citrate agar. The slopes of the media were prepared in bijou bottles as recommended by the manufacturers. A sterile straight wire was used to the slope with a saline suspension of the test organisms before stabbing the butt. The bottles were incubated at 35°C for 48 h. Bright blue colour in the medium indicates positive test while no change in colour indicates negative citrate test.

## 2.5 Statistical Analysis

Analysis of Variance (ANOVA) and Student t-Test at 0.05 (95%) significant level were used to analyze data generated.

## 3. RESULTS AND DISCUSSION

### 3.1 Microbial Load of Untreated Grey Water for Male and Female Hostels

From Table 1, Total Heterotrophic Count (THC) range was  $6.9 \times 10^{10}$  -  $1.2 \times 10^{11}$  cfu/ml; Total Coliform Count (TCC) ranged from  $3.7 \times 10^6$  -  $6.4 \times 10^6$  cfu/ml while Total Fungi Count (TFC) ranged from  $1.5 \times 10^8$  -  $2.2 \times 10^{10}$  cfu/ml. The result is higher than the WHO [47] effluent discharge limits for households. At  $p < 0.05$ , there was no significant difference between male, female and combined hostels, the common source of water, within the same age bracket and limited anthropogenic activities such as food production, may be a factor [48]. From Tables 2, 3 and 4, *Micrococcus* sp, *Staphylococcus* sp, *Enterobacter* sp, *Salmonella* sp, *Shigella* sp, *Bacillus* sp and *Saccharomyces* sp were present in the grey water samples. From Table 5, *Bacillus* sp was observed to be present in the female hostel grey water samples only, while *Micrococcus* sp was observed in sample from the male hostel. Eze et al. [2], carried out microbiological characteristics of grey water samples in Umuahia, and obtained microbial isolates such as *Enterobacter aerogenes*, *Proteus* species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* species, *Staphylococcus aureus*, *Rhizopus* species, *Penicillium* species, *Aspergillus* species and *Trichoderma* species. *Salmonella* sp and *Shigella* sp indicate faecal contamination in water. *Enterobacter* sp (eg *E.coli*) has been implicated as a causative agents of water-borne diseases. Pollutants detected in grey water may arise from four major sources: Household infrastructure (Household

pipes used for supply of water and collection of wastewater), water supply (source of water), materials used in household infrastructure and plumbing (Fittings, such as taps, hot water storage systems, sinks), anthropogenic waste and household practices and products used in daily activities (waste excretion by metabolic processes (faeces, urine, perspiration); waste generation by householder activities and behaviour, such as food preparation, grooming, bathing and cleaning [14]. Contaminants within households also vary with household water consumption, infrastructure and activities [14]. Contaminants can also be influenced by passive transport or migration from household materials to water; the type of products adopted in the household, the amount and frequency used, the householders' habits, their diet and the use of household appliances such as washing

machines and dishwashers, as well as their age groups [2], [14,15].

The high loads of microorganisms from this study showed that the grey water effluent needs to be treated before being discharged into the water bodies. Eze et al. [2], in a study carried out in Umuahia, total heterotrophic plate count range was  $8.1 \times 10^5 \pm 0.04 - 1.11 \times 10^6 \pm 0.40$  cfu/ml. In a related study by Ogah & Ogah [49], carried out in Otamiri River, FUTO (the river where the effluent from the hostels were discharged), total heterotrophic count range of  $1.2 \times 10^5 - 9.0 \times 10^5$  cfu/ml and total coliform count range of  $5.0 \times 10^5 - 9.0 \times 10^5$  cfu/ml, were obtained. Duru et al. [50] also observed microbial load levels of  $4.0 \times 10^4 - 5.1 \times 10^6$  cfu/ml with *proteus* sp and *vibrio* sp, in addition to bacterial isolates as reported in Tables 2, 3 and 4.

**Table 1. Microbial load of untreated grey water from male and female hostels**

Parameter (CFU/ML)	M	F	MF	WHO Limits
THC	$6.9 \times 10^{10}$	$1.2 \times 10^{11}$	$8.9 \times 10^{10}$	$\leq 1.0 \times 10^3$
TCC	$4.9 \times 10^6$	$6.4 \times 10^6$	$3.7 \times 10^6$	$\leq 1.0 \times 10^2$
TFC	$1.5 \times 10^8$	$2.2 \times 10^{10}$	$1.69 \times 10^{10}$	-

All values were expressed as Mean. M – grey water samples from male hostel; F – grey water from female hostel; MF – combined grey water from male and female hostels; THC = Total Heterotrophic Count; TCC = Total Coliform Count; TFC = Total Fungi Count

**Table 2. Biochemical characteristics of bacterial isolates from grey water samples**

Gr	Mo	Cat	Oxi	Coag	In	MR	VP	Cit	Ure	H <sub>2</sub> S	G	S	L	Suggested organism
+	-	+	-	+	-	-	+	-	+	-	+	+	+	<i>Staphylococcus aureus</i>
+	-	+	-	-	-	+	-	+	+	-	-	-	-	<i>Micrococcus luteus</i>
+	+	+	-	-	-	-	+	+	-	-	+	-	-	<i>Bacillus</i> sp
-	-	+	-	-	-	+	-	+	-	+	+	-	-	<i>Salmonella</i> sp
-	-	-	-	-	-	+	-	-	-	-	-	-	-	<i>Shigella</i> sp
-	-	+	-	-	-	+	-	+	-	-	+	+	+	<i>Enterobacter</i> sp

Gr, Gram Reaction; Mo, Motility; Cat, catalase; Oxi, oxidase; Coag, coagulase; In, indole; MR, Methyl Red; VP, Voges Proskauer; Cit, citrate; Ure, urease; H<sub>2</sub>S, hydrogen sulfide production, G, glucose, S, sucrose; L, lactose; M, maltose

**Table 3. Colonial and microscopic characteristics of fungal isolates**

Colonial characteristics	Microscopic characteristics	Identity of isolates
Small circular moist and shiny yellow colonies	Gram positive spherical budding cells	<i>Saccharomyces</i> sp
Small circular moist and shiny cream colonies	Large Gram positive oval budding cells	<i>Saccharomyces</i> sp
Cream butyrous raised dull and mucoid colonies	Gram positive ellipsoidal budding cells	<i>Saccharomyces</i> sp
Dirty green powdery spores enclosed in white mycelium	Septate hyphae, conidia mob like	<i>Penicillium notatum</i>

**Table 4. General Distribution of Bacteria and Fungi in sullage samples**

Samples	Bacterial isolates	Fungal isolates
Male	<i>Micrococcus</i> sp, <i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Female	<i>Bacillus</i> sp, <i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Male + Female	<i>Micrococcus</i> sp, <i>Bacillus</i> sp, <i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Rice husk	<i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Corn cob	<i>Enterobacter</i> sp, <i>Staphylococcus</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Coconut husk	<i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp, <i>Penicillium</i> sp
Corn cob + Coconut husk	<i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Rice husk + Corn Cob	<i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Rice husk + Coconut husk	<i>Enterobacter</i> sp, <i>Salmonella</i> sp, <i>Salmonella</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp
Rice husk + Corn cob + Coconut husk	<i>Staphylococcus</i> sp, <i>Enterobacter</i> sp, <i>Shigella</i> sp	<i>Saccharomyces</i> sp, <i>Penicillium</i> sp

**Table 5. Microbial load of grey water treated with adsorbents**

Media	Rh	Cc	Ch	Rh + Ch	Rh + Cc	Cc + Ch	Rh + Cc + Ch	WHO Limits
THC	5.3x10 <sup>10</sup>	7.3x10 <sup>9</sup>	7.6x10 <sup>10</sup>	3.6x10 <sup>10</sup>	1.9x10 <sup>10</sup>	3.98x10 <sup>10</sup>	1.6x10 <sup>9*</sup>	≤ 1.0x10 <sup>3</sup>
TCC	2.3x10 <sup>6</sup>	7.3x10 <sup>8</sup>	1.1x10 <sup>6</sup>	3.9x10 <sup>5</sup>	3.6x10 <sup>5</sup>	2.2x10 <sup>5</sup>	6.5x10 <sup>5</sup>	≤ 10x10 <sup>2</sup>
TFC	1.67x10 <sup>8</sup>	7.3x10 <sup>8</sup>	1.3x10 <sup>8</sup>	1.0x10 <sup>8</sup>	2.6x10 <sup>8</sup>	1.2x10 <sup>9</sup>	2.0x10 <sup>8</sup>	-

Rh = rice husk; Cc = corn cob; Ch = coconut husk; Rh+Ch = rice husk+ coconut husk; Rh+Cc = rice husk+corn cob; Cc+Ch = corn cob+coconut husk; Rh+Cc+Ch = rice husk +corn cob +coconut husk. THC = Total Heterotrophic Count; TCC = Total Coliform Count; TFC = Total Fungi Count. MF = combined untreated sullage from male and female which was treated using the adsorbents. All values were expressed as Mean (CFU/ML). LSD \*, shows the value with significant different compared with MF at p ≤ 0.05

### 3.2 Microbial Load of Grey Water Treated with Adsorbents

From Table 5, Total Heterotrophic Count after the treatment ranged from 1.69 x 10<sup>9</sup> to 7.60 x 10<sup>10</sup>cfu/ml; Total Coliform Count ranged from 2.2 x 10<sup>5</sup> - 7.3 x 10<sup>8</sup>cfu/ml while Total Fungi Count ranged from 1.0 x 10<sup>8</sup> - 1.2 x 10<sup>9</sup>cfu/ml.

From Table 5, in “Microbial Load of Grey Water Treated with Adsorbents”, it can be observed that the combination of the adsorbents achieved better removal efficiency. Other studies reported that combined carbon from agro-wastes offered better attachment pores (sites) for contaminants [23,20]. Although from Table 4, *Bacillus* sp was not present after treatment with the adsorbent, *Micrococcus* sp, *Bacillus* sp, *Staphylococcus* sp, *Enterobacter* sp, *Salmonella* sp, *Shigella* sp and

*Saccharomyces* sp were observed to be present in some of the grey water samples even after the treatment with the adsorbents. A decrease in the microbial load was observed in the treatments except the treatment with corn cob (CC) for total coliform Count (Corn cob and its derivatives are known to be a good medium for microbial growth [51]. This study suggests that the adsorbents have antibacterial activity. Nuhu, et al., [23] reported that agricultural waste-based activated carbon contains antimicrobial activity against pathogenic *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Nuhu et al., [23] on using the shake flask technique, found that bacterial count reduced after one-hour incubation, while no bacterial growth was detected after 23 and 24 hours using the blended activated carbon from *Melaleuca leucadendron* husk and *Caesalpinia pulcherrima* husk. In a

related work [52], activated carbon derived from rice husk and coconut husk was found to be effective in decontaminating water containing *Escherichia coli* (*E. coli*), the carbons showed > 99% removal of *E. coli*. Further examination [52] using SEM (Scanning Electron Microscope) and BET (Brunauer Emmett Teller, particle surface area measurements) results reveal that the carbons were mesoporous in nature while FTIR (Fourier-transform infrared spectroscopy) showed the presence of functional groups viz. C = O and –OH that might be responsible for adsorption of *E. coli* on the carbon. Leili & Detlef [53] reported that microorganisms attach to activated carbon through strong LiftShitz and vanderWaals forces.

#### 4. CONCLUSION

Microbial properties of grey water samples from student hostels before the treatment exceeded the WHO discharge limits, which pose danger to the receiving water body, posing health risks to communities that depend on such water body as source of water supplies. However, activated carbon from rice husks, corn cob and coconut husk wastes used as adsorbents, were able to reduce the microbial loads (THC, TCC and TFC) with their combination being more efficient in the treatment process. Thus, the adsorbents can provide alternative, cost effective, non-chemical and less toxic way of treating wastewater with high microbial load.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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