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Production of Clay-Based Water Filter Using Biomass of *Bacillus Subtillis*, Sawdust, Activated Charcoal, Periwinkle and Snail Shell as Additives

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Abstract

A Significant proportion of rural households lacked access to improved and safe drinking water due to chemical and microbial contamination. Point-of-use (POU) water filters made from cheap, locally available ceramic materials and additives can achieve quality water parameters. Ceramic water filters were prepared by combining clay minerals with additives. Sawdust was used as a burnout material to achieve porosity and enhance the filtration rate. Silver nitrate, charcoal, periwinkle shell, snail shell, and biomass of *Bacillus Subtilis* were added in different ratios. The filter was formulated with charcoal, sawdust, snail shell, and periwinkle shell to remove microbes and treat heavy metals through the adsorption process. The filters were molded and fired in a temperature range of (700°C - 900°C). Characterization of the clay mineral, physiochemical and Microbial tests were conducted on the ceramic and water. Antimicrobial test was carried out on the biomass of *Bacillus subtilis*. Mineralogical (XRD) and elemental analysis of the clay, snail, and periwinkle shells showed high percentage composition of serpentine (a clay crystal), plagioclase, a mixture of feldspar minerals albite (sodium aluminosilicate - $NaAlSi_3O_8$), anorthite calcium aluminosilicate- $CaA_{l2}Si_2O_8$) and Calcium (70-97 %composition) respectively. The results showed a greater proportion of silica in the clay, suggesting the material is silicate. Filtration rate was estimated at 1.125 L/hr. The result showed the filter has 96.72%, 99.26%, and 66.67% colony removal efficiency for heterotrophic bacteria, coliform, and fungi respectively. The filter showed about 70% - 96% efficiency for the treatment of physiochemical parameters in wastewater.

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1. Introduction

In 2010, United Nation declared access to safe water as a human right [1]. A safe water is one that is free of fecal and priority chemical contamination [2]. Water is therefore considered safe for drinking when it conforms with WHO guidelines on acceptable chemical and microbiological levels [3]. However poor access to safe water around the world remains one of the most challenges facing humanity with Sub-Saharan Africa housing about 48% of global population of people without access to safe water [4]. Rural communities in Nigeria account for 48% of the total population of the country [5]. Yet, these communities are faced with challenge of inaccess to safe water [6]. For instance, as at 2020, approximately 35.8 million Nigerians lack access to safe drinking water [7].

Most rural communities in Nigeria only have access to water resources from rivers, lakes, and streams in a rarely domestically usable form due to pollution [3, 8]. Utilizing such water resources can lead to different disease conditions [9, 10]. Each year, more than 1.8 million cases of diseases related to drinking contaminated water are reported in East, West and Central Africa [11].

Water drawn from rivers, lakes, streams or even underground is hardly clean enough for consumption [3] unless it undergoes some treatments to render it safe for human consumption. Purification of water collected from surface and underground sources, is one of the major challenges of the present-day civilization in the rural communities [12]. Over the years, various water purification technologies have been developed [13], but most of these technologies require high energy input and are capital intensive, thus making them inaccessible to rural populations in the developing countries [14]. The clay-based ceramic water filtration system is fast becoming a low-cost option for rural populations in the developing world, to provide improved potable water [15]. It is a simple, cheap and energy efficient method of water purification.

Ceramic water filters rely on small pore sizes of the ceramic's material to filter dirt, debris, and bacteria out of the water [16], making this technology ideal for use in developing countries [17]. The filter is made of a mixture of clay and sawdust kneaded together, molded, dried and fired to make it a structurally stable vessel [18]. The ceramic water filter can be coated with colloidal silver [15], or Biomass of *Bacillus subtilis* – a combination of *Bacillus subtilis* and Silver nitrate. Like other methods of water filtration, the filter removes particles larger than the size of the pores in the filter material.

Though, this filtration technology is effective in removing dissolved solid particles and microorganisms [19-21], it however presents low ability to remove viruses from contaminated water [15]. This research is therefore aimed at developing a low-cost ceramic water filter with improve efficiency in water purification from mixture of clay, sawdust, silver nitrate, charcoal, periwinkle shell, and snail shell.

2. Materials and Methods

The basic raw material used to make the ceramic water filters were; clay minerals, sawdust, activated charcoal, periwinkle shells, snail shell, and $AgNO_3$ precipitated *BacillusSubtillis*. The equipment used were analytical balance, ball mill, press machine, beaker, conical flask, rotary kiln. The experimental method of agreement was used to determine the causal relationship between the effect E, the change in amount of impurities in water samples; and the presence of factor F, the additives in a clay-based ceramic body composition for water filter production.

2.1. Sample Collection and Preparation

Clay was collected from Okelele deposit at Ilorin, Kwara State Nigeria using hand grabbing method and composite sampling technique. The clay sample was dried, pulverized with mortar and pestle, and sieved with 0.5mm sieve size. The saw dust was collected from a local sawmill. After drying, it was screened through a sieve with 0.5 mm opening. The periwinkle shell was sourced from Bayelsa State. The periwinkle shells were washed to remove extraneous materials, calcined at a temperature of 800°C and pulverized using a ball mill. The Snail shell was sourced from Ilorin. It was calcined at a temperature of 800°C and pulverized using a ball mill. The charcoal was collected in Malete, Moro LGA, Kwara State. It was sun dried and milled to powder fineness of 75% <300μm using a ball mill.

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Table 1. Composition of the Filter Material

Mat.	Sawdust (g)	Charcoal (g)	Snail shell (g)	Periwinkle shell (g)	Clay (g)	Total (g)	Vol of water (mL)
Comp.	875	875	875	875	3500	7000	200

The clay formed the base material in the ceramic body, the sawdust functions as the burnout that creates the required pores in the filter. The higher the proportion of the burn out the higher the porosity of the ceramic filter [22]. Charcoal was also used as a burnout material to create finer pores in the ceramic and to possibly introduce activated carbon into the filter to serve as platform for the adsorption and removal of trace and heavy metals from the water [23]. Periwinkles and snail shell were added to introduce calcium silicate for the removal of heavy metals from the water [24]. The ceramic filters were coated with silver nitrate precipitated *Bacillus Subtillis*, a well-studied antimicrobial agent, that has no effect on the taste, color, and odor of the treated water [25].

2.2. XRD Characterization of the clay sample

Clay sample was crushed and milled to fine particle sizes. The mineral phases in the samples were identified by powered X ray diffractometry method. The sample was first subjected to X-ray scanning using the Philip PW 1830 ray X-ray diffractometry with Cu anode. The X-ray scanning of the sample mineral peaks were identified using XPert high score plus software. The background and peak position were identified and based on the peak position and intensity; a search match routine was performed.

2.3. XRF Characterization of Samples

X-ray fluorescence (XRF) was used to determine mineralogical composition of the clay, the periwinkle shells and the snail shell used for the ceramic body composition. The XRF is a powerful analytical technique used in a wide variety of industries to determine the elemental composition of various materials. The analyzers are widely recognized as a means for accurate, rapid, and non-destructive testing. In this work, the Skyray EDX3600B, which is of a high-end energy dispersive XRF spectrometer (EDXRF) with a large sample chamber which supports most sample sizes was employed.

2.4. Clay Filter Formation

Based on an existing composition, the raw materials – clay, sawdust, periwinkle shells and snail shells were weighed, combined in ratio 4:1:1:1 and thoroughly mixed. 200 mL of distilled water was then added to the mixture (Table 1). This was then mixed to form a homogeneous plastic body. Approximately, 7 Kg of the resultant plastic body was placed on the press machine to form the shape of the filter, using the hydraulic jack (Fig. 1 & 2) to exert pressure. The shaped piece was demolded and dried under natural convection to remove any free water in the sample and then fired in an electric kiln to a temperature of 800°C. Surface water samples were collected from two different sources, filtered and taken to laboratory to test for the efficiency of the filter.

2.5. Biomass Production of B. Subtilis with Silver Nitrate

Bacillus subtilis was sub-cultured in nutrient broth. The nutrient agar was dispensed in five Erlenmeyer flask using 1000ml per flask, and then autoclaved at 15 psi for 15 minutes. After sterilization, each flask was inoculated with 20 mL (10%) of 36-hour old SDW suspense of *B. subtilis.* The culture was shaken for 36 hours. The biomass was harvested by centrifugation at 3500 rpm for 15 minutes. The centrifuged cells were used in the formulation. 0.42g of silver nitrate was weighed into beaker and distilled water was added to dissolve it until it was completely soluble and made up to 1000 mL with distilled water in a round bottom flask. 4 g of sodium hydroxide was weighed into a beaker, and it was dissolved with some amount of distilled water, it was made up to 1000 mL in a round bottom flask. The essence of using sodium hydroxide was to precipitate the biomass of *Bacillus subtilis*.

Figure 1. The Hydraulic Press Machine

2.6. Microbiological Analysis of Water samples

Isolation of microorganism from the water sample was carried out using the pour plate method in duplicate trials. Serial dilution of the water sample was done in 10-folds. 1 mL was taken from the last dilution into sterilized petri dishes. The media used were nutrient agar, eosin metheline blue agar (EMB), and potato dextrose agar (PDA). The agars were prepared according to respective manufacturer's specification. The molten agar was poured on the inoculated plate, mixed gently and allowed to solidify. The nutrient agar and EMB were incubated at 37^o C for 24 hours. While the PDA plate were incubated at room temperature for 5 days. At the end of the incubation period, the cultured plate was observed for the presence of microorganism growth. The growth on the plate were counted with colony counter and expressed in colony forming unit (cfu) per mL. Serial dilution of water sample was carried out using 10-fold serial dilution method. 9 mL of distilled water was poured into test tubes. The mouth of the test tubes was corked with non-absorbent cotton wool. The test tubes were then sterilized in an autoclave at 121^o C for 15 minutes. After cooling, 1 mL of water sample was poured into the first test tube and shaken. From this, 1 mL of the mixture was taken and poured into the second test tube. 1 mL was also taken from the second test tube and transferred into the third test tube till the fifth. After serial dilution, 0.1 mL of the diluent was used as the inoculant and was inoculated for microbial counts through pour plate method in duplicate. About 25 mL of cooled molten agar were then poured and swirl on the bench to mix thoroughly. It was left on the bench to set and solidify. The plates were incubated at ambient temperature for 48-72 hrs for fungi, bacteria at 37 ^{o}C for 24 hrs while coliform were incubated at 37 ^{o}C for 24 hr. At the end of incubation, the microorganisms growing on each plate were counted and recorded in colony forming units (cfu).

Figure 2. Fired Filter Cells

Table 2. XRF Analysis of Clay Sample

Element	Al	Si	S	K	Ca	Fe	Sn	Sb
Conc.(mg)	17.54	27.01	1.44	1.33	7.25	5.09	1.52	1.45
%composition	28.0	43.12	2.30	2.12	11.58	8.13	2.43	2.32

Table 3. XRF Analysis of the Clay Sample in its Oxide Form

Element	Al_2O_3	SiO_2	SO_2	K_2O	CaO	FeO	SnO_2	Sb_2O_3
Conc.(mg)	27.67	42.64	2.27	2.09	11.44	8.03	2.39	2.28
%composition	28.0	43.15	2.30	2.12	11.57	8.13	2.42	2.31

3. Results and Discussion

Figures 3 and 4 show the XRD mineral pattern of the clay samples. The mineral contents are seen at different peak intensities with highest peak corresponding to plagioclase in sample A and quartz in sample B.

Table 2 and 3 showed the characterization of clay sample both in elemental and oxide forms. The results showed large proportion of silicon, indicating that the material is a silicate. The presence of aluminum really confirms the sample to be a clay containing impurities like feldspar because of the potassium, limonite and the Fe content. This result is in agreement with the result of the XRD obtained by [26] which has serpentine (a clay crystal), plagioclase, a mixture of feldspar minerals albite (sodium aluminosilicate - $NaAlSi_3O_8$) and anorthite calcium aluminosilicate - $CaA_{l2}Si_2O_8$)

Table 4 and 5 show the XRF characterization of snail shells in both elemental and oxide forms. Calcium appeared the most predominant element in both elemental and oxide forms accounting for 96.90% and 96.33% respectively

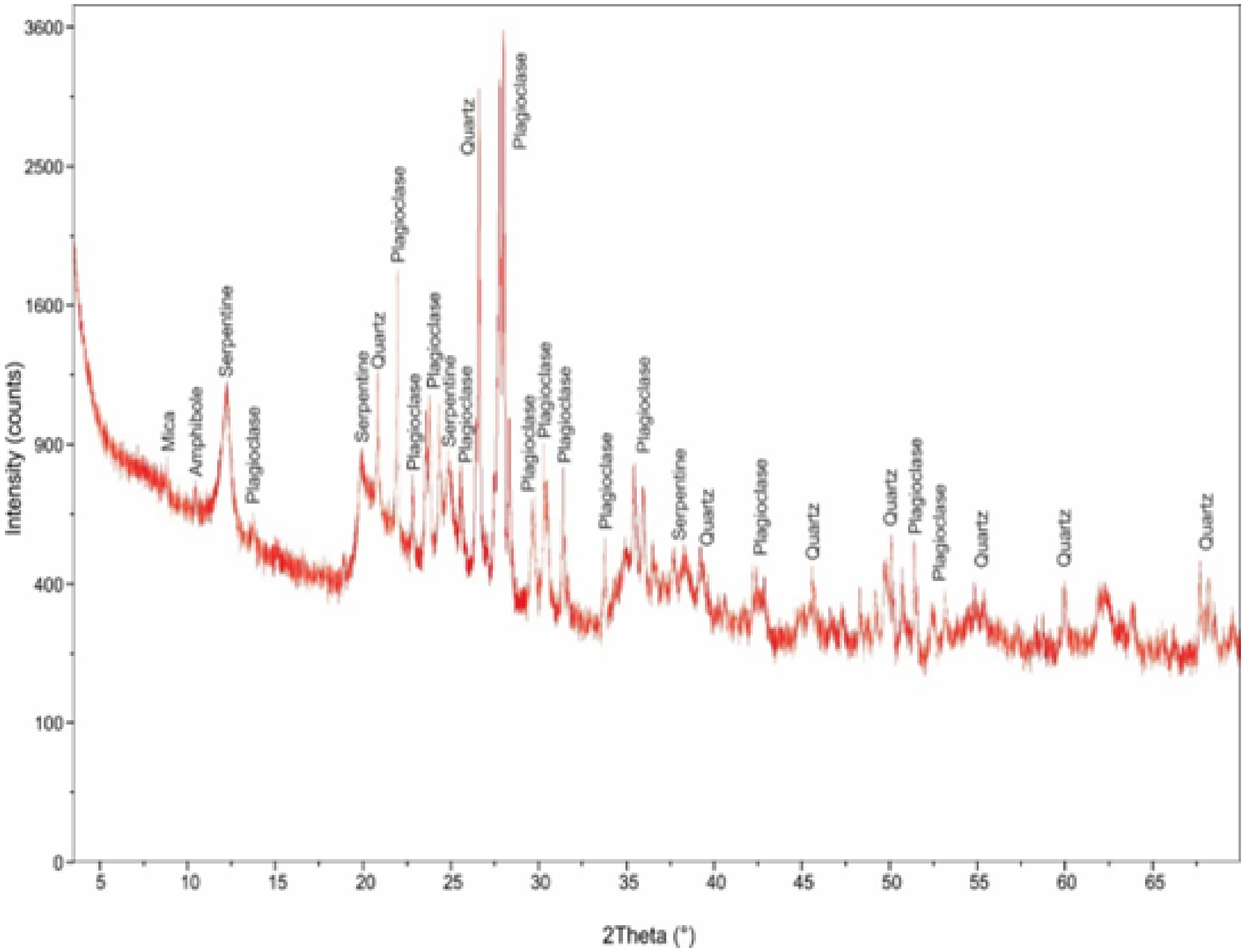

Figure 3. XRD Mineralogy Analysis of Clay Sample A

Table 4. XRF Analysis of Snail Shell Ash

Element	Ca	Al	Si	S	Fe	Sb	Sn
Conc.(mg)	70.01	0.32	0.31	0.69	0.26	0.34	0.32
%composition	96.90	0.44	0.43	0.96	0.36	0.47	0.44

Table 5. XRF Analysis of Snail Shell in its Oxide Form

Element	CaO	Al_2O_3	SiO_2	SO_2	FeO	Sb_2O_3	SnO_2
Conc.(mg)	96.29	0.43	0.43	0.95	0.95	0.47	0.44
%composition	96.33	0.43	0.43	0.95	0.95	0.47	0.44

while silicon is the least with 0.43% elemental and 0.43% oxide. This is in agreement with characterization of the shells of three freshwater snails in which it was reported that $CaCO_3$ content ranges between 87% and 95% [27]. The snail shell composition was used so as to trap heavy metal in the composition by adsorption process [28]. The high percentage composition of calcium in the sample will contribute to the strength of the clay-based filter. Other traces of element present in the samples are: Al_2O_3 (0.43%), SO_2 (0.95%), FeO (0.95%), Sb_2O_3 (0.47%), and SnO_2 (0.44%).

Table 6 and 7 showed the characterization of periwinkle shells in elemental and oxide forms. Calcium constitutes the highest mineral contents of periwinkle shell with 97.68% and 97.45% in both elemental and oxide forms respec-

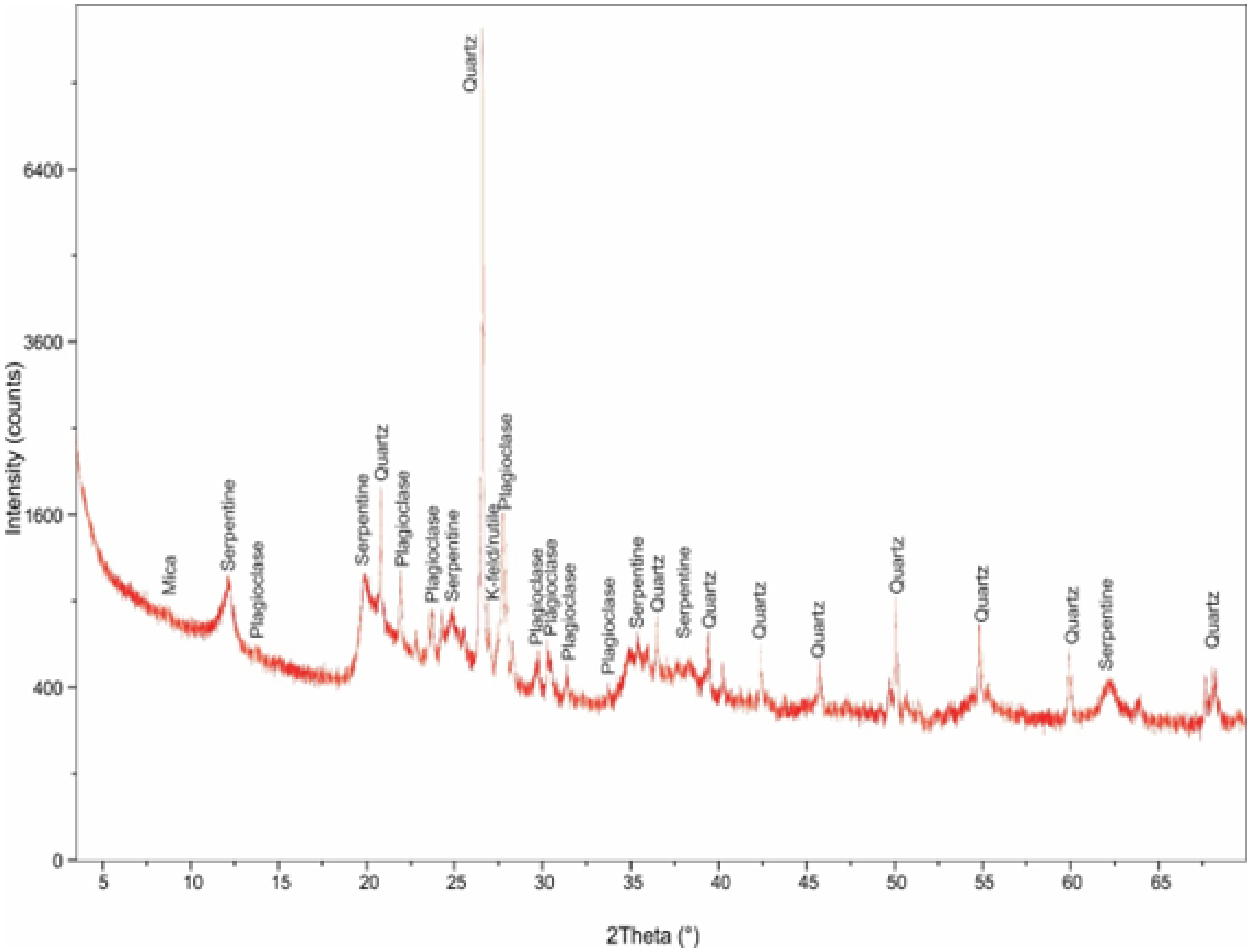

Figure 4. XRD Mineralogy Analysis of Clay Sample B

Table 6. XRF Analysis of Periwinkle Shell Ash

Element	Ca	Al	Si	S	Fe	Sb	Sn
Conc.(mg)	71.93	0.30	0.23	0.49	0.09	0.31	0.29
%composition	97.68	0.41	0.31	0.67	0.12	0.42	0.39

Table 7. XRF Analysis of Periwinkle Shell Ash in Oxide Form

Element	CaO	Al_2O_3	SiO_2	P_2O_5	SO_2	SnO_2	Sb_2O_3
Conc.(mg)	97.04	0.41	0.31	0.34	0.66	0.40	0.42
%composition	97.45	0.42	0.31	0.34	0.66	0.40	0.42

tively. Silicon is the least mineral with 0.31% and 0.31% composition in elemental and oxide forms. Other trace elements in oxide form present in the samples are: Al_2O_3 (0.41%), SO_2 (0.66%), P_2O_5 (0.34%), Sb_2O_3 (0.42%), and SnO_2 (0.40%). This result agrees with earlier report that snail shells contain predominantly $CaCO_3$ in three phases, namely, aragonite, calcite and vatanite by [29].

The quantity of water filtered per time was measured in liter per hour as shown in Table 8. The measurement began when the filter cell had become thoroughly saturated. At that point, the filter is filled, and the filtrate is captured over a period of known time. The volume of the filtrate was then measured with a measuring cylinder. The average

Table 8. Flow rate of Filtered Sample

Volume (Ltrs)	Time (hrs)	Flow rate (L/ hrs)
4.5	4	1.125

Table 9. Physiochemical Parameters of Water Sample Before and After Filtration Compared with WHO Standards

Parameters	Untreated	Treated	*WHO	Filter Efficiency
TDS (mg/L)	1505.00	130.50	500	91%
Alkalinity (mg/L)	2000.00	304.00	300	85%
Conductivity (µS/cm)	3130.00	273.00	1000	91%
Salinity (mg/L)	1514.59	65.58	100	96%
pH	7.6	7.6	6.5 - 8.5	0%
BOD (mg/L)	2.07	1.24	10	40%
COD (mg/L)	1000.00	40.00	40	96%
Phosphate (mg/L)	56.00	16.00	0.5	71%
Sulphate (mg/L)	0.00	0.00	250	
Nitrate (mg/L)	164.31	41.75	50	75%
Chlorine (mg/L)	841.44	36.58	250	96%
Iron (mg/L)	0.27	0.02	0.3	93%
Hardness (mg/L)	1142.40	147.00	100	87%

*[30]

Table 10. Antimicrobial analysis result on Biomass of *Bacillus subtilis*

Extract/Antibiotics	**Zone of inhibition (mm)**			
	Escherichia coli	*Salmonella typhi*	*Klebsiella oxytoca*	*Pseudomonas aeruginosa*
Extract (250mg/ml)	0	0	13.67 ± 0.33	0
Ceftadime (CAZ) 30µg	0	0	0	0
Cefuroxime (CRX) 30µg	0	0	0	0
Gentamicin (GEN) 10µg	0	23	0	18
Ciprofloxacin (CPR) 5µg	0	23	0	32
Ofloxacin (OFL) 5µg	0	24	0	29
Amoxicillin-clavunanic acid (AUG) 30µg	0	0	0	0
Nitrofurantoin (NIT) 300µg	9	27	17	27
Ampicillin (AMP) 10µg	0	0	0	0

filtration was 1.125L/ hrs.

Table 9 showed the values of physicochemical parameters of the water sample before and after filtration. These values were compared with the WHO standards for drinking water. The water sample shows the value of most of the physicochemical parameters higher than the recommended values by the World Health Organization thus indicating the non-suitability of this water sample for drinking purpose. However, the values for the water sample after filtration with ceramic filter shows significant reduction in physicochemical parameters values, even below the recommended standard. This therefore suggests that the ceramic filter effectively showed tremendous efficiency in removing wastewater pollutants, thus making the water suitable for drinking. The efficiency is estimated as the extent to which a particular impurity was removed by the filter. This was calculated using the relation.

$\frac{C_i - C_f}{C_i} \times \frac{100}{1}$ Where C_i = initial concentration of contaminant and C_f = final concentration of contaminant after filtration.

Table 10 above shows the antimicrobial analysis result on Biomass of *B. subtilis*. The biomass (B. *subtilis* and

Table 11. Microbial Contents of Water Sample Before and After Filtration

Total Heterotrophic Count (cfu/ml)			**Total Coliform Count (cfu/ml)**			**Total Fungi Count (cfu/ml)**		
Before	After	CRE (%)	Before	After	CRE (%)	Before	After	CRE (%)
6.1 x 10^6	2.0 x 10^5	96.72	2.7 x 10^4	2.0 x 10^2	99.26	9.0 x 10^2	3.0 x 10^2	66.67

* CRE = colony removal efficiency

silver nitrate), a biocontrol agent was tested against four microorganisms to determine the zone of inhibition. The result showed that the Biomass of *B. subtilis* at 250mg/ml only inhibit the growth of *Klebsiella oxytoca* at zone of 13.67 ± 0.33 mm. This means that a higher concentration of *B. subtilis* is required to inhibit these microorganisms.

The colony removal efficiency of the ceramic filter was determined by the following expression:

$$\text{Colony removal efficiency} = \frac{Cb - Ca}{cb} \times \frac{100}{1}[31]$$

Where Ca = Concentration after, Cb = concentration before.

Table 11 shows the result of the bacterial load in colony-forming units for the water sample. Before and after represent the water sample before filtration and after filtration respectively. The result shows the ceramic filter has 96.72%, 99.26% and 66.67% colony removal efficiency for heterotrophic bacteria, coliform and fungi respectively. This result shows the ceramic filter has a good removal efficiency.

4. Conclusion and Recommendations

The Ceramic water filter developed in this work from mixture of clay, sawdust, silver nitrate, charcoal, periwinkle shell, and snail shell are a low-cost ceramic water filter with improved efficiency in water purification. The ceramic filter showed close to 100% colony removal efficiency for heterotrophic bacteria, coliform and fungi and other chemical contaminants. The filter showed improved physiochemical parameters after filtration of wastewater making it acceptable for water purification to avert the menace of inaccessibility to safe drinking water amongst the less privileged.

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