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Bacterial Removal Efficiency of Crude Oil Polluted Water Using Corn Husk and Charcoal as Filter Material

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Clean drinking water has become a distinguishing factor between developed countries and developing or underdeveloped countries so much so that it is fast becoming the most sought-after initiatives in the public health sector globally. Many people in underdeveloped nations still lack access to safe drinking water, they rely on surrounding rivers and streams for their water supply. Polluted water is not safe for drinking and use in homes for domestic activities as they may pose health risks. It is therefore important to consider effective ways to purify water for domestic use in communities that do not have access to treated water. Microorganisms are key contaminants in unsafe water sources and be of natural or human origin. The frequency of bacterial isolate occurrence showed that the most prevalent were *Staphylococcus species* (12.0 %), *Aeromonas*

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species (10 %), *Klebsiella species* (9.0 %), Clostridium *species* and *Enterobacter species* (8.0 %), while the lowest were *Corynebacterium and Bacillus species* (4 %). The bacteriological quality of the polluted water before and after filtration was analysed to evaluate the efficacy of charcoal and corn husk filters in removing pathogenic isolates. The total heterotrophic bacterial counts (THB) of the contaminated water reduced by 87.0 % and 88.7 %, hydrocarbon utilizing bacterial counts (HUB) reduced by 87.3 % and 95.4 %, faecal coliforms reduced by 94.5 % and 95.8 %, *Salmonella shigella* counts reduced by 69.2 % and 76.0 % for corn husk filter and charcoal filter respectively after filtration with the organic materials. Charcoal filter showed more promise in reducing bacterial isolates than corn husk.

Keywords: Charcoal; corn husk; pollution; pathogens; salmonellosis.

1. INTRODUCTION

Water is an important mineral for all living things. It is required for every process in the human body and in plants, and animals. Water is the most important element in hygiene and sanitation. It is therefore pertinent to ensure access to clean water in homes, schools, farms, and food factories such as beverage productions and fish farming. Farming is fast becoming a major economic activity in growing economies such as Nigeria. Water for production is taken from rivers, streams, and lakes owing to the shortage of water facing over 1.2 billion people (Wasana et al., 2017). Some use underground water in tanks. It is therefore necessary to ensure that clean water is available for everyone. According to Musa et al., (2020), “Research has predicted that by 2025 two-third of the world’s population could be living under water stress and 1.8 billion people may be under extreme water stress”.

Water can be polluted with a wide range of microorganisms of natural and anthropogenic origin (Saxena et al., 2022) while polluted with crude oil. The bacterial genera that have been isolated from varying crude oil contaminated sites include *Nocardia, Pseudomonas, Gordonia, Micrococcus*, *Rhodococcus*, *Arthrobacter*, *Myhuskacterium*, *Flavobacterium*, *Corynebacterium*, *Klebsiella*, *Alcaligenes* and *Bacillus* while the fungal genera include *Penicillium*, *Aspergillus*, *Trichoderma*, and *Fusarium* (Chikere et al., 2016). Apart from bacteria and other microorganisms, water can be contaminated with waste from faulty sewage systems and industrial effluents. Water can also be contaminated with crude oil, heavy metals, toxic chemicals from industries that can be deleterious to living cells through bioaccumulation.

Crude oil has been reported to contain heavy metals and their accumulation in plant or animal tissues can cause mutation or even death depending on the dose and period of exposure when ingested in water. According to (Viviani and Yanopa, 2023; Ekhaise and Nkwelle, 2011), Oil spills have adverse effects on the water environment, productivity of farmlands during irrigation, water bodies and birds as well as microbial community and their distribution in the environment compared to a pristine environment. Crude oil pollution is currently considered to be a great threat to the health of living things in the environment including humans. Crude oil pollution in water bodies is a significant environmental issue that poses serious risks to aquatic life, ecosystems, and human health. Nwakanma et al. reported that Nigerian crude oil could be hepatotoxic and hemotoxic which can cause cancer and infertility (Nwakanma et al., 2016). Nigeria records an average of 300 oil spills in the oil producing States annually, making the Niger Delta regions the most polluted part of Nigeria, affecting the air, soil and water bodies (Adeniji et al., 2017) Apart from the Niger Delta regions, other places that serve as depots for petroleum products such as diesel, premium motor spirit, and kerosene among others have in one way or the other encountered oil spills during transportation or storage either accidentally or due to human error contributing to soil and water pollution (Ubong and Edwin, 2018).The contamination of water sources by crude oil not only degrades water quality but also introduces persistent hydrocarbons that are difficult to remove through conventional treatment methods. In many oil-producing regions, especially in developing countries, the lack of efficient and cost-effective water treatment technologies exacerbates the problem.

As a good solvent essential for social and economic development of humans and environmental preservation (Musa et al., 2020), it becomes pertinent to seek out more effective ways to reduce water pollution. Some techniques

used in water purification are filtration techniques (trickling filter, filter membrane methods), Chlorination, Boiling, Distillation etc.

A trickling filter is a type of wastewater treatment system. It consists of a fixed bed of rocks, coke, gravel, slag, polyurethane foam, sphagnum peat moss, ceramic, or plastic media over which sewage or other wastewater flows downward and causes a layer of microbial slime (biofilm) to grow, covering the bed of media (Ajao et al., 2020). Aerobic conditions are maintained by splashing, diffusion, and either by forced air flowing through the bed or natural convection of air if the filter medium is porous. The treatment of sewage or other wastewater with trickling filters is among the oldest and most well characterized treatment technologies (Kim et al., 2020). Although trickling filters are very good, it makes use of synthetic materials that are not easily accessible to everyone. However, recent studies have explored the potential of natural, biodegradable materials such as corn husk and charcoal as filter media (Ghosh et al., 2017). Corn husk is a readily available agricultural by-product, while charcoal is known for its adsorption properties, making them both viable candidates for low-cost and sustainable filtration systems (Oladipo et al., 2018). Trickling filters operate as biological treatment systems where wastewater is distributed over a medium that supports microbial growth, and the contaminants are biologically degraded. These systems have been widely utilized for treating domestic sewage and industrial wastewater, including crude oil-polluted water, due to their simplicity, cost-effectiveness, and sustainability (Ahlawat et al., 2018).

The use of these natural materials not only enhances the microbial degradation of hydrocarbons but also offers an environmentally friendly solution to filter construction. Corn husk, rich in cellulose, can support microbial growth, while charcoal provides a large surface area for biofilm development and the adsorption of pollutants (Ahmed et al., 2021). When combined in a trickling filter system, these materials may offer a dual function: physical filtration and biological degradation, thus improving the overall efficiency of crude oil-polluted water treatment (Nwaedozie et al., 2021).

The efficiency of fabricated trickling filters is influenced by the characteristics of the filter media. Synthetic materials, such as plastic or gravel, do not support microbial growth as effectively as organic materials. Synthetic media lack the adsorption properties of charcoal, limiting their ability to remove dissolved pollutants from wastewater (Head et al., 2016). Natural materials like corn husk and charcoal are also more sustainable, as they are biodegradable and can be sourced locally, reducing the environmental and economic costs associated with filter construction (Viviani and Yanopa, 2023). In regions where agricultural waste products like corn husk are abundant, using these materials in water treatment systems provides a low-cost, environmentally friendly alternative to synthetic media.

In this study, we will explore the use of indigenous organic materials readily available in sub-Saharan countries such as corn husk and charcoal in place of synthetic materials used in industrial trickling filter in a point of use fabricated trickling filter. The bacteriological quality of the polluted water before and after filtration will be analysed to evaluate the efficacy of the filters in removing pathogenic isolates.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in a river at Woji, Port Harcourt City. It is a harbor for ships carrying petroleum equipment and supplies. The sampling site lies between Latitude 4.81440, and Longitude 7.04732.

2.2 Sample Collection and Transportation

Twenty liters of water was aseptically collected from a crude oil polluted river at Woji, Port Harcourt. Samples were aseptically obtained with the aid of sterile plastic cans, each containing 10 liters. The water was then carefully transported on an ice pack to the laboratory without any delay for further analysis.

2.3 Experimental Design - Fabricated trickling filter set up

The indigenously sourced materials were arranged vertically in a recycled water dispensing bottle from the coarse rocks to the finest sand and filled with organic charcoal as the final layer before the outlet. It was arranged in the order gravel – pebbles – sharp sand – charcoal. The materials were then placed into the recycled bottle aseptically using a funnel which was

Table 1. Fabricated filter setup

Materials	Weight (kg)
Charcoal	3.1
Sharp Sand	7.5
Pebbles	8.1
Gravel	11.5

disinfected using 90% ethanol. The recycled plastic bottle was covered with black duct tape to prevent sunlight from entering the setup leading to the growth of algae or biofilms.

2.4 Sterilization of Materials

Materials such as glassware and media were sterilized before use. The glassware including pipettes, test tubes, beakers, conical plastics, measuring cylinder etc., were washed thoroughly with detergent, rinsed thoroughly with clean tap water and air-dried before sterilizing in the laboratory hot air oven at 160^{o}C for 1 hour. The media were sterilized by autoclaving at 121^{o}C for 15 minutes. Bench working areas were swabbed with dry cotton wool soaked in ethanol to sterilize before any microbiological analysis were carried out to avoid contamination. Sterile disposable hand gloves were worn and changed after each procedure to ensure aseptic conditions.

2.5 Preparation of Culture Media

All the media used for microbial analysis were prepared according to the manufacturer's instruction by weighing the appropriate amount of the powder and dissolved in 1000ml of distilled water into a conical flask and swirled to mix properly. It was homogenized by boiling before sterilizing in the autoclave at 121^{o}C for 15minutes. The sterile media were allowed to cool to 45^{0}C – 50^{o}C before pouring into sterile petri-dishes and allowed to solidify (set).

2.6 Nutrient Agar (NA)

Nutrient Agar was prepared according to manufacturer's instruction. Twenty-eight grams of the powder were properly weighed and dissolved in 1000ml of distilled water into conical flask. It was sterilized by autoclaving at 121^{o}C for 15 minutes. The molten nutrient-agar was allowed to cool to 45^{o}C to 50^{o}C and 20ml was aseptically dispensed/poured into sterile petri dishes and thereafter allowed to solidify and dried in hot air oven at 160^{o}C for 1 hour.

2.7 Eosin Methylene Blue (EMB) Agar

EMB media was prepared according to the manufacturer's standard by dissolving 36g of the powder in 1000ml distilled water. It was autoclaved at 121^{o}C for 15 minutes. It was allowed to cool to 45^{o}C and dispensed into sterile petri dishes, then dried in the hot air oven at 160^{o}C for 1 hour.

2.8 MacConkey Agar (MCA)

MCA media was prepared according to the manufacturer's standard by dissolving 55g of the powder in 1000ml distilled water. It was autoclaved at 121^{o}C for 15 minutes. It was allowed to cool to 45^{o}C and dispensed into sterile petri dishes, then dried in the hot air oven at 160^{o}C for 1 hour.

2.9 Salmonella-Shigella Agar (SSA)

SSA media was prepared according to the manufacturer's standard by dissolving 63g of the powder in 1000ml of distilled water. It was autoclaved at 121^{o}C for 15 minutes. It was allowed to cool to 45^{o}C and dispensed into sterile petri dishes, then dried in the hot air oven at 160^{o}C for 1 hour.

2.10 Normal Saline (Diluent) Preparation

8.5g of sodium chloride was weighed and dissolved in 1000ml distilled water into conical flask and shaken for proper homogenization, using sterile pipette, 9ml was aseptically pipetted into sterile test tubes and the mouth of the test tubes were plugged with cotton wool and it was sterilized by autoclaving at 121^{o}C for 15 minutes

2.11 Enumeration of Bacteria in Water Samples

A tenfold serial dilution was done by adding 1 ml of the water sample to 9 ml of normal saline, it was repeated to 10 $^{-6}$. The content in the pipette was thoroughly mixed to achieve a homogenous solution. An aliquot of 0.1 ml was placed on Nutrient Agar (NA), MacConkey Agar (MCA),

Eosin Methylene blue (EMB), Salmonella Shigella Agar (SSA), and Bushnell Hass (BHA) media in duplicates using the spread plate method and incubated at 37 oC for 24-48 hours. After 24-48 hours, the colonies were counted and recorded.

2.12 Total Heterotrophic Bacteria (THB)

An aliquot of 0.1 ml was placed on Nutrient Agar (NA) using the spread plate method and incubated for 24 hours. the colonies were counted and recorded. Isolates were subcultured and incubated for another 24 hours to obtain pure colonies. The colonies were counted.

2.13 Hydrocarbon Utilizing Bacteria (HUB)

0.1ml of water sample was plated on Bushnell-Haas agar modified with Agar-agar (to aid solidification) using appropriate dilutions of 10^{-5} and 10^{-6}. Hydrocarbon was supplied through the vapor phase transfer technique by placing sterile Whatman No.1 filter paper saturated with 5ml of crude oil in the inside lid of each plate kept in an inverted position and incubated at 37 ^{o}C for 48 hours. Bacteria growth in Bushnell-Haas agar becomes visible from 3-7days after inoculation, colonies formed were counted and expressed as colony-forming units per gram (cfu/g)

2.14 Characterization of Bacterial Isolates

Isolates observed were identified using their cultural, morphology and biochemical characteristics. This includes the elevation, shape, color on media, edges and texture and biochemical characteristics such as gram staining techniques, sugar tests, catalase and oxidase test as described by (Cheesbrough, 2006; Holt and Lawton, 1994).

3. RESULTS AND DISCUSSION

Key:

Using the formula E = Input concentration – Output concentration / Input concentration X 100

E= Efficiency (Von et al, 2020)

Table 2. Mean Microbiological count of polluted water (cfu/ml)

Analysis	Polluted Water
THB (NA)	2.3×10^6
HUB (BHS)	2.6×10^6
(MCA)	2.2×10^6
Faecal Coliforms (EMB)	3.1×10^6
Pathogenic bacteria (SSA)	2.5×10^6

Table 3. Mean Microbiological count of Corn husk filtrate (cfu/ml)

Analysis	Corn husk filtrate
THB (NA)	2.9×10^5
HUB (BHS)	2.1×10^5
(MCA)	2.8×10^5
Faecal Coliforms (EMB)	1.7×10^5
Pathogenic bacteria (SSA)	7.7×10^5

Table 4. Mean Microbiological count of charcoal water (cfu/ml)

Analysis	charcoal Water
THB (NA)	2.6×10^5
HUB (BHS)	1.2×10^5
(MCA)	1.9×10^5
Faecal Coliforms (EMB)	1.3×10^5
Pathogenic bacteria (SSA)	6.0×10^5

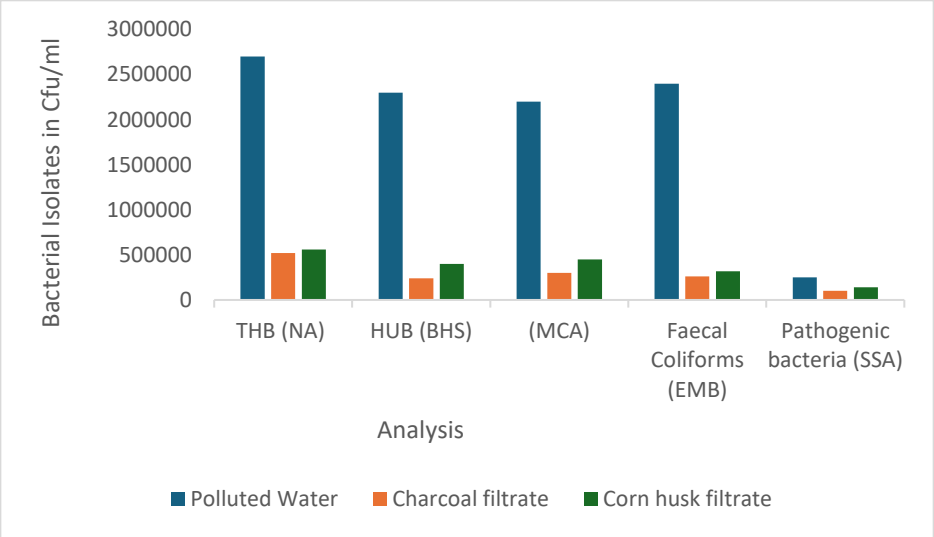

Fig. 1. Comparative microbiological count across water samples (cfu/ml)

Table 5. Bacterial isolates across samples

Bacterial Isolates	Frequency of occurrence
Proteus vulgaris	5
Serratia marcescens	5
Bordetella pertussis	6
Clostridium species	8
Lactobacillus species	9
Bacillus subtilis	4
Staphylococcus aureus	12
Corynebacterium species	4
Aeromonas species	10
Listeria species	8
Klebsiella species	9
Enterobacter aerogenes	8
Rhodococcus spp	7
Escherichia coli	5

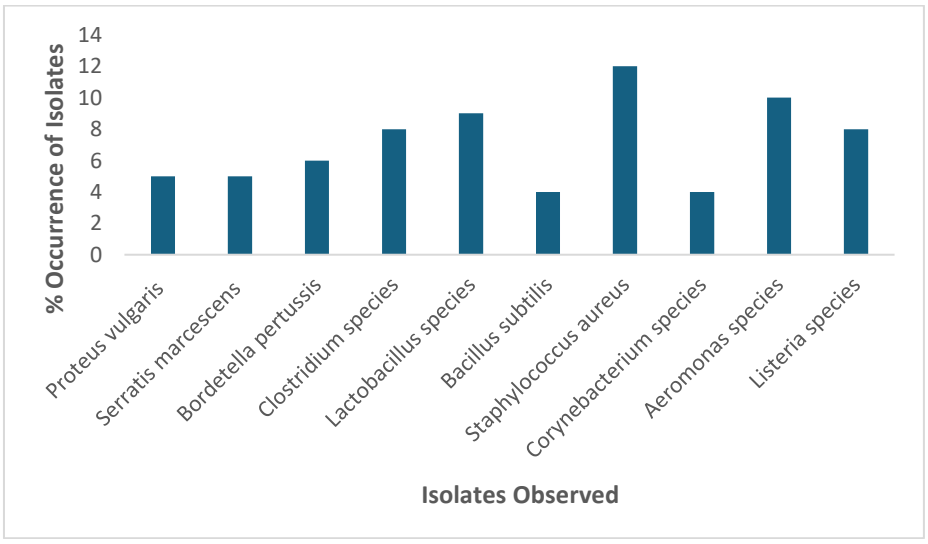

Fig. 2. Percentage Occurrence of Observed Isolates

Table 6. Bacterial Removal Efficiency

Isolates	Corn Husk Filter %	Charcoal Filter %
THB	87.0	88.7
HUB	91.9	95.4
MCA	87.3	91.4
Faecal Coliforms	94.5	95.8
Pathogenic bacteria (SSA)	69.2	76.0

3.1 Bacterial Isolates Obtained in the Study

The total heterotrophic bacteria, hydrocarbon utilizing bacteria, faecal coliforms and pathogenic bacteria from the polluted water were 2.3 x 10^6, 2.6 x 10^6, 3.1x 10^6, and 2.5x 10^6 respectively (Table 2).

The total heterotrophic bacteria, hydrocarbon utilizing bacteria, faecal coliforms and pathogenic bacteria from the corn husk filtrate were 2.9 x 10^5, 2.1 x 10^5, 1.7 x 10^5, and 7.7 x 10^5 respectively (Table 3).

The total heterotrophic bacteria, hydrocarbon utilizing bacteria, faecal coliforms and pathogenic bacteria from the charcoal filtrate were 2.6 x 10^5, 1.2 x 10^5, 1.3 x 10^5, and 6.0 x 10^5respectively (Table 4).

The bacterial species isolated in the water were *Proteus vulgaris, Serratis marcescens, Bordetella pertussis, Clostridium species, Lactobacillus species, Bacillus subtilis, Staphylococcus aureus, Corynebacterium species, Aeromonas species, Listeria species, Klebsiella species, Enterobacter aerogenes, Rhodococcus spp, Escherichia coli* (Table 5). The frequency of occurrence showed that the most prevalent were *Staphylococcus species* (12%), *Aeromonas species* (10%), *Klepsiella species* (9%), Clostridium *species* and *Enterobacter species* (8%), while the lowest were *Corynebacterium Bacillus and species* (4 %) (Fig. 2).

The filtration efficiency of (THB) was 87.0 % and 88.7%, (HUB) reduced by 91.92 % and 95.4 %, 94.5% and 95.8%, *Salmonella shigella* counts 69.2% and 76.0% for corn husk filter and charcoal filter respectively after filtration with the organic materials (Table 6).

The total heterotrophic bacterial counts of the contaminated water reduced considerably after filtration with the organic filters (corn husk and charcoal). Charcoal filter showed more promise in reducing total heterotrophic bacteria than corn husk. The same trend was consistent in faecal coliforms and pathogenic bacterial counts. This shows the efficacy of charcoal and corn husk in removing bacteria from the water sample. This may be associated to the ability of charcoal to absorb pollutants from water This agrees with (Musa et al., 2020; Mukherjee et al., 2020). Corn husk and charcoal have unique properties that enhance the microbial degradation of pollutants in wastewater.

In all analysis the polluted water had more bacterial counts followed by corn husks and then lowest counts were observed in charcoal filtrate. The result also showed that there were high faecal counts in polluted water. The presence of higher *Salmonella shigella*, *Klebsiella sp* (Table 5), counts confirms that the polluted water sample is contaminated with human faecal matter since these are bacterial associated with the human gut. This suggests that there may be open defecation into the river or other streams with open defecation empties into it. This is very dangerous as they can lead to gastrointestinal infections such as salmonellosis if ingested (Cabral, 2010).

The presence of high concentrations of bacteria in water showed the level of contaminants in the river sampled. These high levels of pathogenic bacteria have been associated with enteric diseases of public health concern such as cholera, diarrhoea, enteritis, salmonellosis etc. These are diseases associated with poor sanitation managed such as open defecation and indiscriminate disposal of sewage and organic waste. This agrees with (Musa et al., 2020)

However, the ability of the corn husk filter and charcoal filter to reduce these harmful bacteria confirms their adsorption potential. The removal efficiency of the filters was between 94 to 95 % and 69 to 76% for faecal coliforms and *Salmonella shigella*, although the bacterial level is not of potable standard, the filtrate can be used for other purposes such as agricultural irrigation, washing of drilling machines, and

cooling of heavy drilling equipment (Tagele Haligamo et al., 2022).

This recycled water can be further subjected through charcoal and corn husk filters for further bacterial removal for recreational use and possibly domestic use (Yamina et al., 2013).

4. CONCLUSION

The bacterial isolates indicate organisms of public health of concern which the filter was able to reduce drastically. These pathogens have been implicated in salmonellosis, a common disease associated with poor hygiene and sanitation practices. It is therefore important not to use water from that source for any domestic activities such as washing, cooking, cleaning as it may lead to infection from cross contamination or ingestion.

The fabricated filter was most effective in the removal of faecal coliforms followed by hydrocarbon utilizing bacteria. Charcoal filter was more effective than corn husk filter. The results obtained from individual filters show that a combination of both filters will be very effective in a shorter period of time and hence will be more cost effective.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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