Capacity Evaluation of Local Clay Material in the Removal of some Bacteria from Aqueous Solution

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Abstract
Water pollution is one of the biggest environmental issues causing serious problems to human. There are existing chemical and physical methods of treating contaminated water but they are not indigenous and cheap. Most of the existing methods are not available at the rural areas. This study seeks to address the effectiveness of some local clay materials in removing some bacteria (E.coli, Salmonella typhi and Vibrio cholerae) from aqueous solution. Clay samples collected from Urung Udung in Akwa Ibom State and Uhuala Mbaise in Imo State, Nigeria (labelled AC and MC respectively) were modified thermally and chemically and used to remove some bacteria (E.coli, Salmonella typhi and Vibrio cholerae) from aqueous solution using the spread plate method for quantification and enumeration. Results indicated that alkaline modified clay forms showed 100% removal efficiency for S.typhi in aqueous solution. In the same way, total removal of V.cholerae was observed on all chemically modified clay forms (acid, alkaline and surfactant) except thermally modified clays. In all the treatments, E.coli had the highest resistance followed by S.typhi suggesting that further treatment options should be considered for their removal in aqueous solution. The study therefore revealed that Akwa Ibom clay and Mbaise clay had great capacity for the removal of bacteria (V.cholerea and S.typhi) in aqueous solution but has a reduced capacity for the removal of some bacteria (E.coli) in aqueous solution.

Keywords: *Clay Materials; Bacteria; Urung Udung (Akwa Ibom State); Uhuala Mbaise (Imo State)*

Introduction

Water constitutes the largest proportion (70%) of the human body weight, which makes it a vital component of the body system (Cooper, 2000; Mayer and Bhika, 2015). The importance of water is such that life cannot be sustained beyond few days without water supply. Anthropogenic activities and biological agents have resulted in the contamination of the available water sources thus, limiting the availability of water for human use.

Most of the current outbreaks of diseases in the world are as a result of water and food-borne enteric bacteria, for example cholera is caused by *Vibrio cholera* while diarrhoea and dysentery are caused by *Escherichia coli* and typhoid caused by *Salmonella typhi* (Unuabonah *et al.*, 2018). These pathogens have shown to be the cause of diseases leading to morbidity and mortality in the developing world (Unuabonah *et al.*, 2018). About 88% of diarrhoea disease is linked to unsafe water supply and hygiene (Jyoti and Pandit, 2001). It has been reported (Liu *et al.*, 2013a; Ma *et al.*, 2014) that more than 1.3 million children die every year in the world due to diarrhoea.

Chemical agents such as chlorine and its compounds are most widely used in water treatment because of their effectiveness, low cost and extra protection against re-growth of bacteria and pathogens (Amin *et al.*, 2014). However, the addition of these chemicals to water do alter the

taste of the water and the chemical often react with various constituents in natural water to form disinfection by-products (DBPs), many of which are carcinogens (Villanueva *et al*., 2007).

Membrane filtration, though very effective for disinfection of water, suffers from fouling which results in frequent replacement of membranes which raises the cost of the entire water treatment process.

Adsorption technique is a favourable method for removal of bacteria from potable water and wastewater owing to its simplicity, high efficiency, low-cost of operation, ease of regenerating the adsorbent and up scaling the process (Nassar *et al*., 2012). In addition, adsorption processes do not produce by-products as found in chemical disinfection processes like chlorination which gives it an edge over other purification techniques. Adsorbents used for bacterial removal from water can be organic (Rabea *et al*., 2003; Qi *et al*., 2004), inorganic (Zhang *et al*., 2010) and organo-inorganic (Undabeytia *et al*., 2014) in nature.

Clay minerals are another class of adsorbents that are naturally antimicrobial. They have been used for the treatment of some ailments such as diarrhoea, dysentery, tapeworm, hookworm, wounds, and abscesses (Otto and Haydel, 2013). They are excellent adsorbents for bacteria removal in wastewater treatment (Hrenovic *et al*., 2009) and environmental bioremediation (Muter *et al*., 2012). In spite of its use in other climates, there is lack of use of clay material in the purification of water in Nigeria.

In this study, clay materials from Urung Udung, Akwa Ibom State and Uhuala Mbaise, Imo State in the South – South and South-East geopolitical zones of Nigeria respectively, were thermally and chemically modified and used as adsorbents for the removal of some bacteria (*Escherichia coli*, *Salmonella typhi* and *Vibrio cholerae*) from aqueous solution.

Experimental procedures

Media Preparation

Three media types- Eosin methylene blue (EMB); Salmonella Shigella (SS) Agar and Thiosulfate-Citrate-Bile-Sucrose (TCBS) were prepared according to the manufacturer's instructions and used for the isolation of *Escherichia coli*, *Salmonella typhi* and *Vibrio cholera* respectively.

Preparation of Clay Materials

Each clay material type was oven dried at 120^{o}C for 4hrs to ensure complete evaporation of the moisture content. The dried clay was milled and sieved through a British Standard Sieve (BSS) 100mm mesh. The fine grains obtained were subjected to different modification processes while some parts reserved for analysis and labelled AC and MC (Raw clay materials) according to (Ajemba, 2018).

Modification methods of raw clay

Acid modification

Fine powder of 100g each of the raw clay was thoroughly mixed with 200ml of 0.33M concentrated hydrochloric acid (HCl) solution and the resulting mixture was heated to between 60ºC and 100ºC under continuous stirring for a period ranging from 2-6 h. The mixture was then allowed to cool. The clay suspension was then washed in distilled water before filtration and drying process to recover the solid acid treated clay (Ajemba, 2018).

For the alkaline modified clay material, 100g of the clay sample was mixed with 200ml of 3M sodium hydroxide (NaOH) solution in a beaker and heated to 85ºC for 45 minutes (Khalifa *et al*., 2020).

Into a clean beaker, 6.0g of the clay was mixed with 50ml of 0.05M sodium dodecyl sulphate (SDS) in a clean beaker. The mixture was shaken for 2h. The modified clay was then

separated from the mixture by vacuum filtration, washed several times with deionised water, dried at 110^{o}C for 6h and re-sieved with a 200mm-mesh (Xi *et al*., 2010).
For thermal modification, 10g each of the raw clay was measured in a crucible and heated in the muffle furnace at 400^{o}C for 2h. Each sample was then cooled in a desiccator (Ajemba, 2018).

Preparation and Quantification of bacterial cultures
Pure stock cultures of the microorganisms (*Escherichia-coli, Salmonella typhi* and *Vibrio cholera*) were purchased from the Department of Microbiology, University of Port Harcourt, Nigeria. Loopful of a pure stock culture of the microorganisms (*Escherichia-coli, Salmonella typhi* and *Vibrio cholera*) from bijou bottles were transferred into different prepared nutrient agar plates using the spread plate method of bacteria culture. It was incubated at 37°C for 24h to obtain fresh culture enumerated and then used for further analysis. A loopful of each bacterial type on the different nutrient agar plates were transferred to 10ml of peptone water in test tubes already prepared for the enrichment of the bacteria culture and allowed to stand for 18 – 24h. 10ml of the enriched culture in peptone water was put in 90ml of sterile diluent to make a 1:10 fold serial dilution.

Clay material stimulation test for the microorganisms.
Each clay material (10g) was weighed into a crucible, covered with aluminium foil and was then placed in the hot air oven at 105°C and allowed to dry to constant weight. Sterilized cotton wool was rolled into 50ml separating columns and the different sterile clay samples were introduced into each of the columns and they settled on the cotton wool. Sterile distilled water was ran through the columns containing the test clay sample within 2mins. The water containing the different test organisms were ran through the different columns containing the cotton wool and the test clay sample. The elluent was collected at periodic intervals. The process continued for 18h. To test for the efficiency of the adsorption methods, the eluents from each treated clay, were inoculated on TCBS for enumeration of *V.cholerae*, SSA for enumeration of *S.typhi* and on EMB for enumeration of *E.coli*. 0.1ml of the elluent of the different organism type from the columns was inoculated in duplicate plates on appropriate media for *Vibrio cholerea*, *Salmonella typhi* and *Escherichia coli* respectively using the spread plate method and incubated at 37^{o}C for 24h after which the bacteria were enumerated using the colony counter.

Results
Akwa Ibom clay
The bacterial load on the raw clay before filtration (adsorption process) was as follows: *E.coli* (3.15 x 10^4cfu/ml), *Salmonella typhi* (3.31 x 10^4cfu/ml) and *Vibrio cholerae* (2.65 x 10^4cfu/ml) respectively as shown in table 1.0. After the filtration (adsorption on the different clay types), *S.typhi* had a mean bacterial load of 2.93 x 10^4cfu/ml in the raw clay; *V.cholerae* had 2.57 x 10^4cfu/ml while *E.coli* had 2.89 x 10^4cfu/ml. There was no growth observed on the culture media plates after incubation for *S.typhi* and *V. cholerae* on alkaline modified clay. *E.coli* had a mean bacterial load of 1.0 x 10^4cfu/ml. Similarly, no growth was observed on the culture media plates after incubation for *V.cholerae* on acid and surfactant modified clays. However, *E.coli* and *S.typhi* had mean bacterial load of 1.76 x 10^4cfu/ml and 2.23 x 10^4cfu/ml; 2.18 x 10^4cfu/ml and 2.98 x 10^4cfu/ml for acid and surfactant modified clays respectively. *E.coli, S.typhi* and *V. cholerae* had a bacterial load of 1.95 x 10^4cfu/ml, 1.99 x 10^4cfu/ml and 1.90 x 10^4cfu/ml respectively using thermally modified clay.

Table 1.0 Bacteria load (*E.coli, S.typhi* and *V. cholera)* before Adsorption on the raw Akwa Ibom clay material

***E.coli* on *Emb agar*, cfu/ml**	***Salomella shigella (typhi)* on *S/S agar*, cfu/ml**	***Vibrio cholera* on *Tcbs agar*, cfu/ml**
3.15×10^4	3.31×10^4	2.65×10^4

Table 2: Bacteria load (*E.coli, S.typhi* and *V. cholera)* after Adsorption on raw and modified Akwa Ibom clay

Clay ID	***E.coli, cfu/ml***	***S.typhi, cfu/ml***	***V.cholerae cfu/ml***
AC	2.89×10^4	2.93×10^4	2.57×10^4
ALMAC	1.00×10^4	0	0
AMAC	1.76×10^4	2.23×10^4	0
SMAC	2.18×10^4	2.98×10^4	0
TMAC	1.95×10^4	1.99×10^4	1.90×10^4

Where AC means Akwa Ibom clay (Raw), ALMAC means Alkaline modified Akwa Ibom clay, AMAC means Acid modified Akwa Ibom clay, SMAC means Surfactant modified Akwa Ibom clay and TMAC means thermally modified Akwa Ibom clay

Mbaise Clay

All the bacteria had different degree of adsorption on the raw (unmodified) Mbaise clay. No growth was observed on the culture media plates after incubation for *Vibrio cholera* except for the thermally modified clay (TMMC). *S. typhi* had a mean microbial load of 2.32×10^4cfu/ml while *E.coli* had 2.70×10^4cfu/ml. No growth was observed on the culture media plates after incubation for *S. typhi* and *V. cholerae* on alkaline and surfactant modified clays. *E.coli* had a mean bacterial load of 1.18×10^4cfu/ml and 1.98×10^4cfu/ml for alkaline and surfactant modified clays respectively. *E. coli* had a mean load of 2.11×10^4cfu/ml and *S. typhi*, 2.18×10^4cfu/ml in acid modified clay. *E.coli, S. typhi* and *V. cholerae* had a mean bacterial load of 1.90×10^4cfu/ml, 1.98×10^4cfu/ml and 1.51×10^4cfu/ml respectively on thermally modified clay.

Table 3. Bacteria load (*E.coli, S.typhi* and *V. cholera)* before Adsorption on the Raw Mbaise clay material

***E.coli* on *Emb agar*, cfu/ml**	***Salomella shigella (typhi)* on *S/S agar*, cfu/ml**	***Vibrio cholera* on *Tcbs agar*, cfu/ml**
$2.84 \times 10^4 \pm 0.1$	$2.51 \times 10^4 \pm 0.1$	$2.00 \times 10^4 \pm 0.2$

Table 4: Bacteria load (*E.coli, S.typhi* and *V. cholera)* after Adsorption on raw and modified Mbaise clay

Clay ID	***E-coli, cfu/ml***	***S.typhi, cfu/ml***	***V.cholerae, cfu/ml***
MC	2.70×10^4	2.32×10^4	0
ALMMC	1.18×10^4	0	0
AMMC	2.11×10^4	2.18×10^4	0

SMMC	1.98×10^4	0	0
TMMC	1.90×10^4	1.98×10^4	1.51×10^4

Where MC means Mbaise clay (Raw), ALMMC means Alkaline modified Mbaise clay AMMC means Acid modified Mbaise clay, SMMC means Surfactant modified Mbaise clay and TMMC means thermally modified Mbaise clay

Discussion

The adsorptive capacity of the microorganisms on the different clay types, revealed that the alkaline modification of the clay is more effective than the others, probably due to the homogenous nature of the particle sizes which is likely to be associated with large surface area (Yuan *et al.*, 2013). The varying results of the clay treatments could be as a result of the changes in the clay physical characteristics, texture, particle size and distribution, different cell sizes of the organisms; *Vibrio cholerae* having the highest cell size of 5.0 – 8.0µm by 1.4 – 2.6 µm (Diuret and Delcour, 2010), followed by *Salmonella typhimurium* 0.7 – 1.5µm by 2.0 – 5.0 µm (Arnold, 2009) and then *E. coli* 0.5 µm by 2 µm (Chien *et al.*, 2012). Generally, for all the treatments, *E. coli* had the highest resistance followed by *Salmonella typhi* suggesting that further treatment options should be considered for their elimination. A study by (Koko, 2016) also showed that E.coli was resistant to the prepared composite adsorbents.
The study therefore revealed that Akwa Ibom clay and Mbaise clay had great capacity for the removal of bacteria (*V.cholerae* and *S.typhi*) in aqueous solution but has a reduced capacity for the removal of some bacteria (*E.coli*) in aqueous solution.

Conclusion

This study showed that clay from Urung Udung and Uhuala Mbaise had great potential as adsorbents for the removal of *E-coli, V.cholerae* and *S.typhi* bacteria in aqueous solutions. Alkaline and surfactant modified Mbaise clay showed 100% removal efficiency for *V.cholerae* and *S.typhi*. Microbiological investigation also revealed that for all the treatments, the order of resistance was *E-coli* > *S.typhi* > *Vibrio cholera*. This observation suggests that further treatment options should be considered for *E-coli* and *S.typhi* removal in aqueous solution.

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