

## COMPARATIVE BIOREMEDIATION AND PHYSICOCHEMICAL ANALYSIS OF ABATTOIR WASTE WATER EFFLUENT (A CASE STUDY OF BIRNIN-KEBBI ABATTOIR)

**Yusuf M., Peni D. N. And Danjumma B. J.**

Department of Science Laboratory Technology,  
Waziri Umaru Federal Polytechnic,  
Birnin Kebbi, Nigeria.

*E-Mail: [rahilaishayaganya@gmail.com](mailto:rahilaishayaganya@gmail.com), Phone: +2348066829252*

### **ABSTRACT**

Quality of water is of concern, especially in the developing countries where water lacks proper treatment. Bioremediation, chemical treatments, and physicochemical analysis of the water samples were conducted with the view of curtailing environmental pollution, disease outbreak and enhance recycling of abattoir waste water. The effluent were digested anaerobically for 5 days using *Staphylococcus aureus* and *Streptococcus pyogenes* separately, the digested water samples were divided into six groups (A B C D E F and H), groups A B C and D were subsequently flocculated with alum, moringa seed extract, alum and moringa seed extract and chlorine respectively for 4hr, after which samples were decanted, the filtrates were subjected to physicochemical tests: pH, turbidity, chemical oxygen demand (COD) and biological oxygen demand (BOD), the set ups were subjected to microbiological test such as viable plate count in CFU/ml, which were within the range of  $10 \times 10^2 - 752 \times 10^2$  and  $18 \times 10^2 - 28 \times 10^2$ ), coliform count in ml/l 7-168 and 20 -240. pH average values obtained were 7.24 and 6.90, COD range in mg/l 650 – 750 and 550 – 720, BOD range mg/l 5.8 – 16.3 and 9.6 – 14.6, and turbidity range in NTU 206 – 950 and 80 - 990 were obtained for samples digested by *S. aureus* and *S. pyogenes* respectively, when all the results were compared to that of the WHO standard only the pH range was in conformity, but when compared with sample G they significantly differ, indicating that the treated effluents may not be portable, but may pose little or no harm to the environment. The combination of alum, Moringa seed extract and chlorine yielded the best result for all the parameters tested.

**Keywords:** Bioremediation, Abattoir waste, Anaerobically, Nephelometric unit and Flocculation.

## 1.0 INTRODUCTION

Water is essential for human survival. It has been reported that the total amount of water in the world is about 1400 cubic km ( $10^{18}$  tones) and remains constant. Apparently, more than 97% of this total volume is sea water, the remaining 22% is ground water and 97% is iced block away in the glaciers and the polar ice cap. This obviously leaves less than 1% of the supply of fresh water, which takes in the water hydrological cycle, but half of this is found in rivers, lakes and swamps. Most of the fresh water is polluted. In northern Nigeria, for instance, 95% of the surface water is considerably polluted and this remains true for Sub-Sahara Africa, (Adegbola, 2000).

The ever-increasing prevalence of endemic diseases like diarrhea, dysentery, amoebiasiasis, hepatitis, typhoid fever, jaundice, etc may be suggestive of the severe exposure to harmful effect of water pollution in developing countries. Even in the highly industrialized countries, several children still die of water-borne diseases.(Pink, 2006)Water which is safer for drinking must be free of pathogenic organisms, toxic substances and an excess of minerals and organic debris. It must be colorless, tasteless and odorless in order to be attractive to consumer. (Adegbola,2000)

Waste effluent is a very turbid liquid with an offensive smell in most cases. Its composition varies from large floating or suspended solids to smaller suspended solids, very small solids in colloidal form (microbial and chemical) pollutants (Mora *et al.*, 2005). The quality of organic matter present in waste water determines the strength of waste water (Rosa, et al, 2009). The organic matter in waste water is expressed in terms of the amount of oxygen required by micro-organisms to oxidize the organic matter. It is termed Biological Oxygen Demand (BOD). The different components of waste water are of primary importance as it plays a crucial role in the design of the treatment plant. Waste water generally contains biological components, pathogenic organisms and non-biological substances (Marai, 2004) Waste water effluent is an out-flowing of water or gas from a natural body of water or from a human-made structure. Effluent is defined by the United States Environmental Protection Agency as “waste water – treated or untreated that flows out of a treatment plant, sewer or industrial outfall. Generally refers to wastes discharged into surface waters” (Pink, 2006). Effluent in the artificial sense in general is considered to be water pollution. Such as the outflow from sewage treatment facility or waste water discharged from industrial facilities (West, 2006). Water pollutions occur when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds. Water pollution affects plants and organisms living in the bodies of water (Pink, 2006).

Bioremediation is the use of microbial metabolism to remove pollutants. The technologies can generally be classified as in-situ or ex-situ. The in-situ bioremediation involves treating the contaminated material (water) at the site, while the ex-situ involves the removal of the contamination material to be treated elsewhere (Pink, 2006). In bioremediation of waste water,

microorganisms breakdown the organic compounds in the waste water system. Microorganism can be added to any part of the system including sewers, pump stations and the various components of treatment plants (Jahn, 2003). Bioremediation is proving to be very successful in waste water systems in a range of climates and operating conditions. It is an environment friendly process that is resulting in significant savings for factories. Particularly in reduction of odor and acid attack, reduced cleaning costs and significantly improved performances of existing treatment and plant infrastructure.(West,2006). Industrial waste water treatment systems are inoculated with microorganisms for a wide various reasons, from pre-treatment of sewage, final product quality, bioremediation lead to better oxygen up take solid removal in clarifier, and overall efficiency of removing organic materials such as BOD and suspended solid (Saluja *et al.*, 2007)

The microorganisms work by catabolizing the organic matter; they break it down to simpler compounds in the process multiplying their own numbers. The other process involved in bioremediation in some situations is the out-competing of other, less favorable microbes, to produce a more beneficial result. The success of any bacterial inoculation for waste water treatment systems depends on two very important factors, which are the microbes must have the special capabilities to affect particular waste water problems and be present in high enough concentrations to be effective in the sewage system. High numbers of particular microbes are necessary to ensure the effective digestion of tough organic material (Kurup, 2000).

## 1.2 TEST MICROORGANISMS

*Streptococcus pyogenes* is a spherical grain positive bacterium that is the cause of group of a streptococcal group A antigens on its cell wall. *S. Pyogenes* typically produces large zone of beta-heamolysis (the complete disruption of erythrocytes and the release of hemoglobin) when cultured on a blood agar plate and are therefore called group A (beta-hemolytic) *streptococcus* (Mora *et al.*, 2005). *S. pyogenes* are catalase-negative and in ideal conditions, *S. pyogenes* has an incubation period of approximately 1-3 days. Heamolysis is the lysis of red-blood cells (Erythrocytes), either in vivo, in the host, or in vitro in blood agar artificial medium. Hemolysis the breakdown of red blood cells is the ability of bacteria colonies to induce hemolysis when grown on blood agar or in animal host (Mora, *et al.*, 2005). There are different types of heamolysis, they are the alpha, beta and Gamma heamolysis. *Streptococcus pyogenes* belongs to the Beta-hemolytic group. Beta heamolysis ( $\beta$ -hemolysis), sometimes called complete heamolysis is a complete lysis of red-cells in the media around and under the colonies, the area appears lightened (Yellow) and transparent. Streptolysin, an exotoxin is the enzyme produced by the bacteria which causes the complete lysis of red blood cell. There are two types of streptolysins; Streptolysin, O (SLO), Streptolysin, S (SLS). Streptolysin, O (SLO) is an oxygen-sensitive cytotoxin, secreted by most group A streptococcus and interact with cholesterol in the membrane of eukaryotic cells (mainly red and white blood cells, macrophages, and platelets) and usually result in  $\beta$ -heamolysis under the surface of blood agar. Streptolysin S (SLS) is an

oxygen-stable cytotoxin also produced by most GAS strains which result in clearing on the surface of the blood agar. SLS affect immune cells including polymorph nuclear leucocytes and lymphocytes and is thought to prevent the host immune system from leaking infection (Makkar, 2007).

*Staphylococcus aureus* is a gram positive, round, spherical shaped cell that form grap-like cluster colony; they are catalase and coagulase positive and facultative anaerobic. They are non-motile, non-spore forming, and ferments glucose. *S. aureus* ferments lactose to produce mainly lactic acid, on agar *S. aureus* grows as a golden yellow colony, it is a normal flora of human found on nasal passage. *Staphylococcus aureus* express many potential virulent factors including: Surface protein that promote colonization of host tissue, Invasiveness and invasive factors that promote bacteria spread in tissue (leukocidin, kinases, hyaluronidase), Surface factor that inhibit phagocytic engulfment (capsule protein A), Biochemical properties that enhance their survival in phagocytes (carotenoids, catalase, and coagulase), Immunological disguises (protein A and coagulase), Membranes damaging toxins that lyse eukaryotic cell membrane (hemolysins and leukocidins), Exotoxins that damage host tissue or otherwise provoke symptoms of diseases, Inheritance and acquired resistance to antimicrobial agent. They produce extracellular enzymes e.g. protease, a lipase, a deoxyribonuclease ( $DN_{ase}$ ) and fatty acid modifying enzymes (FAME) and also  $\beta$ -Toxin which is a (sphingomyelinase) which damage the membrane rich in this lipid. A lysogenic bacteriophage is known to encode the toxin. B-toxin belongs to the  $DN_{ase1}$  family, a super family in addition to sphingomyelinase the protein most structurally related to  $\beta$ -toxin, including human endonuclease HAP1. Hemolysis and lymphotoxicity are due to the sphingomyelinase activities of the enzyme. B-toxin has a molecular mass of 35 kDa and appears to function as sphingomyelinase ( $sM_{ase}$ ) which is known as hot-cold toxin because of its unique activity on sheep blood agar plate. At 37°C it interacts with the blood but does not lyse them if the blood is then placed at 4°C, (Mackay, 2007).

### 1.3 MORINGA OLEIFERA

The Moringa tree is grown mainly in semi-arid tropical and subtropical areas. *Moringa oleifera* plants have different importance to humans. The leaves, seeds, roots etc are very nutritious and serve other important purposes (Broin, *et al.*, 2002). The seeds of Moringa have high levels of vitamin C and moderate amounts of B-Vitamins and dietary minerals (Makkar, 2007). Matured seeds yield 38-40% edible oil called Ben oil from its high concentration of Benic acid. The refined oil is clear and odourless and resists rancidity. The seed cake remaining after oil extraction can be used as fertilizer or as a flocculent to purify water (Makkar, 2007). As an alternative to conventional coagulants, *Moringa oleifera* seeds can be used as a natural coagulant (Primary Coagulant) in household water treatment as well as in the community water treatment system, the seed kernels of *Moringa oleifera* contain significant quantities of low molecular weight, (Water-Soluble protein) which carry a positive charge. When crushed seeds are added to raw water, the protein produces positive charges acting like magnets and attracting the

predominantly negatively charged particles (such as clay, silt, bacteria and other toxic particles in water) (Adegbola,2000). The flocculation process occurs when the proteins binds to the negative charges forming flocks through the aggregation of particles. The material can clarify not only highly turbid muddy water, but also water of medium low turbidity. Treatment of raw water with moringa seed powder can leave water clear with 90.99% of the bacteria removed (Makkar, 2007).

#### **1.4 CHLORINE IN THE TREATMENT OF WATER**

Chlorine is one of the most widely used disinfectants. It is very applicable and very effective for the deactivation of pathogenic microorganism. Chlorine can easily be applied, measured and controlled. It is fairly persistent and relatively cheap (West, 2006)

#### **1.5 ALUM IN THE TREATMENT OF WATER**

Alum is a specific chemical, a class of chemical compounds. The specific compound is the hydrated potassium aluminum sulphate (potassium alum) with the formula  $KAL(SO_4)_2 \cdot 12H_2O$ . More widely, alum are double sulphate salts, with the formula  $AM(SO_4)_2 \cdot 12H_2O$ , where A is a monovalent cation such as potassium or ammonium and M is a trivalent metal ion such as aluminum or chromium (III) (Jahn, 2003). Alum is used to clarify water by neutralizing the electrical double layer, surrounding very fine suspended particles, allowing them to flocculate (Stick together). After flocculation the particles will be large enough to settle, and then be easily removed (Yongabi, 2004)

#### **1.6 AIM AND OBJECTIVES**

##### **1.6.1 Aim**

To purify abattoir waste water to a less harmful and less malodorous one prior to release into the environment

##### **1.6.2 Objectives**

- 1 To compare the proteolytic abilities of *Staphylococcus aureus* and *Streptococcus pyogenes*.
- 2 To compare the effect of bioremediation and chemical remediation on abattoir waste water effluent by the use of bacteria, Moringa seed extract alum and chlorine.
- 3 To compare the effect of BOD and COD of the various treated water samples.
- 4 To compare the pH of effluent prior and after digestion.

5 To enumerate and compare coliform count of untreated and treated water samples.

## 1.7 JUSTIFICATION

Due to rapid urbanization of cities in the developing countries remotely sited abattoirs are now engulfed by infrastructural development therefore centralizing the abattoirs in fast expanding cities where they cause grotesque land and acrid air pollution there by exposing the population to the risk of contracting infectious agents. It is against this back drop that this research was carried out to find a novel way of treating this category of waste in our contemporary societies where they constitute monumental urban menace, as well to protect people and the environment by the discharge of less harmful abattoir waste water effluents.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection of waste water effluent sample

The sample for this research was collected in a 2 liter capacity gallon. The gallon was bought at Birnin Kebbi central market. The gallon was washed and sterilized using ethanol (95%) in the laboratory. Waste water effluent was collected from the ultra-modern slaughter house (Abattoir) in Birnin Kebbi metropolis on 16<sup>th</sup> February 2014. And was immediately transported back to the laboratory and stored in the refrigerator at a temperature of +4°C prior to its use for the experiment.

### 2.2 Pre-treatment of sample (waste water effluent)

Large particles present in the waste water were physically removed by hand picking aseptically after which the waste water was further filtered using commercially available sieving cloth to remove buoyant, suspended, and bottom settler particles such as stones, leaves, wood, grass and others physical particles. After which the waste water, was sterilized by heat shocking. This was achieved by heating the waste water at 60°C in a water bath for 10min and immediately cooling the water to 4°C. This was done in order to reduce the microbial load present in the waste water sample. The waste water effluent was pre-treated with antifungal drug clotrimazole, in order to inhibit fungal growth.

### 2.3 Confirmation of isolated bacteria

Clinical samples that contain *streptococcus pyogenes* and *Staphylococcus aureus* were collected from the Federal Medical Centre Birnin Kebbi, on 16<sup>th</sup> February, 2014. The clinical samples collected were cultured on a sterile nutrient agar and were incubated for 24 hours at 37<sup>o</sup>c. The colonies formed were then sub-cultured onto a sterile nutrient agar plates which were also incubated at 37<sup>o</sup>c for 24 hours, Single colony of microorganisms formed on plate were gram-

stained. The microorganisms were found to be gram positive and morphologically spherical in chains and clusters when viewed under the microscope.

## **2.4 Biochemical analysis**

The following biochemical tests were carried out as described by Oyeleke and Manga (2008). Catalase, Lactose/Gas, Citrate, Motility, Indole, Urease, Methyl Red, Vogues Proskuer, Spore Detection, Coagulase Test, Sucrose, Hydrogen Sulfide (H<sub>2</sub>S) Production.

## **2.5 Preparation of *Moringa oleifera* seed**

*Moringa oleifera* seeds that were used in this research were obtained from Birnin Kebbi central market, the seed was de-shelled and crushed using sterile mortar and pestle in the laboratory. 1% solution of the seed powder was prepared by dissolving 10g of the seed powder in 1000ml of distilled water in a volumetric flask, after which the solution was filtered with no.1 whatmans filter paper and then transferred into a sterile container, labeled and stored at 10°C prior to its use.

### **2.5.1 Preparation of chlorine solution**

1% solution of chlorine was prepared by dissolving 10g of Sodium Hypochloride in 1000ml of distilled water in a volumetric flask, after which the solution was transferred into a sterile container, labeled properly and stored at 10°C prior to commencement of the experiment.

### **2.6 Preparation of alum solution**

1% solution of solution was prepared by dissolving 10g of Alum in 1000ml of distilled water in a volumetric flask, after which the solution was transferred into a sterile container, labeled properly and stored at 10°C prior to the commencement of the experiment.

### **2.7 Preparation of nutrient agar**

28g of the dehydrated powder was weighed and poured and dissolved in 1 liter of sterile water in a sterile conical flask, the conical flask was gently warmed on an hot plate so as to make sure that the powder is completely dissolved, and it was ensured that it was not too hot so that it will not alter its nutritional value. Cotton wool and foil was used to cover the conical flask, this is done to prevent water entering into it during autoclaving. It was autoclaved at 121°C for 15minutes. After this the conical flask was then brought out and placed on a sterile bench and allowed to cool to about 60-70°C and then dispensed into sterile Petri dishes.

## **2.8 Secondary treatment of sample (inoculation of test microorganisms)**

*Staphylococcus aureus* and *Streptococcus pyogenes* were aseptically inoculated separately into the sample. This was done using two loopfull of each of the pure isolates of the cultured test microorganism which were dissolved into 5ml of distilled water then these two test tubes were used to inoculate 495ml each of the undigested (crude) waste water. The inoculated sample was incubated for 5 days anaerobically at 37°C.

### **2.8.1 Tertiary treatment of sample**

After 5 days of microbial digestion, 400ml of the digested sample was added into 5 different 500ml capacity conical flasks labeled A-F treatments sample as summarized below:

### **2.8.2 EXPERIMENTAL DESIGN**

Samples

A- Digested Effluent + Alum only

B- Digested effluent + Moringa Seed extract only

C- Digested effluent + Alum + Chlorine

D- Digested effluent + Moringa seed extract + chlorine

E- Digested effluent + Alum + Moringa seed extract

F- Digested effluent + Alum + Moringa seed extract + Chlorine

G- Undigested effluent

H - Digested effluent

### **2.9 TEST CARRIED OUT ON ALL THE EXPERIMENTAL SET UPS**

The following microbiological and physicochemical analysis were carried out on the waste water effluent, before and after digestion by both *S. aureus* and *S. pyogenes* and the treated waste effluents; pH( hydrogen ion concentration), Colony plate counts, coli form count(most probable number)Test for COD,BOD and turbidity.

#### **2.9.1 Microbiological analysis**

**Sterility test:** Sterility test was performed on the 6 experiments set up for *S. aureus* and *S. pyogenes*. This was done by inoculating 1ml of the content in each test tube into a sterile nutrient agar and labeled accordingly. The inoculated plates were then incubated at 37<sup>0</sup>c for 24 hours. This test was conducted to check for any microbial growth on each of the corresponding test tube and numbers of colonies were estimated.



**Coliform test:** This test was carried out on all the 6 experimental set up in order to check for the presence of indicator microorganisms (*Escherichia coli*). As this test will indicate the portability of the treated waste water effluent.

### 2.9.2 Physicochemical analysis

**Turbidity test:** This test is based on the comparison of the intensity of light scattered by a sample under defined conditions with the intensity of the light scattered by a standard reference suspension under the same conditions. The turbidity of the sample is thus measured from the amount of light scattered by the sample taking a reference with standard turbidity suspension. Formalin polymer is used as the primary standard reference suspension. The turbidity of each of the treated sample was measured using a turbidimeter. The procedure involves the calibration of the turbidimeter using a 400NTU standard solution and 0NTU Distilled water before measuring that of the sample. The turbidimeter was recalibrated between each measurement. The result of each measurement was recorded in nephelometric Turbidity Unit (NTU).

**Chemical oxygen demand test:** This test relies on the complete oxidation of organic matter present water sample by potassium dichromate ( $K_2Cr_2O_7$ ) in the presence of sulphuric acid ( $H_2SO_4$ ) and mercury sulphate ( $HgSO_4$ ) to produce  $CO_2$  and  $H_2O$ . The method involves refluxing the sample with a known amount of potassium dichromate in the sulphuric acid medium and the excess potassium dichromate is determined by titrating the mixture against ferrous ammonium sulphate using ferroin as an indicator. The amount of dichromate consumed by the sample is directly proportional to the amount of  $O_2$  required to oxidize the organic matter present in the sample. The C.O.D. of each of the sample was determined by setting up 2 test tubes labeled “test” and “Blank”. 2.5ml each of sample and distilled water was added into the “test” and “blank” tubes respectively after which 1.5ml and 3.5ml of potassium dichromate and sulphuric acid were respectively added into both tubes and incubated at  $150^\circ C$  for 2hours in a C.O.D. digester. After digestion, the tubes were cooled to room temperature ( $25^\circ C$ ) and titrated against Ferrous Ammonium Sulphate using Ferro in as an indicator.

**Biological Oxygen Demand test:** Biochemical Oxygen Demand (BOD) is an empirical test to provide a measure of the level of degradable organic material in a body of water. In this study the BOD of each sample was measured by determining the difference between the dissolve oxygen in each sample before and after incubating 50ml of each sample in an airtight bottle in the dark ensuring that there is no air bubble. The measurement of dissolved oxygen is achieved using commercially available dissolved oxygen measuring kit.

**2.9.3 Test for pH:** Determination of pH is one of the important objectives in biological treatment of waste water. In anaerobic treatment, if the pH goes below 5 due to excess accumulation of acids, the process is severely affected. Shifting of pH beyond 5 to 10 upsets the

aerobic treatment of waste water. In these circumstances the pH values of the samples were measured using a pH meter after calibration using buffer solutions of 4.0, 7.0 and 9.2 respectively. The calibration was repeated after each measurement.

## RESULTS

**Table 1:** The following microbiological and physicochemical analysis results were obtained for the test microorganism (*Staphylococcus aureus*)

Samples	pH	Turbidity(NTU)	BODmg/L	CODmg/L	CFU/ml	MPNTest
WHO Standard	6.5-9.2	5-10	10-30	0-500	0-10 <sup>2</sup>	< 9
A	7.56	250	13.2	650	752x10 <sup>2</sup>	120
B	7.85	950	15.1	750	33x10 <sup>2</sup>	11
C	7.68	220	12.9	700	16x10 <sup>2</sup>	14
D	7.86	850	16.3	650	14x10 <sup>2</sup>	11
E	6.92	206	10.5	670	20x10 <sup>2</sup>	168
F	4.92	210	5.8	650	10x10 <sup>2</sup>	7
G	7.26	1090	19.9	865	742x10 <sup>2</sup>	>1100
H	7.87	980	16.1	820	826x10 <sup>2</sup>	>1100

## KEY

Sample A=Digested Effluent + Alum only. B=Digested effluent + Moringa Seed extract only  
 C=Digested effluent + Alum + Chlorine, D=Digested effluent + Moringa seed extract + chlorine,  
 E=Digested effluent + Alum + Moringa seed extract, F=Digested effluent + Alum + Moringa  
 seed extract + Chlorine, G=Undigested effluent, H=Digested effluent, Standard =WHO.

**Table 2:** The following microbiological and physicochemical analysis results were obtained for the test microorganism (*Streptococcus pyogenes*)

Samples	pH	Turbidity(NTU)	BOD mg/L	COD mg/L	CFU/ml	MPN Test
WHO						
Standard	6.5-9.2	5-10	10-30	0-500	0-10 <sup>2</sup>	< 9
A	6.92	185	9.6	600	752x10 <sup>2</sup>	120
B	7.64	990	14.1	700	33x10 <sup>2</sup>	11
C	7.39	375	13.0	650	16x10 <sup>2</sup>	14
D	7.28	950	14.2	550	14x10 <sup>2</sup>	11
E	7.18	85	13.1	720	20x10 <sup>2</sup>	168
F	4.93	255	7.4	580	10x10 <sup>2</sup>	7
G	7.26	1090	16.8	865	742x10 <sup>2</sup>	> 1100
H	7.67	580	13.9	580	826x10 <sup>2</sup>	> 1100

**KEY**

Sample A=Digested Effluent + Alum only. B=Digested effluent + Moringa Seed extract only  
 C=Digested effluent + Alum + Chlorine, D= Digested effluent + Moringa seed extract + chlorine, E = Digested effluent + Alum + Moringa seed extract, F= Digested effluent + Alum + Maringa seed extract + Chlorine, G=Undigested effluent, H= Digested effluent, Standard =WHO.

**DISCUSSION**

In this study the use of both *S. aureus* and *S. pyogenes* were found to be effective in the digestion of the blood present in the waste water sample from the abattoir which shows significant clearance after five days of incubation at room temperature, which confirm the catabolic activities of enzymes present such as lipase, sphingomisinase and proteolytic protein (beta heamolysins, and alpha heamolysin).

The reduction of the colony forming units 742x10<sup>2</sup> of the raw waste water to 10x10<sup>2</sup> was due to the different treatment the samples (A-F) of the waste water were subjected to. With sample F

(Alum + Chlorine + Moringa + digested effluent) having the least viable plate count of  $10 \times 10^2$  and  $10 \times 10^2$  cfu/ml for *S. aureus* and *S. pyogenes* respectively. Sample A (digested effluent + alum) has the highest viable plate count  $752 \times 10^2$  and  $280 \times 10^2$  for *S. aureus* and *S. pyogenes*. This finding is the same with the result obtained by Agres (2005) who found that *Moringa oleifera* seed extract solution have coagulating and antimicrobial effects which makes it effective for the coagulation and disinfection of the crude waste water. Abuye et al, (1999) reported that the antimicrobial agents in *Moringa oleifera* seed extract include 4-(4-O-acetyl- $\alpha$ -L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-( $\alpha$ -L-rhamnopyrasyloxy) benzyl isothiocyanate, , pterygospermin, benzyl isothiocyanate and 4-( $\alpha$ -L-rhamnopyrasyloxy) benzyl glucosinolate. Similarly, Agres, (2005) showed the antimicrobial activity of *Moringa oleifera* is dependent on the dose of the extract, as the concentration of the extract decreased, the activities also decreased. Also, the result obtained for the bacterial count after treatment with the different samples (B, C, DE and F) agreed with the work of Babu and Chaudhuri (2005).

Also, for the total coli form bacterial count are in agreement with that reported by Babu and Chaudhuri, (2005) and fall within the range given in, 2000 by WHO standards for drinking water. However, alum coagulant gave no significant reduction in the microbial load in both water samples after treatment, as the bacterial count only reduced slightly and had slight effect on the coli form bacteria present, for both digested samples it has showed relatively high number of coli form bacteria. This indicates that only few of the microbial loads settled with the flocks formed by the alum coagulant at the bottom of the beaker after treatment. However alum produces large sludge volumes (Makkar, 2007) and also reacts within natural alkalinity, present in the water leading to pH reduction.

The turbidity of the raw waste water before treatment was 1090 NTU, but after coagulation with moringa seed the turbidity reduced to 950 and 990 NTU for *S. aureus* and *S. pyogenes* respectively (samples B), whereas that of alum reduced the initial turbidity to 250 and 185 NTU (sample A). This result did not correspond to 1.5 NTU residual turbidity reported by Babu and Chaudhuri, (2005) this is probably due to some physiological factors and difference in experimental design and the fact that the species of *Moringa oleifera* used in this work may be different from the species used in the work of Babu and Chaudhuri, (2005) as it is known that different species of *Moringa oleifera* exist and do not have the same coagulation efficiency, (John, 2008). However, Moringa seed extract solution showed considerable turbidity which is not in agreement with the reports of Katayou et al. (2004). It is also observed during the study that the flocs formed by the phytocoagulant (Moringa seed extract), the flocs were tiny and light thus, settles so slowly whereas the flocs formed by the alum coagulant were large enough and the sedimentation rate was higher and faster. The result presented in the table 1 and 2 further indicated that the pH of the original water sample was not significantly affected following digestion, and coagulation with Moringa solution when compared to that of chemical coagulant alum, which affects the pH of the treated water samples A and F This result is in agreement with that reported by Ndabigensere and Narasiah, (2008). Therefore, this offers a significant

advantage to the phytocoagulant as little or no additions of chemical may be required to correct the pH of the finished water. Also the slight decrease in pH following treatment with Moringa seed, solution may be due to hydrogen ions of the weak acidity of *Moringa oleifera* solution which balanced the hydroxide ions in the raw water treated with alum. It was reported by Cikurel *et al* (2006), that *Moringa oleifera* seed extract is effective in reduction of COD of waste water. Also Tambo and Watanabe reported that the combination of Moringa seed extract with alum increased the efficiency of the COD removal from waste water.

## CONCLUSION

This study revealed that *Staphylococcus aureus* and *Streptococcus pyogenes* can be effective in the clarification of abattoir waste water effluent and also the study showed that the seed extract of *Moringa oleifera* seed powder solution possesses antimicrobial properties against microorganisms and these extract could be a promising natural antimicrobial agents and coagulant with potential applications in controlling bacteria that may cause water borne diseases and reduces the number of suspended particles in raw water drastically.

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