BACTERIOLOGICAL EVALUATION OF KWATA ABATTOIR WASTE WATER AWKA, NIGERIA

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ABSTRACT

Abattoir wastes can have a detrimental effect on the environment, public health, animal health, and economy of a country if they are not effectively managed and controlled. The bacteriological evaluation of waste water from Kwata abattoir was carried out to determine the bacterial load present and if the waste water generated is suitable for direct discharge into the environment. A total of two samples were aseptically collected, in which the physicochemical analysis of the waste water showed objectionable color and odor, pH of 7.3 and 6.5, and temperature of 30.2°C and 25.3°C for samples A and B, respectively, of which the pH and temperature were within acceptable limits by WHO. The membrane filter method was used to determine the total coliform and thermotolerant coliform counts present per 100 mL of the samples using MacConkey agar and Eosin Methylene Blue Agar, respectively. The total viable count was obtained for both samples: 5.1×10^5 CFU/mL for sample A and 1.4×10^6 CFU/mL for sample B. Phenotypic and biochemical tests were carried out for four isolates, which include *Escherichia coli*, *Salmonella* spp., *Bacillus* spp., and *Bacillus cereus*. Thus, it can be concluded from the above study that untreated abattoir waste water contains a high level of pollutants, which supports the growth of the microbial population, as evidenced in the microbial study. Therefore, waste water has to be treated before discharge into the environment to protect public health and promote the safety of the environment.

Keywords: Bacteriological evaluation, Kwata, Abattoir, Waste water, Awka.

INTRODUCTION

The environment is a very important and necessary component for the existence of both man and other biotic organisms. In the past two decades, there has been heightened concern over environmental degradation from pollution and the depletion of natural resources. Organic and inorganic substances have been released into the environment as a result of domestic, agricultural, and industrial activities (Ogbomida *et al.*, 2016).

The abattoir industry in Nigeria is an important component of the livestock industry, providing domestic meat supply and employment opportunities to the country's teeming population. The abattoirs generate a lot of waste water, which eventually becomes released into the environment. The release of waste water from abattoirs has increased in recent times, due to the continuous drive to increase meat production to meet the protein needs of the population (Egesi *et al.*, 2019).

The abattoir operations comprise certain major activities, which include receiving and holding livestock, slaughtering and dressing animal carcasses, chilling carcass products, carcass bonding and packaging, freezing finished carcasses and cartooned products, rendering processes, drying of skins, treatment of waste and transport of processed material. Abattoirs are generally known all over the world to pollute the environment either directly or indirectly through their various processes. These processing activities in Nigeria are mostly carried out in unsuitable buildings by untrained personnel or butchers who are mostly unaware of sanitary principles (Ogbomida *et al.*, 2016; Egesi *et al.*, 2019).

The intensity of man's activities has led to an increase in volume and complexity of waste generation worldwide despite the current level of technological advancement (Agu *et al.*, 2014; Agu *et al.*, 2017). Wastewater is usually released from abattoirs directly into the ecosystem without adequate treatment, thereby posing serious threats to surface water quality and general environmental safety and health. Wastewater

from abattoirs contains high amounts of biodegradable organic matter, suspended matter, and colloidal matter such as fats, proteins, and cellulose. Waste water from abattoirs comprises a particularly concentrated source of oxygen-consuming waste, and this directly or indirectly impact on the microbiological and physico-chemical water quality parameters such as pH, temperature, electrical conductivity, salinity, turbidity, total dissolved solids, total suspended solids, dissolved oxygen (DO) and coliforms (Mujere, 2015). Biodegradable organic matter in receiving waters creates high competition for oxygen within the ecosystem, leading to high levels of biochemical oxygen demands and a reduction in DO, which is detrimental to aquatic life (Ogbomida *et al.*, 2016; Dauda *et al.*, 2016).

Nutrients (nitrogen and phosphorus) enrichment in sensitive bodies of water can cause eutrophication by stimulating the growth of algae (called an algal bloom). Blooming and finally collapse of algae may lead to hypoxia/anoxia and hence mass mortality of benthic invertebrates and fish over large areas due to aquatic DO depletion. These effects entail a negative impact on biodiversity; sensitive species may be eliminated, major changes in ecosystem and a number of serious human health hazards may occur (Egesi *et al.*, 2019).

This research studies the bacteriological evaluation of wastewater from Kwata abattoir, Awka, Anambra State, Southeast Nigeria, with a view to determining the bacterial number and diversity, identifying the bacteria present in the wastewater and also determining some physicochemical properties of the waste water, such as, pH, temperature, and organoleptic properties (color and odor).

METHODS

Research design

Experimental research studies were conducted for this work. Experimental research provides a systematic and logical method for a researcher to measure the effect of an experiment that he/she conducts intentionally; hence, it is considered appropriate for this study, which

is aimed at evaluating the microorganisms present in wastewater from Kwata abattoir. Experimental research involves collecting data from several experimental readings, reducing bias, ensuring reliability, and permitting drawing inferences about causalities.

Study area
This study was conducted in Kwata abattoir, Awka, Anambra State, Southeast Nigeria. Awka is located at latitude 8.2069°N and longitude 7.0678°E and has a population of 361,657 (Nigerian Population Commission, 2006). Awka is noted for her iron craft and wood carving, although becoming the capital of Anambra State in 2001 brought about the influence of civil servants in the town. With the increase in population, a few nomadic herds have settled on the outskirts of the town. The abattoir supplies the bulk of the beef consumed in the town, which originates from cattle sourced from local herds, northern parts of Nigeria, and neighboring countries.

Sample size determination
The sterile bottles were individually filled with wastewater effluents from the abattoir, leaving a top space of about 2.5 cm. A total of two wastewater samples were randomly aseptically collected, one from a wash water basin and the other from a butcher's table.

Samples were properly labeled by date of collection, name of the abattoir, and sample type. Containers with the wastewater samples were properly sealed and transported to the laboratory of the Applied Microbiology and Brewing Department, Nnamdi Azikiwe University, Awka, for bacterial analyses. All samples were transported to the laboratory for analysis immediately after collection.

Sterilization of materials and media
Materials used: Autoclave/pressure pot, cotton wool, masking tape, gas cylinder, sponge, test tubes, conical flasks, methylated spirit/70% ethanol, water, detergent, and aluminium foil.

Media used: Nutrient agar (NA) to determine the total viable bacteria; MacConkey agar to detect the coliforms present; Mannitol salt agar to detect *Staphylococcus aureus*; *Salmonella*-Shigella agar (SSA) to detect *Salmonella* and Shigella in the wastewater sample; Eosin methylene blue agar (EMB agar) to distinguish between coliform bacteria and non-coliform bacteria; Mannitol-Egg yolk Polymyxin agar (MYP agar), a selective media for the detection and enumeration of *Bacillus cereus* in food samples, and agar cysteine lactose and electrolyte deficient agar (CLED), a differential media that distinguishes between lactose fermenting and non-lactose fermenting colonies and inhibits the swarming of *Proteus* spp.

Proper sterilization of the sample containers, sample bottles, media and other materials were ensured before use.

Sample preparation
One milliter (1 mL) of the waste water samples were pipetted aseptically and introduced into 9 mL of sterile water for bacteria. It was properly shaken to homogenize the sample. A 10-fold serial dilution of each sample was carried out using peptone water as the diluents. 0.1 mL of appropriate dilutions (10^{-3}) of the samples were inoculated by the pour plate method into sterile plates of NA, MacConkey agar, EMB agar, Mannitol salt agar, CLED agar, MYP agar, and SSA plates for the culture of bacteria. The culture plates were incubated at 37°C aerobically for 24–48 h for bacteria.

Discrete colonies on NA plates were counted to obtain the total viable count.

Determination of total coliform and faecal coliform using membrane filtration technique
This method is used to determine the presence of a member of a coliform group in wastewater and groundwater. 100 mL of the water samples were filtered through a membrane filter (0.45 μm pore size),

which retained the bacteria found in the sample. The filters were then transferred to a Petri dish containing MacConkey Agar and incubated for another 24 h at 35°C+0.5°C. Developing colonies were then counted under magnification and reported per 100 mL of the original sample.

Whereas, for the fecal (thermotolerant) coliform count, 100 mL of the water samples were filtered through a membrane filter (0.45 μm pore size), which retained the bacteria found in the sample. The filters were then transferred to a Petri dish containing EMB agar and incubated for another 24 h at 44°C. Developing colonies were then counted under magnification and reported per 100 metals of the original sample.

The calculations were thus:

$$Water\ Sample: No\ of\ colonies\ per\ 100\ mL = \frac{(No\ of\ colonies\ counted\ on\ plate)}{Volume\ filtered} \times 100$$

Determination of total bacterial count of the water samples
Ten-fold serial dilutions of the water sample was done, and appropriate dilutions were plated on NA. The cultured plates were incubated at room temperature for 24 h. Thereafter, the developing colonies were counted, and the total bacterial count is as follows:

The Total Bacterial Count was calculated thus:

$$TBC\ (CFU/mL) = \frac{N}{VD}$$

where: TBC = Total Bacterial Count

V = Volume of inocula plated

N = Number of colonies, developing on plate, that were counted

D = Dilution factor

CFU/mL = Colony Forming Unit per millilitre.

Characterization and identification of the isolates
Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reactions, and biochemical tests (Chessbrough, 1984; Agu *et al.*, 2014; Anaukwu *et al.*, 2015). The characterization of the isolates were performed by employing the Gram staining reaction, catalase test (Cat), citrate test, coagulase test, motility test, and triple sugar iron test (TSI) as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

Table 1: Total viable count obtained for each sample analyzed

Sample	Total viable count (CFU/mL)
Sample A	5.1×10^5
Sample B	1.4×10^6

CFU: Colony forming unit

Table 2: Physicochemical analysis of waste water samples (pH, temperature, colour and odour)

Samples	pH	Temperature(°C)	Colour	Odour
Sample A	7.3	30.2°C	Light brown	Foul odour
Sample B	6.7	25.3°C	Ox-blood	Putrefied odour

Table 3: Bacterial concentration (number of colonies/100 mL) of waste water from Kwata abattoir

Samples	Thermotolerant *Escherichia coli*	Total coliform count
Sample A	31	123
Sample B	62	250

Table 4: Phenotytic and biochemical test of various bacterial isolates

Isolates	Colour	Form	Opacity	Margin	Elevation	Surface	Coa	A/a	H$_2$S	Gas	Mot	Cit	Gram	Cat	Identity
Isolate A	Whitish	Circular	Translucent	Entire	Convex	Smooth	+	+	−	−	+	−	Negative rod	+	*Escherichia coli*
Isolate B	Greyish/white	Circular	Translucent	Lobate	Convex	Smooth	+	+	−	−	−	−	Negative rod	+	*Salmonella* spp.
Isolate C	White	Circular	Opaque	Irregular	Flat	Smooth	+	+	−	−	+	+	Positive rod	+	*Bacillus* spp.
Isolate D	Off-white	Circular	Opaque	Entire	Convex	Smooth	+	+	−	−	+	+	Positive rod	+	*Bacillus* cereus

Coa: Coagulase test, Mot: Motility test, A/a: Acid/acid production, H$_2$S: Hydrogen Sulphide gas production test, Gas: Gas production test,
Cit: Citrate test, Gram: Gram staining, Cat: Catalase test

Biochemical tests

Cat

Using a Pasteur pipette, add a drop of hydrogen peroxide to a grease-free glass slide. Using a sterile wire loop, pick the pure colony of interest and make a smear on the slide with the hydrogen peroxide, and observe for bubbles. (No bubbles: negative; bubbles seen: positive).

Coagulase test

Using a Pasteur pipette, add a drop of serum to a grease-free glass slide. Using a sterile wire loop, pick the pure colony of interest, and make a smear on the slide with the serum, and observe for clumping.

Motility test

Add 50 mL of water to a beaker, measure 1.15 g of NA, and add to it (based on manufacturer's instructions: 1000 mL of distilled water for 23 g of NA) and mix properly. Pipette 5 mL into each test tube, seal with a foil, and sterilize in an autoclave at 121°C for 15 min. Allow it to solidify in a slanted position. Using a sterile inoculating needle, pick the pure colony of interest and stab it in the solidified nutrient media, Seal and incubate for 24 h. Observe the extension of flagella in a motile organism.

Gram staining

Add a drop of water to a grease-free glass slide. Using a sterile wire loop, pick a pure colony of interest and smear. Allow to air dry; heat fix by passing through the flame.
- Flood with crystal violet stain for 60 s then rinse with water
- Flood with iodine for 60 s then rinse with water
- Add acetone in drops and rinse immediately with water
- Flood with safranin for 60 s then rinse with water.
- Allow the slide to dry in a slanted position
- Add oil immersion and view at ×100 objective lens. (Gram positive appears purple; Gram negative appears pink).

TSI: To observe the ability of the microorganism to produce acid (acid/acid production), gas, and hydrogen sulphide.

Dissolve 6.5 g of TSI powder in 100 mL of water and mix properly. Pipette 5 mL into each test tube, seal with foil and sterilize in an autoclave at 121°C for 15 min. Allow it to solidify in a slanted position. Using a sterile inoculating needle, pick the pure colony of interest and stab it in the solidified nutrient media, Seal and incubate for 24 h. Hydrogen peroxide is produced if there is blackening of the medium; gas is produced if there is the formation of bubbles; and acid/acid is produced if the butt and slant remain yellow.

Citrate test

A 24 h old culture was inoculated into test tubes containing sterile Simmons citrate agar slant and then incubated for 24 h. A positive test was indicated by a change from green to blue color on the surface of the Simmons citrate agar slant. No color change indicated a negative reaction.

RESULTS

Table 1 shows the bacterial count of the two samples in the NA plate to determine the total viable count and the bacterial load in CFU/mL for sample A collected from a wash water pool/basin at Kwata abattoir and Sample B waste water obtained from the washing of butcher's tables in Kwata abattoir.

Table 2 shows the physicochemical analysis of the waste water samples from Kwata abattoir (pH, temperature, color, and odor) to determine if the waste water is suitable for discharge directly into the environment.

Table 3 displays the bacterial concentration (Number of colonies per 100 mL) of waste water from Kwata abattoir to determine the total coliform count and the fecal coliform count.

Table 4 shows the phenotypic and biochemical tests of the bacterial isolates for identification of the organisms isolated.

DISCUSSION

In Table 1, the mean total bacterial count obtained for both samples (sample A: 5.1×10^5 CFU/mL and sample B: 1.4×10^6 CFU/mL) was high. Going by international standards, any water contaminated to this level is neither good for domestic use nor is it supposed to be discharged directly into the environment without treatment, and these values do not conform with the work of Dauda *et al.*, 2016.

In Table 2, the color and odor were objectionable, which could be as a result of direct dumping of raw blood and washing of carcasses without any form of treatment; this was in agreement with the work of Asibor (2019). The untreated abattoir wastewater was slightly warm for samples A (30.5°C) and B (25.3°C). The pH values of the waste water samples were 7.3 for sample A and 6.7 for sample B, of which both the temperature and pH obtained conform with the regulatory standards of WHO (2004) as stated in the work of Asibor (2019).

In Table 3, the total coliform count per 100 mL obtained for both sample A (123) and sample B (250) and the thermotlerant coliform count for both sample A (31) and sample B (62) exceeded the permissible limit by WHO (2004), as stated in the work of Asibor (2019).

In Table4, the morphological characteristics and biochemical tests carried out in this study for four isolates, which were identified as *Escherichia coli, Salmonella* spp., *Bacillus* spp., and *B. cereus,* were in conformation with the work of Egesi *et al.*, 2019. *B. cereus* was distinguished from other *Bacillus* spp. based on its color in the MYP agar from which it was isolated. Pink colonies were observed based on *B. cereus* cannot ferment mannitol, an integral component of the MYP agar, while the other *Bacillus* spp. isolated from the MYP agar were observed as yellow colonies, which shows its ability to ferment mannitol.

CONCLUSION

It is important to understand the need for effective treatment of waste water before discharge to the environment. Abattoirs are important, but as seen in the investigation carried out and other related research works, they are a niche for countless microorganisms. As earlier stated, these pathogenic microbes could be the animals' normal flora or could be present as a result of poor sanitary, health, and hygiene practices during meat handling, slaughtering, processing, distribution, and even consumption. Thus, waste generated in abattoirs can be managed by implementing waste management practices that involves treatment

and processing methods to ensure safe disposal and prevent potential hazards to the environment and public health. By doing so, it is also possible to gain more economic benefit from abattoir waste or by products.

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