Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.51, 2016

Physicochemical and Microbiological Qualities of the Abattoir Wastewater in Part of Minna Niger State

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Abstract
The study was aimed at Physicochemical and Microbiological composition of the abattoir wastewater in parts of Minna, Niger State. Based on morphological and biochemical tests using API kits, the yeast isolates were identified as species of *Candida*, *Cryptococcus* and *Trichosporon*. The most frequently isolated species was *Candida lusitaniae* (29.42%), followed by *Cryptococcus neoformans* (17.76%) while *Candida tropicalis*, *Candida zeylanoides*, *Candida gulliernondii* and *Candida fermata* had 11.72% frequency of occurrence each. *Trichosporon mucoides* had the least frequency of occurrence of 5.8%. Analysis of the abattoir wastewater revealed the mean value of 5257.50 mg/l, 2630.00 mg/l and 5830.00 mg/l for total suspended solids (TSS), Biochemical oxygen demand (BOD) and Chemical oxygen demand (COD) respectively. These values are above WHO standard limits of 20 mg/l, 20mg/l and 1000mg/l respectively. The pH of 7.90 and temperature 26.75 ^{0}C obtained for wastewater are within the limit allowed by the WHO. Besides, the concentrations of the heavy metals, copper, lead, magnesium, nickel and zinc in the samples were higher than permissible limit by the WHO except chromium. The study also revealed that the wastewater had mean total viable bacteria, yeast and coliform counts of 2.16× 10^7, 5.82 x 10^5 and 1.62 ×10^5 cfu/ml respectively. The results observed suggest therefore, the organisms particularly; *C. neoformans* and *C. lusitaniae* can be suitable candidates for bioremediation of abattoir waste water in the tropics.
Keywords: Abattoir; Heavy metal; Wastewater and Yeasts

INTRODUCTION

Isolation of yeasts from abattoir waste waters and related habitats such as contaminated soils have become the subject of research due to economic and public health impacts arising from these sites (Akoma and Olawepo, 2003; Nafarnda, Ajayi, Shawulu, Kawe, Omeiza, and Tags, 2012). The location and operation of abattoirs are generally unregulated, aside, they are usually located near water bodies where access to water for processing is guaranteed (Adelegan, 2004). The animal blood is released untreated into the flowing stream while the consumable parts of the slaughtered animal are washed directly into the flowing water (Adelegan, 2004; Omole and Longe, 2008). World Bank (1995) identified improper management and supervision of abattoir activities as a major source of risk to public health in Northern Nigeria. Wastes from slaughter houses typically contain fat, grease, hair, feathers, flesh, manure, grit and undigested feed, blood, bones and process water which is characterized with high organic level (Nafarnda, Yayi and Kubkomawa, 2006). The total volume of waste produced per animal slaughtered is approximately 35% of its weight (World Bank, 1998).

The organic load from abattoirs could be very high. Omole and Longe (2008) reported a chemical oxygen demand (COD) level as high as 375000 mg L^{-1} for raw bovine blood. Comparatively, in another study conducted by Mittal (2004), on abattoirs in Québec, Canada, typical values for a range of parameters in abattoirs wash down were given: as Total solid (TS) concentrations (2333-8620 mg L^{-1}); Total suspended solid (TSS) (736-2099 mg L^{-1}); while average levels of nitrogen and phosphorus were recorded at 6 and 2.3 mg L^{-1}, respectively. Hence, abattoir effluents could increase levels of nitrogen, phosphorus, total solids in receiving water body considerably. Excess nutrients cause the water body to become choked with organic substances and organisms (American Public Health Authority, APHA, 2005). When **organic matter** exceeds the capacity of the microorganisms in water that break down and recycle the **organic matter,** it encourages rapid growth, or blooms of algae leading to eutrophication (Verheijen, Wiersema, Hulshoff and De Wit, 1996). Equally, improper disposal systems of wastes from slaughterhouses could lead to transmission of pathogens to humans and cause zoonotic diseases such as coli bacillosis, salmonellosis, brucellosis and helminthiasis (Cadmus, Olugasa, and Ogundipe, 1999). Improper management of abattoir wastes and subsequent disposal either directly or indirectly into river bodies portends serious environmental and health hazards both to aquatic life and humans (Coker, Olugasa and Adeyemi, 2001; Hammack and Gill, 2002; Madigan, Martinko and Park, 2003).

Metals persist in the environment and can become concentrated up the food chain (Akpor and Muchie, 2011). The heavy metals emerge into the environments in the form of wastes and effluents from various industrial operations (Ahemad, 2012). An increase of heavy metal pollution in recent years has led researchers to search for the efficient methods for the treatment of heavy metals using biosorbents. Among the most perspective groups of microorganisms which have the ability to sorb heavy metals such as copper, zinc, lead, cobalt and chromium are the yeasts (Ayuanya and Oseghe, 2006). Generally, both quality and quantity of these waste materials coupled with the toxic heavy metals depends upon the types of industries and the raw materials

used for the production processes (Elless and Blaylock, 2000; Alam, Islam, Muyen, Mamun and Islam, 2007; Violante, Huang and Gadd, 2008).

Efforts have been geared towards curbing the menace of pollution around the world, particularly by the United Nations organs e.g., United Nations Environmental Programme (UNEP). There are many international conferences and protocols to this effect. Rio de Janeiro Conference of 1992 was a major effort, collecting previous environmental issues and bringing them to the fore (Oyesola, 1998).

In this work Environmental pollution caused by toxic heavy metals in effluents is one of the most pressing problems of development and is spreading throughout the world along with industrialization has been study. The aim of the present study was to isolate and characterize yeast strains from abattoir wastewater withstand and may be able to tolerate some heavy metals.

MATERIALS AND METHODS

Study Area

The study site was Minna Central Abattoir located at Tayi in Bosso Local Government Area of Niger State, Nigeria. Bosso is a Local Government Area in Niger State, Nigeria with its headquarters at Maikunkele. It has an area of 1,592 km² and a population of 147,359 (National population census, 2006). The abattoir is a small-scale business enterprises and it is managed by an Association of independent butchers and under the supervision of health workers from Ministry of Livestock and Fishery, Minna, Niger State. The slaughtering area measures 150 m^2 in size, fenced with sand Crete blocks while the floor is made of concrete slab. An average number of slaughtered animals per day are 50-80 cows, 20 sheep and 10 goats. Normal abattoir operations are carried out from Monday to Sunday.

The abattoir is provided with tap water but has no slaughter gadgets, cold room and waste treatment facilities. However, there are drainages and channels through which the wastewater leaves the slaughter hall and finally empties into a river in Tayi Village.

Sample Collection

Wastewater samples were collected from Minna central Abattoir, Niger State, Nigeria by the drainage and channels. The samples were collected in sterile glass bottles from four (4) different points designated as P1, P2, P3 and P4. The distance between the points is approximately about 100meters apart, samples were transported in ice box to the laboratory of Microbiology Department, Federal University of Technology (FUT) Minna, Nigeria for analysis. The wastewater were analysed within 6 (six) hours of collection.

Analysis of Wastewater for Microbial Quality

One millilitre (1ml) of serially diluted wastewater was plated on Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA) and MacConkey Agar (MCA) for the enumeration of total viable bacteria (TVB), yeasts and coliforms respectively. Inoculated nutrient agar and macconkey agar plates were incubated at 37^0C for 24-48hours while SDA plates were incubated at room temperature (28 ± 2^0C) for 3-5days. Colonies which developed on the plates were counted and recorded as colony forming units per millilitre (cfu/ml) of the samples. However, the yeast isolates were sub cultured repeatedly on fresh media to obtain pure cultures which were maintained on agar slants for further characterization and identification.

Characterization and Identification of Yeast Isolates

The isolates were characterized and identified using the Analytical Profile Index (API) 20C Test Kits (www. biomerieux.com, 2010). The system provides identification based on a very broad database of several yeast biotypes. The procedure as follows:-

Preparation of the Strip

Following the manufacturer's instruction the incubation box (tray and lid) was prepared by distributing about 5 ml of distilled water into the honey-combed wells of the tray to create a humid atmosphere (www. Biomerieux.com, 2010). The strain reference was recorded on elongated flap of the tray. The strips were placed in the incubation tray.

Preparation of the Inoculum

Actively grown colonies of the isolates (24 old cultures) were picked with an inoculation wire loop, stirred into 2ml of normal saline (NaCl, 0.85%) medium in a test tube and maintained as suspension stock for inoculation experiments. Then, 100µl of the suspension was transferred into ampule of API 20C Medium and gently homogenized with the pipette, avoiding the formation of bubbles.

Inoculation of the Strip

The capsules were filled with the suspension obtained in the ampule of API 20C medium. The formation of bubbles was avoided by placing the tip of the pipette against the side of the cupule. Care was taken not to overfill or under fill the cupules (the surface was flat or slightly convex, but never concave), otherwise incorrect result may be obtained. The lid was placed on the tray and incubated at room temperature (28 ±2 ^{0}C) for 72 hours.

Interpretation

Identification was obtained with the numerical profile

Determination of the numerical profile: On the result sheet, the test was separated into groups of 3 and a number 1, 2, or 4 was indicated for each. By adding the number corresponding to positive reaction within each group, a 7-digit number was obtained which constituted the numerical profile. Identification: was performed using the API machine database (V4.0).

Determination of Physical Properties of Wastewater

pH

Thirty milliliter (30ml) of wastewater was placed in a 100ml capacity sterile beaker and pH meter electrode (EIL 7020; Kent Industrial Measurement Ltd, China) was dipped into it. The wastewater was stirred and pH meter turn on record the value.

Temperature (^{0}C)

Thirty millilitres (30ml) of wastewater was placed in a 100ml capacity sterile beaker and DO_2 meter electrode (WaterTechw² D062, partech, Britain) was inserted into beaker. Then, switch on/off was pressed and reading displayed temperature in degree Celsius. Then the final steady value was recorded.

Biochemical Oxygen Demand (BOD)

Ninety five millilitres (95ml) of wastewater sample was introduced into BOD track sample bottle plus BOD martinet (KOH) powdered pillow. Then, connected to the appropriate tube label and the cup firmly tightened. Then, start (on/off) button was pressed and 0-700mg/L was selected. The setup was incubated at 20 ± 1^{0}C for five days. Results were directly read from BOD track and recorded as it relate to each sample (standard methods 4500-OG and 5210B).

Chemical Oxygen Demand (COD)

Two millilitres (2ml) of wastewater was poured into the digested reagent vials and blank of distilled water was prepared as a control. The vials were placed on COD reactor and heated at 150 ^{0}C for 2 hours. It was cooled to room temperature and COD was measured in milligram per litre (mg/ml) using calorimeter. Then, the final steady value was recorded.

Total Suspended Solid (TSS)

One hundred millilitres (100ml) of the wastewater samples were filtered through a pre weighed filtered paper. The filtered papers were dried at 105^{O}C for one hour and weighed. TSS was determined by using the following formula (Anon, 1992).

$$\text{TSS (mg/l)} = \frac{(B-A-C)}{D} \times 1000$$

Were:

A= weight of pan plus dry paper (g), B= weight of filter paper plus solid (g), C= Blank correction and D= Volume of sample used (litre) (AOAC, 2005).

Determination of Heavy Metals in Abattoir Wastewater

Samples were digested as follows. The samples (100cm^3) were transferred into a beaker and 5ml of concentrated HNO_3 was added for digestion. The beaker with the content was placed on a hot plate and evaporated to about 20ml. The beakers were cooled and another 5ml of concentrated HNO_3 was added. The beakers were covered with watch glass and returned to the hot plate. The heating was continued, and then small portion of HNO_3 added until the solution appeared light colour and clear. The beaker wall and watch glass were washed with distilled water and the samples were filtered to remove some insoluble materials that could clog the atomizer. The volume of the samples was adjusted to 100cm^3 with distilled water according to Radojevic and Bashkin (1999). A blank sample was digested so as to allow a blank correction to be made. This was done by transferring 100ml of distilled water into a beaker and digested as described above. Determination of Cu^{2+}, Zn^{2+}, Mg, Fe^2, Cr^{2+}, Cd, As, Ni, and Pb^{2+} were made directly on each final solution using an Atomic Absorption Spectroscopy (AAS) (Perkin-Elmer A Analyst 300, Oahus, USA).

RESULTS AND DISCUSSION

Physicochemical and Microbiological Qualities of the Abattoir Wastewater

The result of physicochemical and microbiological properties of the abattoir waste water is presented in (Table

1.1). The results for the mean values of Biochemical Oxygen Demand (BOD) and Chemical Oxygen demand (COD) were 2630.00 mg L^{-1} and 5,830.00 mg L^{-1} which were higher than WHO recommended standard limits of 20mg L^{-1} and 1000mg L^{-1} respectively for the wastewater discharged into surface water and connotes the toxic level of the effluent. Increase in BOD may have been due to heavy discharge of industrial waste water effluent, animal and crop wastes and domestic sewage. BOD values have been widely adopted as a measure of pollution effect. It indicates the amount of putrescible organic matter present in water (Kumar *et al*., 2011). Low BOD content is an indication of good quality water, while a high BOD indicates polluted water. BOD directly affects the amount of dissolved oxygen (DO) in rivers and streams. The greater the BOD, the more rapidly oxygen is depleted in the stream.

Table 1.1: Microbiological and physicochemical parameters in the abattoir wastewater

Parameters	**Standard mean deviation**
TSS (mg/l)	5257.50 ± 2221.72
BOD (mg/l)	2630.00 ± 246.22
COD (mg/l)	5830.00 ± 2009.34
pH	7.90 ± 0.60
Temperature (ºC)	26.75 ± 1.30
Total viable Bacterial (cfu /ml)	2.16×10^7
Yeast (cfu/ml)	5.82×10^5
Coliforms (cfu/ml)	1.62×10^5

This means less oxygen is available to higher forms of aquatic life (Harrigan and McCance, 1990; Prescott *et al*., 2002; Akpor and Muchie, 2011).

COD test is used to measure the load of organic pollutants in the industrial waste water. Both COD and BOD values are a measure of the relative oxygen depletion effect of a waste contaminant (Harley and Prescott, 1996). Both have been widely adopted of pollution effect. COD is similar in function to BOD, in that both measure the amount of organic compounds in water.

The mean value of the Total suspended solid level (TSS) of abattoir wastewater was 5257.50 mg L^{-1}. Value was higher than WHO (2011) standard of 20 mg L^{-1}. Such elevated value for TSS in the abattoir wastewater might be attributed to the high organic load and dissolved solid wastes originating from the slaughtered animals. High concentrations of suspended solids can cause many problems for stream health and aquatic life (Dan-AZumin and Bichi, 2010). High TSS can block light from reaching submerged vegetation. As the amount of light passing through the water is reduced, photosynthesis slows down. Reduced rates of photosynthesis causes less dissolved oxygen to be released into the water by plants. If light is completely blocked from bottom dwelling plants, the plants will stop producing oxygen and will die. As the plants are decomposed, bacteria will use up even more oxygen from the water (Mueller and Helsel, 1999). Low dissolved oxygen can lead to fish kills. High TSS can also cause an increase in surface water temperature, because the suspended particles absorb heat from sunlight. This can cause dissolved oxygen levels to fall even further (because warmer waters can hold less DO), and can harm aquatic life (Giller and Malmqvist, 1998).

The pH of the abattoir wastewater sample was near alkaline with pH mean values of 7.90 (Table 1.1). However, the mean pH was within the WHO (2011) permissible limits of 6.0-9.0 for wastewater discharged from industries into river. Temperature is one of the most important environmental features in waste water. It controls behavioural characteristics of organisms, solubility of gases and salts in water (Joanne *et al*., 2011). The basis of all life functions is complicated set of biochemical reactions that are influenced by physical factors such as temperature. The mean value of temperature in the abattoir wastewater was 26.75 ^{O}C the moderate temperature could be as a result of waste not from thermal pollution or a power plant (Ram, Pravin, and Deepali, 2011).

The microbiological analysis revealed that the abattoir wastewater samples were highly contaminated by both bacteria and fungi. Mean coliform counts was 1.62×10^5 cfu/1mL mean total viable bacteria counts of 2.16×10^7 cfu/mL and yeast counts of 5.82×10^5 cfu/mL. Attested to this fact (Table 1.1), these results were higher than Federal Environmental Protection Agency (FEPA) permissible limit of (500.0 cfu/100mL). The high microbial contents may have been due to high organic content, sufficient organic biological nutrients, adequate alkalinity of wastewater and its components such as blood, fat, manure, animal and undigested feeds of abattoir effluent stream (Rajaram and Das, 2008; Nafarnda *et al*., 2012).

The heavy metal content and concentration of the abattoir wastewater is presented in Table 1.2. Mean value of 1.61mg/l for copper (Cu) was observed which was above the acceptable limit of 0.05mg/L set by the WHO (2011). Copper is a very common substance that occurs naturally in the environment and spreads in the environment through natural phenomena such as wind blown dust, decaying vegetation (Do¨nmezand Aksu, 1999). Humans widely use copper. For instance itapplied in the industries and in agriculture which includes mining, metal production, wood production and phosphate fertilizer production. Copper is highly toxic to most fishes, invertebrates and aquatic plants than any other heavy metal except mercury (Berdicevisky *et al*. 1993). It

reduces growth and rate of reproduction in plants and animals. Aquatic plants absorb three times more Cu than plants on dry lands (Centre for Ecological Sciences, CES, 2001). Excessive Cu content can cause damage to roots, by attacking the cell membrane and destroying the normal membrane structure; inhibit root growth and formation of numerous short, brownish secondary roots (Nafarnda *et al*., 2006). Cu becomes toxic for organisms when the rate of absorption is greater than the rate of excretion, and as Cu is readily accumulated by plants and animals, it is very important to minimize its level in the waterway (Nur *et al*., 2011).

Table 1.2: Concentrations of heavy metals in abattoir wastewater

Heavy metals mg/l	Mean value
Copper	1.61 ± 0.04*
Chromium	0.02 ± 0.04
Zinc	79.50 ± 15.71
Nickel	19.10 ± 7.32
Magnesium	5.15 ± 4.90
Lead	0.16 ± 0.13

*Mean standard deviation (±) of four determinations

The mean value of chromium (Cr) in the effluent samples was found to be 0.02mgL^{-1} (Table 1.2), which was less than the permissible limit of 0.05mg/L set by the WHO (2011). This is probably because of the abattoir wastes water was not polluted by textile industries and tannery wastes which is major source of Cr (Rajaram and Das, 2008). Chromium enters the air, water and soil in forms of chromium (III) and chromium (VI) through natural processes and human activities. The main human activities that increase the concentrations of chromium (III) are steel, leather and textile manufacturing while chromium (VI) concentrations are due to chemical, leather and textile manufacturing, electropainting and other chromium (VI) application in the industry (Badar *et al*., 2000).These applications will mainly increase concentration of chromium in water (Suranjana and Manas, 2009). Through coal combustion of chromium also end up in air and through waste disposal chromium ends up in soils (Jeyasingh and Ligy, 2005).

The average concentration of Lead (Pb) in the wastewater samples was found to be 0.16mg L^{-1} (Table 1.2) which is higher than the permissible limit for Pb in drinking water (<0.05mgL^{-1}) according to the WHO (2011) drinking water standards. Pb occurs naturally in the environment. However, most lead concentrations that are found in the environment as a result of human activities. In car engines lead is burned, so that lead salts will originate (Simone, Fernando and Maria, 2012). These lead salts enter the environment through the exhausts of cars. Lead is one out of four metals that have the most damaging effects on human health (Chikere and Okpokwasili, 2003). It can enter the human body through uptake of food (65%), water (20%) and air (15%). Lead can end up in water and soils through corrosion of leaded pipelines in a water transporting system and through corrosion of leaded paints (Nur *et al*., 2011).

The mean value of Nickel (Ni) in the wastewater was 19.10mg L^{-1} (Table 1.2). The value is considerably higher than the maximum limit of 0.1mg/L set by W H O (2011). Nickel is released into the air by power plants and trash incinerators. It will then settle to the ground or fall down after reactions with raindrops (Bergman and Dorward-King, 1996). It usually takes a long time for nickel to be removed from air (Coppoolse, Schwartz, Annema and Quarles, 1993). Nickel can also end up in surface water when it is a part of wastewater streams. In small quantities nickel is essential, but when the uptake is too high it can be a danger to human health. Short-term exposure to Ni on human being is not known to cause any health problems, but long-term exposure can cause decreased body weight, heart, liver damage and skin irritation (Tiwana *et al*., 2005; Suranjana and Manas, 2009).

In the current study the mean value of Zn in the waste water was 79.5mg L^{-1} (Table1.2), which is also higher than the permissible limit of 5.5mg L^{-1} set by WHO (2011). Water is polluted with zinc due to the presence of large quantities of zinc in the wastewater of industrial plants. Zinc may increase the acidity of water bodies. Some fish can accumulate zinc in their bodies, when they live in zinc-contaminated waterways (Alam and Maughan, 1992). When zinc enters the bodies of these fishes it is able to biomagnify up the food chain (Coppoolse *et al*., 1993). Large quantities of zinc can be found in soils. When the soils of farmland are polluted with zinc, animals will absorb concentrations that are damaging to their health. Water-soluble zinc that is located in soils can contaminate groundwater (Bergman and Dorward-King, 1996). Zn cannot only be a threat to cattle but also to plant species. Plants often have Zn uptake that their systems cannot handle due to the accumulation of zinc in soils. Extreme concentration of Zn may result in necrosis, chlorosis and inhibited growth of plants

(Suranjana and Manas, 2009; Raut, Charif, Amal, Shinoona and Abrar, 2012).

The mean concentration of magnesium in the wastewater was 5.15mg L^{-1} which is slightly above the acceptable limit of 5.0mgL^{-1} set by WHO (2011). This is probably because magnesium is commonly included in dietary mineral preparations, include many multivitamin preparation of animal feeds, other sources of magnesium are plant products such as cereal, nuts, vegetables and green leaf (Teigen and Boes, 2014). High magnesium in water is capable of reducing water to highly flammable hydrogen gas; as a result, water cannot extinguish magnesium fires. The hydrogen gas produced only intensifies the fire (Arnaud, 2008).

CONCLUSION AND RECOMMENDATIONS

Conclusion

Heavy metal contamination constitutes an important environmental problem due to the toxic effects of metals, and their build-up throughout the food chain leading to serious ecological and health problems. The wastewater from Minna Central Abattoir located at Tayi in Bosso Local Government Area of Niger State, Nigeria harboured various yeast includes species of *Trichosporon*, *Cryptococcus* and *Candida* which may have potential to exhibit remarkable biotolerance and biosorption towards different concentrations.

Recommendations

It is recommended that:

i. Abattoir wastewater should be collected, transported, recycle, treated and disposed of to avoid adverse effects on the environment and public health.

ii. The Butchers and personnel of abattoir should be enlightened on the need for safety, precaution and proper hygiene in their activities in the abattoir.

iii. The role of bacteria and moulds in bioremediation of abattoir waste water having heavy metals should be studied.

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