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Biotreatment of abattoir waste water using selected microorganisms at Mandate market Ilorin, Kwara state

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Abstract

Slaughterhouse wastewater has a complex composition and very harmful to the environment. The abattoir wastewater at mandate market Ilorin, Kwara State was studied for possible pollutants and biotreatment using indigenous microorganisms. Microbial count was done using pour plate techniques and the physicochemical analyses were done using standard method. Total bacteria count was between 1.60×10^4 - 2.43×10^7 Cfu/ml while fungi count was 1.63×10^3 - 1.8×10^6Cfu/ml. Findings showed that the various water samples were contaminated with E. coli and other enteric bacteria. This result revealed that the temperature, pH and electric conductivity ranged from 27.8 - 28.40^{o}C, 7.07 - 7.19, and 103 - 887µS/cm respectively, Total Dissolved Solid (TDS) and Total Suspended Solid (TSS) were between 1350.20 - 880 and 76.5 - 2266.0 mg/L respectively, the Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemically Oxygen Demand (COD) ranged between 4.5 - 0.2, 54.4 - 130 and 700 - 1240 mg/L respectively. The presence of E. coli and Streptococcus faecalis in the abattoir wastewater may be attributable to the high load of animal excreta in the wastewater.. Most of the fungal isolates were also soil-inhabiting microorganisms as well as common spoilage organisms associated with beef industry.

Keywords: Abattoir, Biochemical Oxygen Demand (BOD), Biotreatment, Chemically Oxygen Demand (COD), Physicochemical, analysis

1. Introduction

In Nigeria, the abattoir industry is an important component of the livestock industry providing domestic meat supply to over 150 million people and employment opportunities for teaming population (Adeyemi and Adeyemo, 2007). Slaughtering of animals result in significant meat supply, a good source of protein and production of useful by-products such as leather, skin and bones. The processing activities involved sometimes result in environmental pollution and other health hazards that may threaten animal and human health. Animals are slaughtered in abattoirs for sale to the public. An abattoir has been defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, processing and effective preservation and storage of meat products for human consumption (Alonge, 1991).

Adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites (Adebowale, *et al.,* 2010) unlike in developed countries where these facilities are adequately provided. These abattoir wastes could be a source of embarrassment since conventional methods of waste management have been grossly neglected (Adeyemi and Adeyemo, 2007).

Waste water or effluent generated from the abattoir is characterized by the presence of a high concentration of whole blood of slaughtered food animals and suspended particles of semi- digested and undigested feeds within the stomach and intestine of slaughtered and dressed food animals (Coker, *et al.,* 2001). In addition, there may also be the presence of pathogenic microorganisms such as *Salmonella, Escherichia coli* (including serotype 0157:H7), *Shigella*, parasite eggs and amoebic cysts (Adebowale, *et. al.,* 2010) are of public health importance. Also, several pathogenic bacteria and fungi species has been isolated from abattoir wastewater and surface water; including *Staphylococcus, Escherichia coli, Streptococcus, Salmonella, Aspergillus, Mucor, Saccharomyces and Penicillium* species (Coker, *et al.,* 2001, Adesomoye, *et. al.,* 2006, Adebowale, *et. al.,* 2010). These pathogens might threaten public health by migrating into ground water or surface water; wind or vectors like animals, birds and arthropods can transmit diseases from these microorganisms (Raheem & Morenikeji, 2008).

The continuous drive to increase meat production for the protein needs of the ever increasing world population has some pollution problems attached. Pollution arises from activities in meat production as a result of failure in adhering to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) (Akinro, *et. al*., 2009). In the Nigerian livestock industry, slaughter houses are littered with non-meat products and wastes that need to be recycled into useful by-products for further agricultural and other industrial uses (Osibanjo & Adie, 2007).

Effluent from slaughterhouses has also been known to contaminate both surface and groundwater because during abattoir processing, blood, fat, manure, urine and meat tissue are lost to the wastewater streams (Bello and Oyedemi, 2009). In Nigeria, many abattoirs dispose their effluents directly into streams and rivers without any form of treatment and the slaughtered meat is washed by the same water. Leaching into groundwater is a major part of the concern, especially due to the recalcitrant nature of some contaminants (Muhirwa, *et. al*., 2010). This study therefore evaluates the distribution of microorganisms in the abattoir wastewater and also the bio-treatment of the abattoir wastewater with selected indigenous microorganisms.

2. Materials and Methods

2.1. Sample Area and Collection

Effluent samples were collected from the abattoir at Mandate market in Ilorin metropolis, Kwara State. Investigation showed that many cows are slaughtered in this abattoir. Normal abattoir operations are carried out from Monday to Saturday. The blood wash and the process water from the abattoir are channelled directly into a close by river.

Wastewater samples were collected using the method of Adesemoye, *et. al.* (2006). Sterile 2.0 litres sample bottles were used to aseptically collect the abattoir waste water by placing the sterile container at the end of the effluent passage or drainage. The samples were collected at four different points as the waste water was running off the drainage system. About 500ml of the sample collected from each point were pooled together to obtain composite sample. Control samples were collected from water stored in buckets used for washing meat and utensils in the abattoirs. The samples were transported immediately to the laboratory for analysis.

2.2. Determination of Physical and Chemical Properties

2.2.1. pH and temperature determination

The pH meter (Hanna HI9024 Microcomputer pH) was calibrated with two buffer standard solutions (pH 4 buffer and pH 9 buffer). After that, the pH reading of the water sample was taken on the spot. The temperature of the water sample was taken on the spot using a mercury thermometer.

2.2.2. Electrical Conductivity

The electrical conductivity was measured with conductivity meter (Hanna HI9024 Microcomputer pH/ev-) at 25^{o}C.

2.2.3. Total Suspended Solids (TSS)

Hundred (100) millilitre of each water sample was filtered through a pre-weighed filter paper. The filter paper was dried at 103- 105°C. TSS was determined using the formula of (Anon, 1992).

2.2.4. Total Dissolved Solids (TDS)

This was by the evaporation method. Evaporation dish was weighed and later 100 ml of the water sample introduced into the weighed dish and dried in an oven operated at 103°C for one hour to a constant weight. After drying, it was transferred to a desiccators and left to cool for one hour. The dish was finally weighed with its content. The difference in mass gives a measure of the total dissolved solids of the sample (Hach Water Analysis Hand book, 1983)

2.2.5. Dissolved Oxygen (DO)

Dissolved oxygen (D.O.) was determined by a titration method; 2ml of manganese sulphate and 2 ml of alkali Iodide azide reagents were added and a brownish colour was obtained. The solution was then made to stand until it formed clear supernatants. Concentrated sulphuric acid was added for preservative purpose and was shaken to distribute iodide evenly. Sodium thiosulfate was used in titrating to get a pale yellow colour and 1 ml of 1% starch was added to get blue-black colour. At a point, the blue-black colour disappeared which is referred to as the end point, and this coincided with the amount of D.O. in the water samples methods described by (Ademoroti, 1996).

$$\text{DO (mg/l)} = \frac{16000 x M x V}{V1 - 2.0}$$

where M = Molarity of the thiosulphate solution, V = Volume of thiosulphate used for titration, V1 = Volume of the bottle with the stopper in place.

2.2.6. Biochemical Oxygen Demand (BOD)

The BOD was determined using Winkler titration method. The water sample was collected in the BOD bottle and incubated at 20°C in the dark for 5 days. The BOD on day five was determined using the same procedure for DO above. The mass of oxygen obtained in day 5 was subtracted from the mass of oxygen on day 1 to determine the BOD (mg/L) using the formula (Ademoroti, 1996).

BOD5 (mg/L) = (DO1 – DO5)

2.2.7. Chemical Oxygen Demand (COD)

Titrimetric method was employed in the determination of COD. A 10 ml of 0.125 M $K_2Cr_2O_7$ was added to 20ml of the water sample using a pipette in a refluxing flask. Glass beads or anti-bumping chips were added. Then 30ml of concentrated H_2SO_4 was added slowly and with gentle swirling. The flask was connected to the condenser and refluxed for 2h.

After that, the flask was cooled and the condenser washed with distilled water into the flask and diluted to about 150 ml. The excess dichromate was titrated with 0.05 M ferrous ammonium sulphate (FAS) using 2 drops of ferroin as indicator. A blank mixture was prepared and treated using the same procedure (Ademoroti, 1996).

$$\text{COD (mg/l)} = \frac{(Vb-Vs)xMx16000}{mlsample}$$

where: Vb = mL FAS used for blank, Vs = mL FAS used for sample, M = molarity of FAS.

2.3. Microbiological Analysis

2.3.1. Sterilization of Materials

Glassware such as conical flasks, test tubes pipettes, measuring cylinder and Petri dishes were washed thoroughly with detergent, rinsed with water and sterilized in the oven at 140^{o}C for 180 minutes. Petri dishes, pipettes and measuring cylinders were wrapped properly with aluminum foil before sterilization in an oven at a temperature of 100^{o}C for one hour (Fawole & Oso, 2004).

2.3.2. Preparation of culture media

Nutrient agar (NA) was used for the isolation of bacteria, Eosin Methylene Blue (EMB) for coliform organisms and potato dextrose agar (PDA) used for the isolation of fungi. Sterilized glass wares were used during preparation. Appropriate weights of the nutrient agar, eosin methylene blue and potatoes dextrose agar were transferred to separate conical flask, following the manufacturer's instructions. The media were sterilized in the autoclave at 121^{o}C for 15 minutes.

2.3.3. Isolation and enumeration of associated microorganisms

The method used was that of Obuekwe & Ogbimi (1998). One millilitre of each of the sample (waste water and source water control) was first measured and diluted in 9 ml. of sterile distilled water prior to serial dilution. One millilitre aliquot was diluted with 9 mL of sterile water in different test tubes to give 1:9 dilutions.

From this, ten-fold serial dilutions were made up to 10^{-6}. One millilitre of the 10^{-4} dilution of the sample was pour plated on nutrient agar for bacteria, dilution of 10^{-5} was pour plated on PDA for fungi count. All the plates in duplicates were incubated at 37°C for 24 h for bacteria, while the plates for fungi were incubated at 30°C for 24-72 h.

Colonies of microorganisms that developed on the plates after incubation were counted, recorded and expressed as standard numbers of colony forming unit per millilitre (cfu mL^{-1}) for bacteria and spore forming unit per millilitre (sfu mL^{-1}) for fungi. The discrete colonies (3) that grew on NA and PDA were subculture on fresh media to obtain pure cultures. The pure cultures were maintained at 4°C as stock culture for further tests.

2.3.4. Characterization of the bacteria isolates

Colonial characteristics of the bacteria isolates were determined using parameters such as size, elevation, pigment, surface, opacity, edge and shape. Cellular and biochemical characteristics of the isolates were determined as follows; gram staining, motility test, spore staining, capsule staining, catalase, oxidase, indole, starch hydrolysis, citrate utilization, oxygen relationship and sugar fermentation.

2.3.5. Characterization of Fungal Isolates

The fungal isolates were characterized using their colonial morphology on the plates. Parameters such as colour of the colonies, nature of the hyphae, appearance of the colonies and the growth rates were considered for proper characterization of the isolates. Microscopic examinations of the isolates were also carried out.

2.3.6. Preparation of Inocula

Each of the bacterial isolates which include *Pseudomonas* sp., *Bacilli* sp. and *Klebsiella* sp. and *Staphylococcus aureus* were subculture on a separate fresh, sterile nutrient broth medium followed by incubation at 37℃ for 24 hours.

The cells were then harvested by centrifuging at 2000rpm for 30 minutes, after which the cells were individually suspended in sterile physiological normal saline and further washed by centrifuging at 2000rpm for another 30 minutes to obtain neat cells which were suspended in sterile physiological normal saline and further diluted with sterile physiological normal saline to a low density cell suspension of concentration of over 10^6 using the heamocytometer. The resultant cell suspension was diluted serially, then 0.1ml from 10^{-2} dilutions, which served as inoculum was added to the experimental flasks containing 1 litre of waste water. Sample were taken at regular interval of 48hr from the 1 litre flask for BOD analysis (Prasad & Manjunah, 2011).

3. Results

Table 1 showed the mean bacterial count from Mandate market abattoirs. The mean total bacterial count was 1.60 $x10^4$cfu/ml for the source water while that of the abattoir wastewater was 2.43$x10^7$cfu/ml. Meanwhile, mean coliform count of the source water and abattoir wastewater is 2.30 $x10^2$cfu/ml and 2.3 x 10^6 cfu/ml respectively. Mean total fungi count from the abattoir source water and wastewater was 1.63 $x10^3$sfu/ml and 1.8 $x10^6$sfu/ml respectively. The result obtained is an indication that abattoir effluent contains high level of organic pollution

The morphological, microscopic and biochemical characterization of the isolated organisms are on Table 2. The bacterial isolated and identified from the abattoir wastewater were *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus feacalis, Bacillus subtilis, Klebsiella pneumonia,* and *Escherichia coli.* The fungi isolated and identified were *Aspergillus niger, Aspergillus flavus, Penicillum* sp., *Fusarium* sp.*, Sacharomyces cervisiae and Rhizopus stolonifer. Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli. Aspergillus flavus, Penicillum* sp and *Sacharomyce scervisiae*are common to both water (source water and wastewater).

Table 3 showed the antibiotics sensitivity pattern of isolates while table 4indicated the physico-chemical properties of abattoir wastewater from Mandate market abattoir. Monitoring the biodegradation process after seeding with indigenous microorganisms for twelve days is indicated in Table 5.

Table 1: Total viable counts of Bacteria, coliform and fungi isolated from mandate market abattoir wastewater and source water used at the abattoir

Organism	Source of water (Cfu/ml)	Abattoir waste water(Cfu/ml)
Bacterial	1.60×10^4	2.43×10^7
Coliform	2.30×10^2	2.3×10^6
Fungi	1.63×10^3	1.8×10^6

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolates from wastewater and source of water used

Parameter	Isolate1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6
Cellular shape	Cocci	Rod	Rod	Rod		Rod
Colonial elevation	Raised	Flat	Raised	Raised	Raised	Raised
Colonial edge	Entire	Lobate	Entire	Lobate		Entire
Colonial opacity	Opaque	Opaque	Translucent	Translucent		Opaque
Colonial surface	Smooth	Dull	Smooth	Dull		Mucoid
Colonial pigmentation	Creamy white	White	Yellowish cream	Yellow	Dark centred	Pinkish on EMB
Cellular arrangement	Cluster	Clusters	Chain	Chain		Scattered
Gram's staining	+ve	+ve	-ve	+ve	-ve	-ve
Motility test	-ve	+ve	+ve	+ve	-ve	-ve
Spore staining	-ve	+ve	-ve	+ve	-ve	-ve
Capsule staining	-ve	+ve	-ve	-ve	-ve	-ve
Catalase test	+ve	+ve	-ve	+ve	+ve	+ve
Methyl red test	-ve	+ve	+ve	-ve	-	+ve
Indole	-ve	-ve	-ve	-ve	+ve	+ve
Voge-prokauer	-ve	-ve	-ve	-ve	+ve	-ve
Starch hydrolysis	+ve	-ve	-ve	+ve	-	-ve
Citrate utilization	+ve	-ve	-ve	-ve	+ve	-ve
Oxygen reaction	FAN	FAN	AE	AE		
Lactose	AG	AG	-ve	-ve	AG	A
Glucose	A	A	A	AG	AG	A
Sucrose	A	A	A	A	AG	A
Maltose	A	A	A	AG	AG	A
Fructose	-ve	A	AG	AG	-	A
Probable identity	*Staphylococcus spp*	*Bacillus spp.*	*P. aureginosa*	*Micrococus spp.*	*Salmonella pneumonae*	*E coli*

Keys; Negative = -ve, Acid production = A, Positive = +ve, Acid and Gas production = AG, Facultative anaerobe = FAN, Aerobic = AE

Table 3: Antibiotics sensitivity pattern of isolates

Isolate	Zone of inhibiton (mm)							
	AUG (30ug)	OFL (5ug)	CXM (5ug)	CRX (30ug)	CAZ (30ug)	GEN (10ug)	CRP (5ug)	NIT (300ulg)
Staphylococcus aureus	R	R	14.0	R	R	4.0	19.0	R
Psuedomonasaureginosa	10.0	12.0	R	R	R	9.0	18.0	R
Streptococcus. Feacalis	R	19.0	R	R	R	23.0	18.0	18.0
Klebsiella pneumonia	R	12.0	R	R	R	16.0	R	12.0
Escherichia coli	4.0	26.0	16.0	15.0	19.0	12.0	25.0	16.0

AUG=Augmentin, OFL=Ofloxacin, CXM=Cefixime, CRX=Cefuroxine, CAZ=Ceftazidine, GEN=Gentamicin, CRP=Ciprofloxacin, NIT=Nitrofurantim

Table 4: Physico-chemical properties of abattoir wastewater from Mandate market abattoir

Parameters	Source water	Waste water(Before treatment)	Waste water (After treatment)
Temperature $T^{O}C$	27.8	28.4	28.2
Colour	Colourless	Reddish brown	Light brown
Odour	No odour	Dung	No odour
pH	7.07	7.19	7.04
Conductivity		887	2, 420
Dissolved oxygen mg/l	4.5	0.2	3.86
Biological Oxygen Demand (BOD) mg/l	54.5	130	0.0
Chemical Oxygen Demand (COD) mg/l	700	1240	440
Total Suspended Solid (TSS) mg/l	76.50	2266.82	350
Total Dissolved Solid (TDS) mg/l	1350.20	880	750
Total Solid (TS) mg/l	1426.70	3116.82	1100
Chloride Cl^{-}	8	15	10
Sulphate SO_4^{-}	15	28	25

Table 5: Monitoring the biodegradation process after seeding with indigenous microorganisms for twelve days

Organism	BOD result after seeding the organisms in the wastewater						
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
Bacillus subtilis	130	90	53	32	17	4	0
Staphylococcus aureus	130	81	42	23	12	0	0
Pseudomonas aeruginosa	130	85	45	27	11	0	0
Streptococcus faecalis	130	100	60	35	18	2	0

4. Discussion

The presence of *E. coli* and *Streptococcus faecalis* in the abattoir wastewater may be attributable to the high load of animal excreta in the wastewater. It is also an indication of recent faecal pollution as shown in table 1 above. In similar findings, *Streptococcus faecalis, Escherichia coli, Staphylococcus* sp, *Clostridium* sp, and *Salmonella* sp were reported by Ezeronye & Ubalua (2005), from Aba River as a result of contamination in the abattoir.

Most of the fungal isolates were also soil-inhabiting microorganisms (Atlas & Bartha, 2007) as well as common spoilage organisms associated with beef industry (Alonge, 1991). The presence of these organisms is a pointer to possible pollution and may have an effect on the soil ecological balance. These findings were in conformity to that of Adesemoye, *et. al.* (2006), as well as Ogbonna & Igbenijie (2006).

The level of resistance and sensitivity in table 3 of the isolated bacteria to antibiotics differs. Levy (1992), reported that antimicrobial resistance in bacterial pathogens is a major impediment to successful therapy, and bacterial strains have arisen that are resistant to most available antimicrobial treatments. The multiple nature of drug resistance of these bacteria creates an extremely serious public health problem and it has always been associated with the outbreak of major epidemic throughout the world (Prescott, *et. al*., 2008).

Table 4 reveals the TDS are an indication of the degree of dissolved substances such as metal ions in the water (Efe, 2005). DO have a range of 0.2-4.50 mg/L with a mean value of 3.50 mg/L. This value is lower than WHO permissible limit of 4 mg/L and also, lower than the control 4.5 mg/l. Low DO may result in anaerobic conditions that cause bad odour. BOD and COD ranges between 54.5-130 and 440-1240 mg/L with mean values of 92.25 and 840 mg/L, respectively. The mean value for BOD is higher than the control and WHO allowable limit of 20 mg/L (WHO, 2006). COD has a mean value lower than the permissible limit of 1000 mg/L (WHO, 2006). BOD and COD are indices of organic pollution. BOD is not a specific pollutant indicator, but rather a measure of the amount of oxygen required by bacteria and other microorganisms engaged in stabilizing decomposable organic matter over a specified period of time.

A high oxygen demand indicates the potential for developing DO sag as the microbiota oxidizes the organic matter in the water. Since nearly all organic compounds are oxidized in the COD test, Sulphate concentration ranges from 15-28 mg/L with a mean value of 23.0 mg/L. This is lower than the maximum permissible limit of 250 mg/L set by WHO but higher than the control value of 15 mg/L in table 4 above. This implies that the activities in the abattoir are contributing to the pollution load of the stream and long term effect may subsequently lead to contamination of the surrounding water body. Chloride concentration ranges between 8 and 15 mg/l. chlorine can affect BOD measurement by inhibiting or killing the microorganisms that decompose the organic and inorganic matter in a sample.

In the biodegradation study, table 5 indicate *Staphylococcus aureus* showed the highest reduction potential for BOD value than other organisms used for degradation while *Streptococcus feacalis* had the least BOD reduction potential. BOD directly affects the amount of dissolved oxygen in body of water. The greater the BOD, the more rapidly oxygen is depleted in the body of water. This means that less oxygen is available to higher form of aquatic life as shown table 5 above and this correlate with the carried out by Adesemoye, *et. al.* (2006).

5. Conclusion

Although abattoir operation could be very beneficial to man because it provides meat for human consumption and other useful by-products, still, it can be very hazardous to public health with respect to the wastes that is generated. The high pollution strength of the abattoir wastes as revealed in this study further confirmed the danger associated with discharging untreated wastes to the environment, hence the need for adequate treatment to ensure decontamination.

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