ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 6, pp.053-057, July, 2010

RESEARCH ARTICLE

ENUMERATION AND CHARACTERIZATION OF MICROORGANISMS INVOLVED IN THE DEGRADATION OF ABATTOIR WASTE IN PORTHARCOURT

[1]Eze, V.C.*and [2]Ikeri, E.P

[1]Department of Microbiology, Michael Okpara University of Agriculture, Umudike, P.M.B.7267, Umuahia, Abia State, Nigeria

[2]Department of Microbiology, Madonna University Okija, Elele Campus, Rivers State, Nigeria

ARTICLE INFO

Article History:
Received 11th May, 2010
Received in revised form
24th May, 2010
Accepted 19th June, 2010
Published online 1st, July, 2010

Key words:
Microorganism,
Degradation,
Abattoir and wastewater,
Soil

ABSTRACT

The enumeration and characterization of microorganisms involved in the degradation of abattoir waste in Port Harcourt was investigated. Soil and wastewater samples contaminated with abattoir wastes were collected. The mean counts of the soil samples showed that the total aerobic plate count ranged from 3.2 ± 1.1 to 7.3 ± 2.3 Log_{10}cfu/g; proteolytic count ranged from 1.9 ± 0.3 to 3.0 ± 0.9 Log_{10}cfu/g; amylolytic count ranged from 1.1 ± 0.30 to 3.85 ± 0.8 Log_{10}cfu/g; cellulolytic count ranged from 0.35 ± 0.15 to 0.95 ± 0.30 Log_{10}cfu/g and lipolytic count ranged from 0 to 0.06 ± 0.01 Log_{10}cfu/g.The fungal count ranged from 2.2 ± 1.3 to 4.5 ± 1.5 Log10cfu/g. The mean counts of the wastewater samples revealed that the total aerobic plate count ranged from 2.0 ± 0.9 to 8.6 ± 3.6 Log_{10}cfu/m; proteolytic count ranged from 1.6 ± 0.7 to 4.7 ± 2.1 Log10cfus/mL; amylolytic count ranged from 0.45 ± 0.1 to 4.0 ± 1.7 log10cfu/mL; cellulolytic count ranged from 0.28 ± 0.06 to 0.35 ± 0.11 Log10cfu/mL while the lipolytic count had no count. The fungal count ranged from 2.2 ± 1.4 to 3.2 ± 1.6 Log10cfu/mL.The microorganisms isolated were Staphylococcus aureus, Cytophaga species, Streptococccus species, Pseudomonas species, Bacillus species, Micrococcus species, Klebsiella species, Vibrio cholerae Aspergillus species, Cladosporium species and Rhizopus species This indicates that microorganisms can actively degrade abattoir waste.

INTRODUCTION

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. Abattoir operations like slaughtering, processing and meat packaging produce a characteristic highly organic waste into the environment (Alonge, 1991).

Livestock waste spills can introduce enteric, pathogens and excess nutrients into surface waters (Meadow, 1995). Livestock production, which is perceived by the public to be potential food for the world's needy people, is a major pollutant of the countryside, where the animals are raised and cities, if processors do not manage slaughter wastes properly with dung and slurry washed into water ways. Other environmental problems include pollution of soil with dung and the atmosphere with methane (a greenhouse gas) from decomposing waste (Chukwu *et al.,* 2008). Wastes could be hazardous or non hazardous. Hazardous waste is defined as the wastes that possess substantial harm to human health or the environment when not properly treated, stored, transported or disposed off or otherwise managed.

***Corresponding author:** *vin13eze@yahoo.com*

While non hazardous waste refers to the waste that is converted into economical use either by analysis or treatment (Gilbert, 1998). Abattoir waste are hazardous waste and is another form of agricultural waste which includes intestinal content, rumen, scraps of tissues, horns, bones, blood, faecal matter, fatty and proteinous materials. Abattoir waste contains small quantities of components which are dangerous or potentially dangerous to the environment. It is not pleasant statistics that a 100 cow dairy herd can produce as much waste as 2,400 people. But it is not the only unpleasant fact, in certain types of soil this waste can seep through the ground and reach groundwater, polluting it with nitrate and bacteria. Meat processing industries (abattoirs) are generally less developed in developing countries like Nigeria unlike advanced countries where waste generation, analysis and treatment are considered before constructing the abattoir (Ogbonnaya, 2008).

The elimination of a wide range of pollutants and wastes from this environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versality of microorganisms to degrade or convert such compounds (Madigan and Markinko, 2008).

The process for the microbial degradation of waste comprises determining the significant constituents of a waste, providing one or more microorganisms able at least partially to degrade each determined constituent of the waste, optionally providing one or more other microorganisms capable of partial degradation of products, growing one or more mixed cultures of at least some of the microorganisms on a synthetic mixture of at least some of the determined constituents of the waste and utilizing the adapted population of microorganisms substantially to degrade the actual waste (Ryan and Ray, 2004). Microorganisms are excellent recyclers, breaking down animal and plant matter into molecules that can be re-used by other organisms. These organisms produce enzymes that allow them to break up complex compounds into pieces that can enter the cell to be used for growth and reproduction (Willey *et al.,* 2008). The aim of this work is to isolate, enumerated and characterize the microorganisms involved in the degradation of abattoir waste.

MATERIALS AND METHODS

Background of Study Site

Port Harcourt is the capital of Rivers State, Nigeria and is located in the South-South part of Nigeria. Abattoirs in Port Harcourt are located in Rumuokoro, Eliozu. Rumuokurusi, Trans-Amadi and Ogbunabali. They are all constructed differently in such a way as to accommodate up to ten slaughtering of cattle.

Collection of Samples

Samples collected were the soil and wastewater contaminated with abattoir waste. The contaminated soil samples were collected with sterile cellophane while the wastewater samples were collected with syringes and then transferred into sterile containers and properly labeled. They were transported to the laboratory in an ice parked cooler and immediately analyzed on reaching the laboratory.

Chemical Reagents

The chemical reagents employed in the study were of analytical grade and were products of BDH chemicals, Pooles England and Sigma Chemical Company St. Louis Missouri, USA. The microbiological media used were products of Oxoid and DIFCO Laboratories, England. They included nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification of isolates and for stock culture; Sabouraud dextrose agar used for the isolation of fungi; thiosulphate citrate bile salt agar for the isolation of Vibrio cholerae and MacConkey agar for the isolation of coliforms. The proteolytic, amylolytic, celluloytic and lipolytic media were compounded and used for the isolation of proteolytic, amylolytic, cellulolytic and lypolytic microorganisms.

Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the soil and wastewater contaminated with the abattoir wastes were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined using pour plate technique. Then the molten nutrient agar, TCBS, MacConkey and Sabouraud dextrose agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi, Vibrio cholerae and coliforms respectively. They were swirled to mix and colony counts were taken after incubating the plates at room temperature for 48h and preserved by sub culturing into nutrient agar slants which were used for biochemical tests.

Enumeration of Proteolytic, Amylolytic, Cellulolytic and Lipolytic Bacteria

The medium for the enumeration of proteolytic organisms was prepared as recommended by Cruickshank et al. (1980). The skimmed milk was added after autoclaving to avoid its denaturation thereby retaining its nutritive value. The medium was composed of $MgS0_4.7H_20$, 0.3g; $FeS0_4.7H_20$, 0.2g; 0.02g; $ZnS0_4.7H_20$, 0.02g; $MnS0_4.4H_20$, 0.02g; yeast extract, 5g; skimmed milk,10g; agar, 15g and IL of distilled water. The medium for the enumeration of amylolytic organisms comprised Nacl, 2g; K_2HP0_4, 2g; $MgS0_4.7H_20$, 0.06g; $CaC0_3$, 0.02g; $FeS0_4.7H_20$, 0.01g; agar,15g in IL of distilled water was used. It was adapted from Cruickshank et al. (1980). The cellulolytic medium according to Cruickshank et al.(1980) was used for the enumeration of the cellulolytic organisms It comprised $CaC0_3$,2g; $MgS0_4.7H_20$, 1g; K_2HP0_4,1g; $(NH4)_2$ $S0_4$,1g; Cellulose powder, 5g and agar, 15g in IL of distilled water.

The lipolytic medium prepared according to Demain and Solomon (1986) and composed of peptone, 5g; Nacl, 2g; Olive oil, 10ml and agar, 20g in IL of distilled water was used for the enumeration of lipolytic organisms. The proteolytic, amylolytic, celluloytic and lipolytic organisms were then enumerated after plating in duplicate using pour plate technique, 1mL of the appropriate dilution of the samples on Petri dishes. The molten media were poured accordingly in the respective Petri dishes for the isolation of these organisms. They were swirled to mix and allowed to solidify. Enumeration of these organisms performed after incubation at room temperature for 2 days. Colonies of proteolytic, amylolytic, cellulolytic and lipolytic bacteria growing on agar plates were counted, isolated, purified by streaking on their respective media and kept on the media slants as stock cultures for characterization and identification.

Characterization and Identification of Isolates

Bacteria isolates were characterized and identified after studying the Gram reaction as well as cell micromorphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, oxidative/fermentation (O/F) utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges Proskaur reaction and urease production. The tests were performed according to the methods of (Cheesbrough, 2005; Adeoye, 2007; Agwung-Fobellah and Kemajou, 2007; Ochei and Kolhatkar, 2007). Microbial identification was performed using the keys provided in the *Bergeys Manual of Determinative Bacteriology* (1994).

Fungal isolates were examined microscopically and macroscopically using the needle mouth technique. Their identification was performed according to the scheme of Cheesbrough (2005). .

RESULTS

The results of the mean count of microorganisms isolated from the soil samples are shown in Table 1. The total aerobic plate mean count ranged from 3.2 ± 0.8 - 7.3 ± 2.3Log_{10}cfu/g.The Rumuokoro slaughterhouse had the highest count of 7.3 ± 2.3Log_{10}cfu/g while the Eliozu slaughterhouse had the least count of 3.2 ± 0.8 Log10cfu/g. The ANOVA, $P < 0.05$ showed that there was significant difference in the mean count among the locations.

The mean count of proteolytic bacteria ranged 1.9 ± 0.4 to 2.65 ± 1.2Log_{10}cfu/g. The highest count of 2.65 ± 1.2Log_{10}cfu/g came from Ogbunabali slaughterhouse and the least count of 1.9 ± 0.4Log_{10}cfu/g came from Eliozu slaughterhouse. The mean count of amylolytic bacteria ranged from 1.10 ± 0.30 to 3.85 ± 0.75log_{10}cfu/mL. The Rumuokoro slaughterhouse had the highest count of 3.85 ± 0.75Log_{10}cfu/mL while the Trans-Amadi slaughter house had the least count 1.10 ± 0.30Log_{10}cfu/L. The celluloytic and lipolytic bacteria mean count ranged from 0.35 ± 0.15 to 0.95 ± 0.30Log_{10}cfu/g and 0 to 0.06 ± 0.01Log_{10}cfu/g respectively.

The Rumokoro slaughterhouse had the highest count of 0.95 ± 0.30Log_{10}cfu/g and Trans-Amadi slaughterhouse

Table 1. The Mean Counts of Microorganisms isolated from the Soil Samples containing Abattoir Waste.

Location	Log_{10}cfu/g					
	TAPC	PC	AC	CC	LC	FC
Rumokoro	7.3 ± 2.3	2.5 ± 1.25	3.85 ± 0.75	0.95 ± 0.30	0	2.8 ± 1.16
Eliozu	3.2 ± 0.8	1.9 ± 0.4	1.25 ± 0.65	0	0	3.2 ± 1.8
Rumuokurusi	4.7 ± 1.5	3.0 ± 0.9	1.65 ± 0.55	0	0	4.5 ± 1.5
Trans-Amadi	3.45 ±1.0	2.0 ± 0.5	1.10 ± 0.30	0.35 ± 0.15	0	2.2 ± 1.3
Ogbunabali	5.0 ± 1.8	2.65 ± 1.2	1.50 ± 0.40	0.85 ± 0.20	0.06±0.01	2.5 ± 1.0
Control	2.1 ± 0.5	0	0	0	0	1.4 ± 0.6

KEYS: TAPC= Total aerobic plate count, AC = Amylolytic count , CC = Cellulolytic count, PC = Proteolytic count, LC = Lipolytic count, FC = Fungal count

Table 2. The Mean Count of Microorganisms isolated from Wastewater Samples containing Abattoir Waste

locations	Log_{10}cfu/g					
	TAPC	PC	AC	CC	LC	FC
Rumokoro	7.0 ± 3.8	4.7 ± 2.1	2.2 ±1.6	0.95 ± 0.30	0	2.16±1.2
Eliozu	5.5 ± 2.3	3.6 ± 1.6	1.9 ± 0.7	0	0	3.0 ± 1.5
Rumuokurusi	2.0 ± 0.8	1.6 ± 0.7	0.45 ± 0.05	0	0	2.5 ± 1.8
Trans-Amadi	8.6 ± 3.56	4.3 ± 1.65	4.0 ± 1.65	0.28 ± 0.06	0	3.2 ± 1.6
Ogbunabali	5.7 ± 1.86	1.55 ± 0.45	1.55 ± 0.45	0.31 ± 0.11	0	2.2 ± 1.4
Control	1.2 ± 0.1	0	0	0	0	1.4 ± 0.3

KEYS: TAPC= Total aerobic plate count, AC = Amylolytic count , CC = Cellulolytic count, PC = Proteolytic count, LC = Lipolytic count; FC = Fungi count

Table 3. **Microorganisms isolated from Abattoir Wastes and their Percentage Occurrence**

Isolates	RS	ES	RKS	TS	OS	TNI	%Occurrence
Bacteria							
Pseudomonas species	2(28.6)	1(14.3)	1(14.3)	2(28.6)	1(14.3)	7	10.6
Streptococcus species	2(25)	1(12.5)	2(25)	2(25)	1(12.5)	8	12.1
Staphylococcus aureus	4(30.8)	2(15.4)	2(15.4)	3(23.1)	2(15.4)	13	19.7
Cytophaga species	3(25)	1(8.3)	1(8.3)	4(33.3)	3(25)	12	18.2
Bacillus species	2(33.3)	1(16.7)	1(16.7)	1(16.7)	1(16.7)	6	9.1
Micrococcus species	1(33.3)	-	1(33.3)	1(33.3)	-	3	4.5
Vibrio cholerae	1(33.3)	1(33.3)	-	-	1(33.3)	3	4.5
Klebsiella species	2(25%)	2(25%)	2(25%)	1(12.5%)	1(12.5%)	8	12.1
Escherichia coli	1(16.7)	1(16.7)	1(16.7)	2(33.3)	1(16.7)	6	9.1
Total	18	10	11	16	11	66	100
Fungi							
Aspergillus species	4(44.4)	1(11.1)	1(11.1)	2(22.2)	1(11.1)	9	42.9
Rhizopus species	1(14.3)	2(28.6)	1(14.3)	2(28.6)	1(14.3)	7	33.3
Cladosporium species	2(40)	1(20)	1(20)	1(20)	-	5	23.8
Total	7	4	3	5	2	21	100

KEYS: RS = Rumuokoro Slaughterhouse; ES = Eliozu Slaughterhouse; RKS = Rumuokwurusi slaughterhouse; TS = Trans-Amadi slaughterhouse; O = Ogbunabali Slaughterhouse; TNI = Total number of isolates

had the least count 0.35 ± 0.15 Log_{10}cfu/g for cellulolytic count while Ogbunabali slaughter house had the highest count and all the other locations had the least count. The ANOVA, $P < 0.05$ showed that there was significant difference in the mean counts of all the bacterial count among the location. The fungal count ranged from 2.3 ± 1.3 to 4.5 ± 1.5Log_{10}cfu/g. The Rumuokurushi slaughterhouse had the highest count of 4.5 ± 1.5 Log_{10}cfu/mL while the Trans-Amadi slaughter house had the least count of 2.3 ± 1.3Log_{10}cfu/mL. The ANOVA $P<0.05$ showed that there was significant difference in the mean count among the locations.

Table 2 shows the mean count of microorganisms isolated from the abattoir wastewater. The total aerobic plate mean count ranged from 2.0 ± 0.8 to 7.0 ± 3.8Log_{10}cfu/mL with the Rumuokoro slaughterhouse having the highest count of 7.0 ± 3.8Log_{10}cfu/mL while Rumuokwurushi slaughterhouse had the least count of 2.0 ± 0.8 Log_{10}cfu/mL. The proteolytic count ranged from 1.55 ± 0.45 to 4.7 ± 2.1Log_{10}cfu/mL. The highest count came from Rumuokoro slaughterhouse and the least count came from Ogbunabali slaughterhouse. The amylolytic and cellulolytic organisms ranged from 0.45 ± 0.05 to 4.0 ± 1.7Log_{10}cfu/mL and 0.28 ± 0.06 to 0.95 ± 0.30 Log_{10}cfu/mL respectively.

The Transamadi slaughterhouse had the highest count of 4.0 ± 1.7 Log_{10}cfu/mL and the Rumuokwurushi slaughterhouse had the least count of 0.45 ± 0.05 Log_{10}cfu/mL for amylolytic organisms. The Rumuokoro slaughterhouse had the highest count of 0.95 ± 0.3 Log_{10}cfu/mL and the Transamadi slaughterhouse had the least count of 0.28 ± 0.06 Log_{10}cfu/mL for cellulolytic organisms. There was no lipolytic count in all the locations. The ANOVA, $P < 0.05$ showed that there was significant difference in the mean counts for the parameters in all the locations. The fungal mean count ranged from 2.2 ± 1.4 to 3.2 ± 1.6 Log_{10}cfu/mL. Transamadi slaughterhouse had the highest count of 3.2 ± 1.6 Log_{10}cfu/mL while the Rumuokoro slaughterhouse had the least count of 2.2 ± 1.4 Log_{10}cfu/mL. The ANOVA $P < 0.05$ showed that there was significant difference in the mean count among the locations. Table 3 shows microorganisms isolated from the abattoir wastes and their percentage occurrence. They included Staphylococcus aureus, 16.3%; Cytophaga species, 24.6%; Streptococcus species, 16.3%; Pseudomonas species, 14.3%; Bacillus species, 12.2%; Micrococcus species, 6.1%; Vibrio cholerae, 4.5%; Klebsiella species, 4.5%; Escherichia coli, 12.1% Aspergillus species, 42.9%; Cladosporium species, 33.3% and Rhizopus species, 23.8%.

DISCUSSION

The mean bacterial counts and fungi were high for samples from the five abattoirs. Any water contaminated to this level is neither good for domestic use nor is it supposed to be discharged directly in the environment without treatment. This could be attributed to the failure of adhering to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). Consideration is hardly given to safety practices during animal transport to the abattoir, during slaughter and during dressing. For example, during dressing, the oesophagus of cattle and sheep should be sealed to prevent leakage of animal contents. These ineptitudes often lead to contamination from hides, hooves and contents of alimentary tract during evisceration and negatively impact on the environment, including microbes in the soil and surfaces and groundwater (Amisu *et al.,* 2003; Adesemoye *et al.,* 2006). The high count also facilitates the degradation of the waste from that environment

Total bacterial population obtained from the contaminated abattoirs soil was more than that in the soil without wastewater contamination. Environmental stresses brought about by the contamination could be adduced for the reduction in microbial diversity but increasing the population of few surviving species. Previous reports have proved extensive microbial diversity with population estimated between approximately 4 x 10^3 to 10^4 species per g of uncontaminated soil (Bornenman *et al.,* 1996; Adesemoye *et al.,* 2006). A possible explanation on what transpired leading to the change in population pattern is that the organisms in the wastewater and organisms autochthonous to the soil engaged in competition and other negative microbial interactions such as antibiosis after the water was discharged into the soil. Guided by the law of survival of the fittest (Madigan *et al.,* 2003), those that were able to survive the new condition were probably excluded.

The microorganisms isolated were Staphylococcus aureus, Cytophaga species, Streptococcus species, Pseudomonas species, Micrococcus species, Bacillus species, Vibrio cholerae, Klebsiella species, Escherichia coli Aspergillus specie, Rhizopus species and Cladosporium species. This result is in line with works of Ezeronye and Ubalua (2005) and Adesemoye et al. (2006). They are autochthonous microbial communities and have the potential of waste degradation (Demain and Solomon, 1986; Eze and Okpokwasili, 2010). Bartha and Atlas (1977) reported that when natural environments are contaminated with pollutants the indigenous microbial communalities are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading the contaminating waste. Degradation of macromolecules in waste to smaller molecules is enhanced by soil microorganisms which produce a tremendous range of potentially useful enzymes that help in breaking down or decomposition of these macromolecules. Calomiriss (1976) reported that the medium employed for isolation of degrading microorganisms may have a significant selective effect on the microbial population being investigated. amylolytic, proteolytic, cellulolytic and lipolytic microorganisms were isolated from the waste and were responsible for the decomposition of starch, protein, lipid and cellulose materials respectively. Metcalf and Eddy (1978) showed that many of these substances present in waste are organic materials and serve as food for saprophytic organisms which in turn produce enzymes that enhance the degradation process.

Most of these microorganisms are pathogenic. It is therefore very necessary that wastes in abattoirs are properly disposed and treated to avoid the release of spores or organisms thus preventing the spread of diseases such as aspergillosis, anthrax and food poisoning and gastroenteritis (Piet, 2009). Better inspection of abattoir and strict enforcement of the law are needed to be able to

reduce environmental contamination and related diseases especially zoonotic diseases. Attempts to control the hygiene of slaughterhouse should include visual assessment of premises and animals themselves and those that are "visibly unacceptably dirty" or are affected by diseases should not be allowed for slaughter (Amisu *et al.,* 2003; Adesemoye *et al.,* 2006).

REFERENCES

Adeoye, A. 2007. Medical Laboratory Practice, 1st edition FEMCO Publishers Limited, Lagos, Nigeria, p.153

Adesemoye, A.O., Opere, B.O. and Makinde, S.C.O.2006. Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria, Afri. J. Biotech., 5(20):1963–1968

Agwung-Fobellah, D. and Kemajou, S.T. 2007. Laboratory Microbiology and Activity Manual, Ark of Wisdom Publishers Aba, Nigeria, pp. 12 – 37.

Alonge, D.O. 1991. Textbook of Meat Hygiene in the Tropics. Farmcoe Press, Ibadan, p. 58

Amisu, K.O., Coker, A.O., On, S.L.W., Isokpehi, R.D. 20003. *Azotobacter butzlieri* strains from poultry abattoir effluent in Nigeria, East Afri. Med. J., 80: 218 - 221

Bartha, R. and Atlas, R.M.1977. The Microbiology of Aquatic oil Spills, Adv. Appl. Microbiol. 22: 225 - 226.

Bergrey's Manual of Determinative Bacteriology. 1994. 9th edition, Holt, J.G. (Ed.) Williams and Wilkins Co. Baltimore: 783.

Borneman, J., Skroch, P.N., O'sullivan, K.M., Palus, J.A. Rumjarek, N.G, Jansen, J.I., Nienhuis, J. and Triplett, E.W. 1996. Molecular microbial diversity of an agricultural soil in Wiscosin, Appl. Environ. Microbiol. 82: 1935 - 1943.

Calomiris, J.J.B. Austin, J. Walker and Colwell, R.R.1976. Enrichment for Estuarine: Petroleum Degrading Bacteria using Liquid and Solid Media, J. Appl. Bacteriol. 41:135-144.

Cheesbrough, M. 2005. District Laboratory Practice in Tropical Countries. Cambridge University Press, United Kingdom, pp. 30-41.

Chukwu, O. H. I. Mustapha and Abdul Gafar, H.B. 2008.The effect of Minna Waste on Surface Water Quality, Environ. Res. J., 2(6): 339-342.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H. 1975. Medical Microbiology, 12th edition, Church-Hill Livingstone Edinburgh, p. 587.

Demain, L and Solomon N. A. Solomon. 1986. Manual of Industrial Microbiology and Biotechnology, American Society for Microbiology Washington. D.C, p.75.

Eze, V.C. and Okpokwasili, G.C. 2010. Microbial and other related changes in a Niger Delta River sediment receiving industrial effluents, Continent. J. Microbiol., 4: 15 –24

Ezeronye, O.U. and Ubalua, A.O.2005. Studies on the effects of abattoir and industrial effluents on the heavy metals and microbial quality of Aba River in Nigeria, Afri. J. Biotechnol., 4(3): 266 – 272.

Gilbert. M .M. 1998. Introduction to Environmental Engineering and Science 2nd edition, Prentice-Hall, New Jersey, p.275.

Madigan, M. and Markinto J.M. and Parker, J. 2003.Brock biology of microorganisms, International edition, Prentice Hall International Ltd, London , pp. 1020 – 1040

Madigan, M. and Markinto J.M.2003.Brock biology of microorganisms, 11th edition, Prentice Hall International Ltd, USA, pp. 78 – 89.

Meadows. 1995. Livestock Legacy, Environ. Health Perspect. 103(3):1096 -1100.

Metcalf, and Eddy, R.1979. *Waste Water Engineering Treatment Disposal Re-Use* 2nd edition McGraw-Hill Companies, New York, p.52.

Ochei, J.O. and Kolhatkar, A.A.2007. Medical Laboratory Science: Theory and Practice, Tata McGraw-Hill Publishing Company Limited, New York, pp. 637 – 745.

Ogbonnaya, C. 2008. Analysis of Ground Water Pollution from Abattoir Waste in Minna Nigeria, Res. J. Dairy Sc., 2(4): 74-77.

Piet, K. 2009. Waste Disposal Technology, Mpumalanga. South Africa, pp. 24-27.

Ryan, K.J. and Ray, C.G. 2004. Shem's Medical Microbiology, 4th edition, McGraw-Hill New York, pp. 58 - 67.

Willey, J. M. Sherwood and J.Woolverton. 2008. Prescott, Harley and Klein's Microbiology, 7th edition, McGraw-Hill Companies, Inc, USA, pp. 571-589.

****