Original Research Article

Comparison of the effectiveness of Alum, Chlorine, Sodium Hypochlorite and *Moringa*'s seeds in reducing bacterial loads in the treatment of restaurant wastewater

ABSTRACT

The effectiveness of different wastewater-treating agents were individually analysed and compared to one another in reducing bacterial counts (total bacterial and total coliform counts) during the treatment of restaurant wastewater. These agents include alum, chlorine, sodium hypochlorite and seeds of *Moringa oleifera.* Wastewater samples were collected at interval and analysed for bacteriological and physiochemical properties. Bacteriological analyses include total bacterial and coliform counts, while physiochemical analyses include pH, total titratable acidity (TTA), biochemical oxygen demand (BOD), total hardness, alkalinity and mineral components. *Moringa oleifera* seeds, was found to be very effective as a sedimentation agent, but least effective in reducing bacterial counts. Also, it was discovered that the seeds of *M. oleifera* aid the increase in the bacterial population. Alum, a non-bactericidal, sedimentation agent, was found to reduce total bacterial and coliform counts mainly by the use of flocculation. Chlorine was found to be bactericidal against all bacteria except *Pseudomonas aeruginosa*, while sodium hypochlorite was found to be most effective in reducing bacterial growth during the study.

UNDER PEER REVIEW

INTRODUCTION

Wastewater is any water that has been adversely affected in quality by anthropogenic influence (Beychok, 1999). It comprises of liquid wastes discharged by domestic residences, commercial properties like restaurants, industries and agricultural facilities, and it can encompass a wide range of potentially contaminants and concentrations (Guerrero and Calpe, 1998).

According to a 2015 United Nation's water analytical brief, wastewater is defined as a combination of one or more of:

- domestic effluent consisting of blackwater (excreta, urine and faecal sludge) and greywater (kitchen and bathing water);

- water from commercial establishments and institutions, including hospitals and restaurants;
- industrial effluent, stormwater and other urban run-off;
- agricultural, horticultural and aquaculture effluent, either dissolved or as suspended matter (Corcoran *et al.*, 2010).

Unless properly treated, wastewater can harm the health of the public, the environment, local economy, recreation, residential and business development, and also other aspect of everyday life. According to Tchobanoglous *et al.* (2003), before the mid-1800s, human wastes and other kinds of waste were usually dumped or conveyed to the nearest body of water without treatment. Due to this, groundwater and other sources of drinking water were regularly contaminated, and epidemic of cholera, typhoid, dysentary and other water-borne disease were common, with outbreaks especially devastating.

While the treatment of wastewater is to primarily reduce the biodegradable pollutants and disease-causing agents in the water, efforts are also being made to make such treated water useable again. According to United Nation World Water Development Report (2015), 780 million people lack access to clean, usable water in developing countries. While there are many factors responsible for this, a singular factor has been discovered to have the most disastrous effect on the accessibility of people to fresh, clean water in developing countries: pollution and contamination. Therefore, several attempts are regularly being made to recycle wastewater in order to reuse them.

According to Beychok (1999), there are three stages in the treatment of wastewater. The primary stage, in which large solid particles and trashes are screened off or mechanically removed from the rest of the wastewater; the secondary stage, in which combination of biological and physical processes such as filtering are employed to reduce the amount of organic wastes such as oil, and; the tertiary (or advanced) stage, in which several methods such as microbial denitrification and ozone treatment, are used to reduce nutrients, toxic substances and excessive amount of dissolved materials.

Several chemical and biological agents have been identified over the years for the treatment of wastewater (Nester *et al.*, 2005). Four of these agents were compared for effectiveness in reducing the bacterial load and population during the treatment wastewater in this study to

UNDER PEER REVIEW

obtain relatively safe and useable water. These include Alum (aluminium sulphate), *Moringa oleifera*'s seeds, chlorine and sodium hypochlorite.

MATERIALS AND METHODS

Sample Collection: Wastewater samples were collected from Falegan Restaurants, a large eatery along Banks/State Ministries Secretariats Road in Ado-Ekiti in Ekiti State, Nigeria that carters for the many staffs of over fifteen banks and the state ministries located along that road. The wastewater samples collected were those used for washing and rinsing of dishes and plates used for eating. They were labeled as follows:

Sample A: Soap solution used for dishwashing

Sample B: Initial water used for first rinsing

Sample C: Final water used for second rinsing

1 litre of each sample was collected once in a week for four weeks between the hours of 9:00am and 3:00pm. They were transported to the laboratory for immediate analyses, while the remaining samples were kept in the refrigerator for later analyses.

Bacteriological Analyses: Microbial analyses were carried out as described in Olutiola *et al.* (1991). Attempts were made to isolate microorganisms in the wastewater samples. Ten-fold serial dilution was carried out using 1ml of the each of the samples; dilutions 9 and 10 were inoculated on MacConkey Agar and Standard Plate Count Agar (SPCA). The samples were cultured in duplicates and incubated in an inverted format. Total bacterial count (TBC) and total coliform count (TCC) were done after 24 hours using colony counter. Stock cultures from distinct colonies on the plates were prepared using Nutrient Agar. Identifications of the microorganisms were carried out using cultural and morphological characteristics and confirmed using physiological and biochemical tests.

Physiochemical Analyses: Some physiochemical parameters of the wastewater samples such as pH, biochemical oxygen demand, total titratable acidity and others were determined during the course of the research as described in the Encyclopaedia of Chemical Technology (2005).

Preparation of Purifying Agents: *Moringa oleifera*'s seeds were prepared as described in N'Dabigengesere and Narasiah (1998). 1g, 2g and 3g of the powdered seeds were dissolved in 100ml of distilled water, shaken vigorously and left for 24 hours, after which they were

UNDER PEER REVIEW

filtered to give clear, colourless liquids of concentrations 1%, 2% and 3% respectively. Also, for alum, chlorine and sodium hypochlorite, 1g, 2g, and 3g of their powdered forms were each dissolved in 100ml of distilled water to give concentrations 1%, 2% and 3% respectively.

Treatment and Purification of Wastewater Samples: 1000ml of each wastewater sample was filtered using a filter-bed constructed using sterilized granite stones of decreasing sizes and washed sand to remove big solid particles in the wastewater samples. The samples were then filtered using a pre-weighed filter paper, which was then dried and weighed again to determine the total soluble solid (TSS) of each sample of wastewater. 1ml of each concentration of the purifying agents was then used to treat 1000ml of each wastewater sample. The treated wastewater samples were then kept for 24 hours and analysed microbiologically to determine the TBC and TCC. Isolates that survived the treatments were cultured and identified using morphological, physiological and biochemical methods.

RESULTS: The bacteriological analyses of the wastewater samples were similar, showing distinctly formed colonies on both SPCA and MA used. The sets of samples, together with the total bacterial and total coliform counts are shown in **Table 1** below.

Table 1: **Bacterial Counts of Wastewater Samples (CFU/ml)**

		Total Bacterial Count		**Total Coliform Count**	
Set	**Sample**	**10^{-9}**	**10^{-10}**	**10^{-9}**	**10^{-10}**
I	A	120	20	121	96
	B	178	89	115	35
	C	14	4	106	34
II	A	2	15	80	29
	B	59	22	105	24
	C	25	11	49	12
II	A	40	7	43	14
	B	60	32	98	87
	C	50	19	80	37
IV	A	150	140	74	27
	B	168	142	112	99
	C	128	72	54	40

The physiochemical analyses of the wastewater samples also showed a similar trend in all parameters tested for. The results are shown in **Table 2**.

Table 2: **Physiochemical Analysis of Wastewater Sample (mg/l)**

UNDER PEER REVIEW

Set	Sample	pH	Alkalinity	TTA	Total Hardness	Phosphate	Chloride	BOD	TSS
I	A	9.0	136.0	26.0	84.0	1.8	67.5	300	400
	B	5.8	6.0	4.0	76.0	1.0	12.4	280	300
	C	5.2	4.0	3.0	80.0	0.8	8.9	250	300
II	A	10.3	225.0	80.0	90.0	2.0	69.0	230	300
	B	6.6	62.0	5.0	58.0	1.7	11.6	210	200
	C	5.6	31.0	3.0	85.0	1.4	9.0	200	200
II	A	12.4	286.0	91.0	96.0	2.4	74.6	300	300
	B	9.8	70.0	15.0	60.0	1.6	10.7	270	100
	C	8.9	42.0	10.0	80.0	1.2	8.9	230	100
IV	A	9.2	168.0	42.0	84.0	2.6	69.1	350	400
	B	6.7	40.0	11.0	72.0	1.6	12.4	350	300
	C	6.4	10.0	9.0	84.0	1.2	11.2	200	200

Forty distinct microorganisms were isolated from the wastewater samples before and after treatment, identified using morphological and biochemical tests. These isolates and their counts were given in **Table 3** and **Table 4**.

Table 3: Isolated Organisms and Percentage of Occurrence before Treatment

Microorganisms	Bacterial Colony Count	Percentage of Occurrence (%)
Citrobacter freundii	3	7.5
Escherichia coli	6	15
Enterobacter aerogenes	3	7.5
Staphylococcus aureus	4	10
Klebsiella pneumoniae	3	7.5
Shigella dysenteriae	8	20
Proteus spp	4	10
Salmonella spp	2	5
Pseudomonas aeruginosa	2	5
Streptococcus faecalis	5	12.5
Total	**40**	**100**

Table 4: **Bacterial Counts of Wastewater Samples after Treatment**

UNDER PEER REVIEW

Treatment Agents	Concentration (%)	Total Bacterial Count (cfu/ml)		Total Coliform Count (cfu/ml)	
		Untreated wastewater	**Treated wastewater**	**Untreated wastewater**	**Treated wastewater**
M. oleifera seeds	1	28	38	81	98
	2	28	57	81	168
	3	28	TNTC	81	TNTC
Alum	1	19	09	67	01
	2	19	08	67	17
	3	19	14	67	14
Chlorine	1	40	TNTC	106	TNTC
	2	40	TNTC	106	TNTC
	3	40	TNTC	106	TNTC
Sodium Hypochlorite	1	71	24	164	45
	2	71	15	164	14
	3	71	NG	164	NG

Keys: TNTC – Too Numerous To Count; NG – No Growth

DISCUSSION

The wastewater sample sets showed varying numbers in the total bacterial count (TBC) and total coliform count (TCC). The use of detergent is suspected to be responsible for the reduced TBC and TCC observed in Sample A, since detergents are known to have antimicrobial ability (Wang *et al.* 2012). Sample B sets showed the highest TBC and TCC which might be due to the reduced effect of the detergents diluted concentration.

Physiochemical properties of the samples showed high alkalinity values for samples A, which was also indicated in the pH values. This could be majorly attributed to the detergent used in the washing (Pipeline, 2001). Also, phosphate, a major constituent of detergent, has a corresponding decreasing value similar to those of alkalinity. The total soluble solid (TSS) is mostly similar in samples B and C because most of the food particles would have been removed into sample A. There is a direct proportionality in the relationship between TSS and BOD, and the values gotten in this study are in the same range with some past studies on domestic wastewater (Tchobanologlous *et al.*, 2003).

UNDER PEER REVIEW

The bacteriological analyses of the wastewater samples in Table 3 showed the presence of bacteria, especially coliform bacteria, with *Shigella dysenteriae* and *Escherichia coli* showing the highest percentage of occurrence. *S. dysenteriae* is the major causative agent of dysentery in man, and it is spread and transmitted by houseflies (Nester *et al.*, 2001). Also, water contaminated with *S. dysenteriae* has been implicated in reported cases of diarrheal outbreaks in nearby communities (Adegunloye, 2006). Though most restaurants tried to be hygienic in their food preparations, the ubiquity of houseflies coupled with observed carelessness of some workers makes the contamination of prepared food possible. The recorded high occurrence of *E. coli* is probably due to its versatility and ability to survive almost anywhere (Thompson, 2007). Although most strains of *E. coli* do not cause disease, the virulent strains are prominent causative agents of gastroenteritis, urinary tract infections and neonatal meningitis (Todar, 2007), and the same factors responsible for contamination of food in the incidence of *S. dysenteriae* may be accorded to the high occurrence of *E. coli* in restaurant-related gastroenteritis (CFSAN/FDA, 2006)

Also, as shown in Table 3, *Salmonella spp* and *Pseudomonas aeruginosa* are the least occurring bacteria. While *Salmonella* is also a well-known cause of gastroenteritis, their few numbers of colony observed may be due to the fact that high temperature associated with cooking easily inactivate the bacterium (Frazier *et al.*, 2002). *P. aeruginosa* is versatile due to its ubiquity and ability to exist in soil, water, skin flora, and many natural and artificial environments (Balcht and Smith, 1994). Although *Pseudomonas spp* are known to be spoilage of many foods types as reported by Franzetti and Scarpellini (2007), the presence of *P. aeruginosa* here as a food contaminant is probably incidental or as a result of the water used.

In the initial stage of treatment of the wastewater samples, the soil bed used was constructed with washed and sterilized granite stones of different sizes. This is to prevent the introduction of Fe^{3+} which is common if ordinary pebbles are used. Hence, granite stones, which contain minute amount of iron and are relatively resistant to leaching (Burks and Minnis, 1998) are used. The soil bed reduced the amount of solid particles present in the wastewater, achieving the primary treatment. However, oil particles passed through soil bed which would require other method of treatment as reported in Pipeline (2001) and Nester *et al.*, (2001).

For the treatment of the wastewater samples with the purifying agents, extract of *Moringa oleifera*'s seeds showed remarkable sedimentation ability. However, it had positive effect on

UNDER PEER REVIEW

the microbial population, surprisingly aiding the proliferation of the microorganisms. This is in line with the experiment carried out by Kalogo *et al.* (2000), where the effect of a continuous supply of water extract of *Moringa oleifera*'s seed (WEMOS) on the hydrolytic microbial population of biomass grown in mesophilic up-flow anaerobic sludge blanket reactor treating wastewater was investigated. In the experiment, it was found out that various hydrolytic bacteria could degrade WEMOS and that a continuous supply of it increased the diversity of the bacteria.

During the course of the present study on *M. oleifera*'s seeds effect on wastewater, it was found out that with the increasing concentration of the seeds' extract, there is are relative increases in the total bacterial and coliform counts. This corroborates the earlier reports of Kalogo *et al.* (2000) and Howgrave-Grahm *et al.* (1994) where it was indicated that the extract is a source of substrate for the microorganisms present in the wastewater.

In the treatment with alum, there were decreases in the TBC and TCC in the treated wastewater samples. Although alum is not bactericidal in action, it has high sedimentation ability and it produces 'flocs' when dissolved in water which eventually settle at the bottom. Flocs collect suspended particles, including microorganisms, as they are settling down, reducing the numbers of the 'free' microorganisms. Alum also produced more sludge than the extract of *M. oleifera*'s seeds as reported by N'Dabigengesere and Narasiah (2005).

Chlorine was found to have bactericidal effect on all the microorganisms except *Pseudomonas aeruginosa*. This was discovered when there was a single type of growth on the media used after culturing the chlorine-treated wastewater samples. The growth was identified to be *P. aeruginosa* which had proliferated and spread uninhibited due to the absence of competition from other microorganisms. This indicated that the organism was resistant to chlorine.

At the 3% concentration, sodium hypochlorite showed inhibitory effects on all the microbes present in the wastewater, including *P. aeruginosa* which had shown resistance to chlorine at 3%. This confirmed the strong bactericidal effect of sodium hypochlorite as stated by Kuroshima *et al.*, (2007), and its effectiveness as a better disinfectant than chorine for bacteria, especially coliforms (Tsai and Lin, 1999). At lower concentrations, however, the bactericidal effect was reduced as few microbial growths were observed.

UNDER PEER REVIEW

CONCLUSION

The analyses carried out on wastewater indicated that if untreated, wastewater could be a source of contamination and, subsequently, infections and diseases to the public. The bacteriological analyses (total bacterial and coliform counts) showed the need for wastewater to be treated before being reused or released to the environment.

Pertaining to reduction of the bacterial load of wastewater during treatment, the research showed that different agents could be used to treat wastewater, either singly or synergistically. Although *Moringa* seeds and alum showed strong coagulating and sedimentation ability, while sodium hypochlorite and chlorine showed strong bactericidal effect on the wastewater samples treated, the combination of alum and sodium hypochlorite is recommended due to their pronounced effectiveness when compared to the remaining two agents.

Generally, it is recommended that treatment of wastewater should involved combination of coagulating and bactericidal agents as shown above, in addition to pre-treatment processes for removal of those constituents such as solid particles, soluble particles and oil particles which these agents cannot remove.

REFERENCES

Adegunloye D.V. Carrier rate of enteric bacteria associated with diarrhoea in children and pupils in Akure, Ondo State, Nigeria. *Afr. J. Biotechnol.* Short Communication November 24, 2005.

Balcht, Aldona and Smith, Raymond (1994). *Pseudomonas aeruginosa*: Infections and Treatment. *Informa Health Care*. pp. 83–84. ISBN 0-8247-9210-6.

Beychok, M.R. (1999). Aqueous Waste from Petroleum and Petrochemical Plants 4th Edition. John Wiley & Sons. LCCN 67-19834

Burks, B.D., and Minnis, M.M. (1998) *Onsite Wastewater System*. Madison, WI: Hogart House Ltd.

Encyclopaedia of Chemical Technology (2005).

UNDER PEER REVIEW

Franzetti, Laura and Scarpellini, Mauro (2007) Characterisation of *Pseudomonas spp*. isolated from foods. *Annals of Microbiology* 57 (1) 39-47

Frazier, W.C., and Westhoff, D.C. (2002) *Food Microbiology* McGraw-Hill Publishing Company, 4^{th} Edition. Pp 477-494.

Guerrero, R and Calpe, A. (1998) Water Resources Management: A Global Priority. National Academy of Science and Technology, Manila, Philippines.

Howgrave-Grahm, A.R., Ishewood, H.A. and Wallis, F.M. (1994) Evaluation of two up-flow anaerobic digesters purifying industrial wastewater high in organic matter. *Water Science and Technology* 29: 225 – 229.

Kalogo, Y., Rosillonn, F., Hammes, F. and Verstraete, W. (2000) Effect of Water Extract of *Moringa oleifera* seeds on the Hydrolytic Microbial Species Diversity of a UASB Reactor Treating Domestic Wastewater. *Letter in Applied Microbiology* 31: 259 – 264.

Kuroshima, K.N., Barreriros, M.A., Lacava L.A. and Costodio, P.S. (2007) The influence of dioxide chloride on water quality. *Environmental Monitoring and Assessment* 129: 1 – 3.

N'Dabigengesere, A and Narasiah, S. (1998). Use of *Moringa oleifera*'s seed as a primary coagulant in wastewater treatment. *Environmental Microbiology* 19:789-800.

Nester, E., Anderson, D., Robert, C., Pearsall, N. and Nester, M. (2001) Microbiology: A Human Perspective, McGraw-Hill Inc., USA 4^{th} Edition. Pp 787-798.

Olutiola, P., Famurewa, O. and Sonntag, H. (1991). An Introduction to General Microbiology: A Practical Approach. 1^{st} Edition, Bolabay Publications, Nigeria.

Pipeline (2001) Basic Wastewater Characteristics. 8(4): 2 – 6.

Retail Establishments; Annex 3 – Hazard Analysis". Managing Food Safety: A Manual for the Voluntary Use of HACCP Principles for Operators of Food Service and Retail Establishments. U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition. April 2006. Archived from the original on 2007-06-07. Retrieved 2007-12-02.

Tchobanoglous, G., Burton, F and Stensel, H. (2003) Wastewater Engineering (Treatment, Disposal and Reuse), Metcalf & Eddy Inc., 4^{th} Edition. McGraw-Hill.

UNDER PEER REVIEW

Thompson, Andrea (2007-06-04). "E. coli Thrives in Beach Sands". Live Science. Retrieved 2007-12-03.

Todar, K. "Pathogenic E. coli". Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology. Retrieved 2007-11-30.

Tsai, C.T. and Lin, S.T. Disinfection of hospital waste sludge using hypochlorite and chlorine dioxide. *Journal of Applied Microbiology* 86(5): 827 – 833.

United Nations World Water Development Report (WWDR), 2015 Water for a Sustainable World

Wang Z., Shen Y., Ma J., Haapasalo M. The effect of detergents on the antibacterial activity of disinfecting solutions in dentin. *J Endod.* 2012 Jul;38(7):948-53. doi: 10.1016/j.joen.2012.03.007. Epub 2012 Apr 24.

UNDER PEER REVIEW