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Incidence of Lipolytic Mycoflora in Domestic Wastewater

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Abstract

The decomposition of domestic effluent by mycoflora was investigated. The microbial load enumerated for the period of 13 days, using standard microbiological techniques revealed the average total bacterial count between the range of 1.97 x 10^6 CFU/ml and 1.25 x 10^7 CFU/ml, the total coliform count have a mean range between 1.29 x 10^6 CFU/ml and 0.56 x 10^7 CFU/ml while the total fungal count showed a mean range of 3.17 x 10^6 CFU/ml to 2.14 x 10^7 CFU/ml. One hundred and twenty fungal isolates were obtained from the wastewater with the highest occurred organism as *Fusarium moniliforme* (19.2% occurrence), followed by *Fusarium oxysporium* (14.2%) and the least occurred organism *Aspergillus versiculor* (0.8%). The acidic pH and turbidity values obtained ranged from 3.41 - 5.98 and 1.63 - 1.79 respectively. Only 39 (32.5%) of the fungi isolates showed ability to degrade lipids with varying potentials; of which four (10.3%) were grouped among high and slight lipolysis. Among the lipolytic fungal isolates, *Aspergillus* spp. showed the highest occurrence of 79.5%, followed by 5.1% occurrence of *Penicillium* spp., *Fusarium* spp. and *Rhizopus* spp. while *Absidia* spp. and *Thermophillus* spp. showed the laeast occurrence (2.6%). It is significant that fungi associated with oil-rich wastewater also attribute the potentials of degrading the lipid component of sewages, an advantage in the treatment process.
Keywords: Mycoflora, oil-rich wastewater, lipolytic, fungi

1. INTRODUCTION

Indiscriminate disposal of untreated wastewater generated from domestic households to the environment is taken as normal thing in Nigeria (Odeyemi *et al*., 2011; Adeyemi, 2003). This attitude consequently causes gradual depletion of biological life and health risks to the body of water (Odeyemi *et al*., 2011). Lipids (fats, oil and greases) are one of the major components in a municipal and restaurant wastewater which can cause severe environmental pollution. Oil-rich wastewaters were used to be treated physically, which has proven ineffective especially when the fat is in its dispersed form.

However biological treatment has been found to be the most efficient method for removing fat, oil and grease by degrading them into miscible molecules (Odeyemi *et al*., 2010). The use of microorganisms for treatment and bioremediation purposes have hence been proven to afford a very efficient tool for purifying contaminated effluents and natural water (Glazer and Nikaido, 1995). The use of lipase enzymes produced by all microorganisms is an alternative measures to solve this problem, as they tend to catalyze the synthesis or hydrolysis of fat (Shabtai, 1991). Odeyemi *et al*. (2013) recently reveal the potency of genera of *Klebsiella*, *Pseudomonas* and *Staphylococcus* spp. in producing active lipolytic enzymes that tends to hydrolyze lipid content of wastewater.

Likewise fungi are found in virtually every habitat on the earth where organic materials exist, most especially decay compounds in wastewater. They secrete wide varieties of enzymes such as cellulase, pectinase, lipase which assist them in degrading organic matters in wastewater. Various species of fungi involved in the decomposition of wastewater include species of *Rhizopus*, *Aspergillus*, *Alternaria*, *Trichoderma* and *Thermoactinomyces*, which grow best at low pH level and optimal temperature between –6^{o}C to 50^{o}C for optimal biological activities (Maier *et al*., 2003). Hence this paper aimed at reporting the lipolytic potency of some mycoflora associated with wastewater obtained from a restaurant in Ado-Ekiti, Ekiti State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sources and collection of wastewater samples

Wastewater samples were collected aseptically from different sampling points including wash-hand basin, dish washing and dish rinsing bowls of Falegan restaurant situated along the Ekiti State Secretariat road, Ado-Ekiti, Nigeria. The wastewater collected contains food remnants and cleaning soap solution. The samples were collected using sterile sampling bottle, kept in ice (25^{o}C±1^{o}C) and transported to the Microbiology laboratory of the Ekiti State University, Ado-Ekiti until when needed.

2.2 Enumeration and identification of fungal loads

All samples collected from each of those outlets were mixed together to make up a volume of about 4liters, shunned and separated into 13 conical flask with 250ml of the sample each. Each of these sample-contained conical flasks was labeled according to days of analysis day (0 - 12) which were kept under aerobic condition throughout the days of experiments. Serial dilution of each of these samples was carried out as described by Olutiola *et al.* (1991) for the enumeration of total bacterial, coliform and fungal count using Nutrient agar (NA), MacConkey agar and Potato Dextrose agar (PDA) respectively. The plates were incubated aerobically at 37 ^{o}C. After 48-72h of incubation, the colonies were counted using colony counter, after which these colonies were purified by sub-culturing.

Each of the fungi growths on the plates were identified and named based on comparison and similarities in their documented morphological appearance in accordance with Leslie and Summerel (2006).

2.3 Evaluation for lipolytic activity

The lipase-producing microorganisms among the isolated microbial cells were further confirmed by inoculating plates containing a sterile Tributyrin agar (TBA) according to Odeyemi *et al*., (2013), and incubated for 48-72 h. The lipolytic microbes were observed having cleared zones around their growth. The cleared zones were measure in millimeter.

2.4 Physicochemical analysis

About 5ml of concentrated HCl was added to 250ml of wastewater sample and evaporated to 25ml. The concentrate was transferred to a 50cm^3 standard flask and diluted to the mark with distilled de-ionized water (APHA, 1995). The pH was measured with a KENT EIL 7020 pH-meter (Kent Industrial Measurement Limited, Surrey, England) while turbidity was determined with a colorimeter (MODEL 6025, JENWAY, UK) at wavelength of 470nm (Okonkwo *et al*., 2008).

3. RESULTS AND DISCUSSION

The microbiological and physicochemical analyses carried out on the wastewater sample obtained from Falegan restaurant, Ado-Ekiti, Ekiti State, is a reflection of the rate of contamination and the tendency of isolating lipase producing fungal isolates which could also be applied in the treatment of the oil-rich wastewater generated from homes and restaurants. The pH values obtained ranged between 3.41 and 5.98, which indicated an acidic medium throughout the period of experiment. The most acidic pH (3.41) was however obtained on day 2. The turbidity had slight decreasing values ranging between 1.63 and 1.79 (Table 1). Microorganisms have been reported by Sheryl *et al*., (1994) to fill their energy needs by catalyzing the oxidation of organic compounds containing reduced carbon and nitrogen which requires the concurrent reduction of electron acceptors-redox reaction; these affects the pH of the wastewater. This justifies the gradual reduction in pH values observed from day 0 to day 3 with little hike towards day 6 which thereafter decreased through the days of the experiment. The effect of degradation process in the wastewater was hence significant of the pH values as well as the turbidity, which showed decreasing value throughout the days of experiment.

The average total bacterial and coliform counts ranged from 1.97 x 10^6 CFU/ml to 1.25 x 10^7 CFU/ml and 1.29 x 10^6 CFU/ml to 0.56 x 10^7 CFU/ml respectively. While the average total fungal count showed a mean range of 3.17 x 10^6CFU/ml and 2.14 x 10^7CFU/ml (Table 1). The high value of microbial load observed is rather not surprising as wastewater has been noted for high microbial composition ranging from 100,000 - 1,000,000 microorganisms per millimeter (Prescott *et al*., 2004). The high value obtained for microbial load of the wastewater samples could be attributed to food debris contained in the wastewater which accumulates, serving as nutrient for organisms involve in microbial biodegradation of the wastewater (George *et al*., 2003). However, the bacterial composition of this wastewater is an advantage to achieving a favorable condition for hydrolyzing the organic contents in the water according to George *et al*. (2003) who reported that aerobic and facultative bacteria majorly oxidize the organic matter in wastewater to stable and unobjectionable end products like methane, carbon dioxide and ammonia. This eventually encourage anaerobiosis as a prevailing condition in the degradation of wastewater. The high microbial load, especially the coliform, can therefore be attributed to the presence of suspended/particulate solids (Mburu *et al*., 2008). The result of microbial count got indicates a gradual increase for six days. This is in agreement with the report of MohdKhairul (2008) that, microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines.

Table 1: pH, Turbidity and Microbial counts of restaurant wastewater samples

Days	pH	Turbidity	TBC		TCC		TFC	
			10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}
0	3.97	1.79	0.97	0.48	0.81	0.30	1.41	1.62
1	3.94	1.78	1.36	0.89	1.04	0.38	1.96	1.54
2	3.41	1.74	1.73	1.03	1.16	0.42	2.69	2.11
3	3.55	1.74	1.93	1.24	1.21	0.48	3.48	2.87
4	4.23	1.71	2.64	1.76	1.77	0.79	5.12	3.41
5	4.24	1.70	2.48	1.58	1.79	0.84	3.96	2.24
6	5.98	1.69	2.79	1.83	1.94	0.99	2.87	1.22
7	5.76	1.66	2.71	1.66	1.86	0.93	3.00	1.61
8	5.56	1.75	2.43	1.53	1.39	0.59	2.71	1.71
9	5.44	1.72	2.01	1.20	1.07	0.49	2.84	1.98
10	5.05	1.65	1.87	1.13	1.01	0.41	3.12	2.18
11	4.45	1.65	1.59	1.02	0.90	0.36	3.98	2.56
12	4.46	1.63	1.12	0.91	0.86	0.33	4.02	2.73

Keys: TBC- Total bacterial count
TCC- Total coliform count
TFC- Total fungal count

A total of 120 fungal isolates were obtained from the wastewater for the period of 13 days showing the highest percentage (12.5%) of the isolated organisms at day 3 and the least (4.2%) isolated on day 12. *Fusarium moniliforme* appeared as the highest occurred isolate (19.2%), followed by *Fusarium oxysporium* (14.2%). The least occurred organism was *Aspergillus versiculor* with just 0.8% occurrence. It could be deduced that the utmost availability of organic content in the wastewater occurred within day 0 and day 3, serving as nutrient which results to increase in microbial load but as the nutrient tends to decrease, the number of fungi also retards; since to obtain energy and construct new cellular components, organisms must have a supply of raw materials or nutrients (Prescott *et al*., 2004).

Only 39 (32.5%) of all the isolates showed the ability to produce this lipid-hydrolyzing enzyme, although in a varying potentials. The degrees of potency of the enzyme produced by each of these isolates are enumerated by measuring (in millilitre) the zones of clearance around each isolate. Four (10.3%) of these lipase-producing fungi were highly lipolytic, nine (23.1%) were strongly lipolytic, 12 (30.8%) were moderately lipolytic while 14 (35.9%) proved to be slightly lipolytic (Table 3). *Aspergillus* spp. showed the highest occurrence of 79.5%, followed by 5.1% occurrence of *Penicillium* spp., *Fusarium* spp. and *Rhizopus* spp. while *Absidia* spp. and *Thermophillus* spp. showed the least occurrence of just 2.6% (Table 4). The isolation of lipase producing microorganisms capable of degrading fat and oil in wastewater and their degradable efficiency in both single culture and mixed culture formula have been studied (Bhumibhamon *et al*., 2003). Isolation of lipolytic fungi from these wastewater samples also conforms to Sharma *et al*., (2001) who stated that lipase production by fungi are typically extracellular and therefore are relatively easy to recover after the fermentation. According to Hala *et al*. (2010); among the lipolytic microorganisms that are significant in wastewater are aerobic and anaerobic bacteria of the genera; *Pseudomonas, Clostridium* and also fungi of the genera; *Penicillium, Aspergillus, and Fusarium* thus supporting the detected/isolated fungi as regards this research. This is also supported by Sharma *et al*. (2001) in his statement that; many genera as *Penicillium, Fusarium and Rhizopus* have been noted as producers of lipases with desirable properties. However, this experiment has revealed the ability of *Absidia corymbifera* to degrade the lipid composition of oil-rich wastewater.

Table 2: Percentage distribution of fungal isolates from restaurant wastewater

Days	*Fusarium oxysporium*	*Fusarium moniliforme*	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus candidus*	*Aspergillus ochraceus*	*Aspergillus fumigates*	*Aspergillus versiculor*	*Aspergillus glaucus*	*Rhizopus arrhizus*	*Penicillium citrinium*	*Absidia corymbifera*	*Thermophillus chaetrinium*	Total
0	1	1	1	1	-	-	1	-	-	1	-	1	1	8
1	2	2	1	1	1	-	1	-	-	1	-	1	-	10
2	2	2	1	1	1	-	1	-	-	-	-	1	1	10
3	2	2	1	1	1	1	1	1	-	2	1	1	1	15
4	2	2	1	1	1	-	-	-	1	1	1	1	1	12
5	-	3	1	1	-	2	-	-	-	2	-	2	-	11
6	2	1	1	1	-	1	-	-	-	1	1	-	1	9
7	1	1	1	1	-	-	-	-	1	-	1	1	1	8
8	1	1	1	1	1	-	-	-	-	-	-	1	1	7
9	1	2	-	1	-	-	-	-	-	-	1	2	1	8
10	1	2	1	1	-	-	1	-	-	1	-	1	-	8
11	1	3	1	1	1	-	-	-	-	1	-	1	-	9
12	1	1	-	1	-	-	1	-	-	1	-	-	-	5
Total	17	23	11	13	6	4	6	1	2	11	5	13	8	120
% of occurrence	14.2	19.2	9.2	10.8	5.0	3.3	5.0	0.8	1.6	9.2	10.8	4.2	6.7	100

Furthermore, it was observed from this research that; the rate of occurrence of the lipolytic fungi at the earlier days were relatively higher than the later days, this could be assumed as a resultant effect of decrease in the availability of oil (lipid) content in the wastewater thus supporting the previous observations. This is in agreement with MohdKhairul (2008) that, microbes capable of degrading a particular substance increase in numbers when the substance is present, meanwhile when the substance is exhausted; the biodegradative populations declines.

This work has however emphasized on the need for proper and adequate treatment of wastewater, which may involve multi-stage processes aimed at reducing or removing all organic matters/solids, nutrients, diseases-causing organisms, ensuring conservation of water by re-usage of wastewater after treatment and others. It also supports previous researches that has aid the detection of microorganisms which could be employed in the biological treatment (bioremediation) of oil-rich wastewater generating from our homes, kitchens, restaurants, farms and factories; without any hazardous product (i.e. ensuring 0% threat to lives and the entire biosphere). Therefore the populace should be conscious of possible risk associated with discharging untreated wastewater into the water body. Hence when wastewater receives inadequate treatment, the overall quality of the world water-supply suffers.

Table 3: Lipolytic activity of fungal isolates from restaurant wastewater

S/N	Isolate codes	Zone of clearance/ degradation (mm)			
		0.00-0.15	0.15-0.25	0.25-0.35	0.35 and above
1	A_2	-	-	0.26	-
2	A_8	-	0.18	-	-
3	B_2	0.11	-	-	-
4	B_3	0.13	-	-	-
5	B_4	0.09	-	-	-
6	B_9	0.10	-	-	-
7	B_{10}	-	-	0.27	-
8	C_1	-	0.24	-	-
9	C_2	-	0.22	-	-
10	C_3	-	-	0.33	-
11	C_4	0.12	-	-	-
12	C_7	-	-	-	0.38
13	D_9	0.08	-	-	-
14	D_{10}	0.14	-	-	-
15	D_{11}	0.11	-	-	-
16	D_{12}	-	0.23	-	-
17	D_{15}	0.09	-	-	-
18	E_1	-	-	-	0.37
19	E_2	-	-	-	0.40
20	E_6	-	0.21	-	-
21	E_7	-	0.19	-	-
22	E_8	-	-	0.28	-
23	E_{11}	-	-	0.25	-
24	E_{12}	-	0.17	-	-
25	F_6	0.13	-	-	-
26	F_9	-	-	0.26	-
27	F_{11}	-	-	-	0.35
28	G_3	-	0.19	-	-
29	G_4	-	0.22	-	-
30	G_5	0.13	-	-	-
31	G_7	0.14	-	-	-
32	H_1	0.10	-	-	-
33	H_4	-	-	0.27	-
34	J_4	-	-	0.28	-
35	J_6	-	0.20	-	-
36	K_2	0.11	-	-	-
37	K_4	-	0.21	-	-
38	L_4	-	-	0.29	-
39	M_5	-	0.24	-	-
Total	**Number**	14	12	9	4

Keys: 0.00-0.15mm- slightly lipolytic 0.15-0.25mm- moderately lipolytic
0.25-0.35mm- strongly lipolytic 0.35mm and above- highly lipolytic

Table 4: Distribution of lipolytic fungal isolates in restaurant wastewater

S/N	Isolates	Number of isolates	Frequency
1	*Aspergillus* spp.	31	79.5
2	*Penicillium* spp.	2	5.1
3	*Fusarium* spp.	2	5.1
4	*Rhizopus* spp.	2	5.1
5	*Absidia* spp.	1	2.6
6	*Thermophillus* spp.	1	2.6
Total		**39**	

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REFERENCES

Adeyemo, O. K. (2003): Consequences of pollution and degradation of Nigerian aquatic environment on fisheries resources: *The Environmentalist*, **23**: 297-306.

American Public Health Association (1995). Standard methods for the examination of water and waste water, 19th ed.

Bhumibhamon,O., Jinda, J. and Fungthong, S. (2003). Isolation and Characterization of Pseudomonas sp. KLB1 Lipase from High Fat Wastewater. *Kasetsart Journal of Natural Science*, 37: 176 – 185.

George, T., Frank, L. B. and Stensel, H. D. (2003): Wastewater Engineering: Treatment and Reuse. New York: McGraw-Hill Companies, Inc., 753-775.

Glazer, A. N. and Nikaido, H. (1995): Microbial Biotechnology: Fundamentals of Applied Microbiology. New York: W. H. Freeman and Company.

Hala, M. R., Adel, A. E., Hassaan, A. E. and Samah, A. D. (2010): "Production, Optimization and Partial Purification of Lipase from *Fusarium oxysporium*". *Journal of Applied Science in Environmental Sanitation*, 5(1): 39-53.

Leslie, J. F. and Summerell, B. A. (2006): Techniques and methods, In: *Fusarium* laboratory manual. Blackwell Publishing Ltd., Oxford, UK, pp: 7-8.

Maire, R. M., Pepper, I. L. and Gerba, C. P. (2003): "Important constituent in wastewater". *Journal of Environmental Microbiology*, 3: 149-173.

MohdKhairul Nizam Bin MohdZuhan, (2008): Bioremediation of oil from domestic wastewater using mixed culture; Effect of inoculum concentration and agitation speed. Thesis submitted to the Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang.

Mburu, N., Thumbi, G. M. and Mayabi, A. O. (2008). Removal of bacterial pathogens for domestic wastewater in a tropical subsurface horizontal flow constructed wetland. Proceedings of Taal 2007: The 12th World Lake Conference; 1010-1015.

Odeyemi, A. T., Aderiye, B. I. and Bamidele, O. S. (2013): Lipolytic activity of some strains of *Klebsiella*, *Pseudomonas* and *Staphylococcus* spp. from restaurant wastewater and receiving stream, *Journal of Microbiology Research*, 3(1): 43-52.

Odeyemi, A. T., Aderiye, J. B and Adeyeye, E. I. (2011): Changes in the microflora and chemical components of domestic oil-rich wastewater. *Journal of Microbiology, Biotechnology and Food Sciences*, 1(1): 126-147.

Odeyemi, A. T., Dada, A. C., Ogunbanjo O. R and Ojo, M. A. (2010): Bacteriological, physicochemical and mineral studies on Awedele spring water and soil samples in Ado Ekiti, Nigeria. *African Journal of Environmental Science and Technology*. 4(6): 319-327.

Okonkwo, I. O. Adejoye, O. D, Ogunnusi, T. A., Fajobi, E. A. and Shitti, O. B. (2008): Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State Nigeria. *African Journal Biotechnology*, 7(5): 617 - 621.

Olutiola, P. O., Famurewa, O. and Sonntag, H. G. (1991): An Introduction to Microbiology. A Practical Approach; Printed in the federal Republic of Germany.

Prescott, L. M., John, P. H. and Donald, A. K. (2004): "Microorganism in aquatic environmental microbiology". McGraw Hill Higher Education, New York. 576 - 626.

Shabtai, Y. (1991): Isolation and characterization of a lipolytic bacterium capable of growing in a low-water-content oil-water emulsion. *Applied Environmental Microbiology*, 57: 1740 - 1745.

Sharma, R., Chisti, Y. and Banerjee, U. C. (2001): Production, purification, characterization and application of lipases. Biotechnology Advances, 19: 627 - 662.

Sherryl, R. W., Sherryl, L. S. and John, A. C. (1994): Biogeochemical evolution of domestic wastewater in septic system: 1. *Conceptual Model*, 30(6): 905 - 916.

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