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Biodegradation of detergents by aquatic bacterial flora from Otamiri River, Nigeria

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The utilization of Omo[®], Jet[®] and Persil[®] detergents by aquatic bacteria isolates from Otamiri River at Nekede in Owerri North, Imo State, Nigeria was investigated. Identification tests for bacteria isolates from Otamiri River revealed them to belong to the genera Bacillus, Micrococcus, Escherichia, Enterobacter, Klebsiella, Psendomonas, Actinomyces, Corynebacterium, Serratia and Staphylococcus. Detergent utilization studies revealed total heterotrophic count of 3.38, 3.40, 3.36 and 5.35 log cfu/ml and 2.08, 2.20, 1.95 and 3.48 log cfu/ml obtained at 0 and 48 h for Omo[®], Jet[®], Persil[®] and control experiment, respectively. At 96 and 144 h, 2.37, 2.35, 2.25 and 2.47 log cfu/ml and 2.39, 2.37, 2.35, and 2.46 log cfu/m were obtained. While counts of 1.70, 2.37, 1.38 and 2.4 log cfu/ml were obtained at 192 h for Omo[®], Jet[®], Persil[®] and the control experiment, respectively. Of the nine bacterial isolates obtained from the river water, only Pseudomonas, Bacillus, Actinomyces, Corvnebacterium and Staphylococcus were found to survive in the detergent water and possibly utilize the test detergents. Isolate specific detergent utilization test revealed these isolates to be capable of utilizing the test detergents in single and combined forms with Pseudomonas showing the highest ability while the least was observed for Staphylococcus. Statistical analysis revealed significant changes in optical density of detergent broth challenged with the test organisms, with the organism showing more ability to utilize, Omo[®] and Jet[®] than Persil[®] detergents. The result obtained, however, reveals the ability of natural aquatic bacterial Isolates to degrade detergents in aquatic ecosystem.

Key words: Aquatic bacterial flora, detergents, biodegradation, Otamiri River, Nigeria.

INTRODUCTION

Detergents play an important role in any modern society. They are any soap or non-soap powder, which can be used in both industrial and domestic premises as cleaning agent (Swisher, 1972; Holding, 2005). They usually contain Alkyl Benzene Sulphonates (ABS), which forms lather with hard water (Swisher, 1972; 1987). An increased rate of technological development has brought about the production of these synthetic materials, a factor that has supported the need for an enhanced biodegradability studies. Indeed, the need for these studies has become important especially as a result of consumer use and disposal pattern of these detergent chemicals, prior to discharges into streams, rivers and estuaries. Actually, considerable level of detergents biodegradation activity takes place in the environment (Lawson and Payne, 1980), but only the easily degradable detergent chemicals are used up by microorganisms while the hardto-degrade types bioaccumulate and concomitantly cause adverse environmental problems (Swisher, 1973). Such problems have been reported to include: destruction of the external mucus layer that protects fish from bacteria and pathogens, severe damage to the gills, lowering of the surface tension of the water, algal blooms that releases toxins and decreases oxygen in waterways and decrease in the breeding ability of aquatic organisms (Holding, 2005). The main contributors to the toxicity of these detergents are the sodium silicate solution and the surfactant used as part of the compositions (Holding, 2005).

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Biodegradability test for assessing the environmental acceptability of synthetic compound usually employ mixed culture of various organisms (Oba et al., 1968, Stainer et al., 1971; Okpokwasili and Nwabuzor, 1988). These tests could be such that indicate the susceptibility of chemicals to microbial degradation and simulation test, which provide information about rates of biodegradation under relevant experimental conditions (Gilberts and Watson, 1977). Thus, to ascertain the fate of different detergent in our natural environment, especially the aquatic ecosystem, this study was designed to determine the susceptibility of some different brands of comer-cially available detergents to degradation by aquatic bacterial isolates with a view to determining the fate of these detergents in the aquatic ecosystem.

MATERIALS AND METHODS

Detergents

The detergents used for the study were Omo[®] manufactured by Unilever Nigerian Plc, Jet[®] manufactured by PZ Nigerian Plc and Persil[®] manufactured by Lever Company Saudi Arabia. The detergents were all labeled and stored in a water free polyethylene bags until they were needed for the study.

Source of microorganism

The microorganisms for the biodegradation study were naturally occurring bacteria flora from Otamiri River at Nekede, where washing, laundry and other human activities requiring detergents take place. The river water sample was collected aseptically as described by APHA (1998) and was taken to the laboratory where it was analyzed within 30 min.

Detergent utilization test

Three (3) grams of each of the 3 test detergent sample were dissolved in 100 ml of water from Otamiri River in 150 ml capacity conical flask, while the fourth, 150 ml capacity flask contained only 100 ml of Otamiri River water and was used as a control. These were set up in shaker (Parker Model No 7633 PLV). And 1 ml of each of this samples was analyzed microbiologically every 48 h for six days by plating on mineral salt detergent medium prepared by adding 3% of the detergent as carbon source and nutrient agar using spread plate technique as described by APHA (1998). The inoculated plates were incubated at 37°C for 48 - 72 h and 24 h for the detergent mineral salt and nutrient medium, respectively. After incubation, detergent utilizing and total heterotrophic bacterial population was isolated and identified using Bergeys Manual of Determinitive Bacteriology (Holt, 1984).

Turbidity test

Mineral salt broth was prepared as in APHA, (1998) with 0, 0.5 and 1% of Omo[®], Jet[®] and Persil[®] detergents and 100 ml of the broth, dispensed into 250 ml capacity conical flask and sterilized. After

sterilization, the medium was allowed to cool and each of the isolates obtained from Otamiri River was inoculated into the tubes in single and combined forms. The OD was ascertained to determine the absorbance of each batch at Oh at 576 nm wave length using spectrophotometer (Model Spectronic 21 D). The microbial population was equally determined using the plate count method of Chesbrough (1994) on Oxoid trypton soy agar prepared according to the manufacturers instructions and incubated at 37°C for 24 - 48 h. This was repeated every 24 h intervals for five days.

RESULTS

Isolation and identification test as shown in Table 1 revealed the presence of Escherichia, Staphylococcus, Klebsiella, Serratia, Pseudomonas, Bacillus, Corynebacterium, Enterobacter and Actinomyces from Otamiri River. The level of occurrence of bacterial population (Figure 1) shows a higher recovery of 2.4 x 10³, 2.54 x 10^3 , 2.3 x 10^3 and 2.26 x 10^3 , and 2.76 x 10^5 cfu/ml from Omo[®], Jet[®], Persil[®] and control experiment at 0 h. Where as at the 48 h, a sharp decrease of 1.2×10^2 , 1.6×10^2 , 0.9×10^2 and 3.0×10^2 cfu/ml were recorded for Omo[®], Jet[®], Persil[®] and control experiments, respectively. However at the 96 h the population of bacterial isolates in the control sample stabilized at 2.94 x 10² and remained virtually at that level till the end of the experiment at 192 h. On the other hand, the test sample showed a slight but progressive increase until the 192 h when a sharp decrease of 0.5 x 10^2 and 0.24 x 10^2 were obtained for Omo[®] and Persil[®] while the population of bacterial genera stabilized at that level for the Jet® sample. The detersive property of detergents examined, such as foaming was found to be noticeable until at 144 h except Jet detergent that was not noted at the 144 h.

Treatment specific and bacterial succession studies (Table 2) revealed the isolation of 100% of the isolates from the control experiment (D) (Otamiri River without detergent treatment) at 0 h, while 88.89 and 77.78% of the isolates were obtained from Omo® and Jet® and Persil[®] treated samples, respectively. After 48 h, a progressive decrease in the number of species in both treated and control samples were observed with the isolation of 77.78% of the isolates from the control samples. Sample treated with Jet[®] (B) haboured 55.56% of the isolates whereas Omo® and Persil® treated samples haboured 44.44% of the isolates. Bacterial recovery revealed the isolation of 55.56, 44.44 and 33.33% from Jet[®], Omo[®] and Persil[®] treated samples and the control experiment respectively after 96 h. At 144 h of treatment, 44.44% of the isolates were recovered from Omo[®], Jet[®] and Persil® while the least percentages of 22.22% were obtained from the control experiment. At the end of the experiment at the 192 h, 33.33% of the isolates were obtained from Omo[®] and Jet[®] treated samples, while 22.22 and 11.11% were obtained from Persil® treated

Colony morphology	Gram stain	MR	V.P	Citrate	Oxidase	Catalase	Indole	Glucose Oxidation	Possible isolate
Milky brown flat colony with a glistering surface rods	- rods	-	+	+	+	-	+	+	Psuedomonas sp.
Irregular creamy flat wavy colony with glistering surface	- rods	+	-	-	-	+	+	+	E. coli
Round creamy wavy colony with a glistering surface rods	- rods	+	-	+	-	+	-	+	Citrobacter sp.
Round creamy flat with a glistering surface	+ cocci	+	-	+	+	+	-	+	<i>Staphylococcus</i> sp.
Irregular cramy flat colony with a dull surface	+ rods	-	+	-	-	+	+	+	<i>Bacillus</i> sp.
Irregular flat pinkish with a glistering surface	- rods	-	+	+	+	+	-	+	<i>Serratia</i> sp.
Roundish, flat milky brown colony with glistering surface	+ rods	+	-	+	+	-	-	+	Actinomyces sp.
Roundish wavy flat creamy colony with a glistering surface	- rods	+	-	+	-	+	-	+	Enterobacter sp.
Irregular wavy flat creamy colony with glistering surface	+ rods	-	-	-	+	+	+	+	Corynebacterium sp.

Table 1. Morphological and biochemical characteristic and probable identities of bacterial isolates.

- rods = Gram negative rods; + rods = Gram positive rods; + cocci = Gram positive cocci; sp. = species; + = positive reaction; - = negative reaction.

sample and the control experiment.

Isolates specific succession revealed that at 0 h, *Klebsiella* and *Serratia* were lost in the entire detergent treated sample except in the control sample. However at 48, 96, 144 and 92 h, *Klebsiella, Enterobacter, Serratia* and *E. coli* were not recovered from the entire sample. Generally *Pseudomonas* predominated in all the samples throughout the period of analysis and was followed in that order by *Bacillus, Actinomyces, Corynebacterium* and *Staphylococcus* species.

The ability of these aquatic bacteria isolates to degrade detergents in single and combined forms is as shown in Tables 3, 4 and 5. The result revealed increased in OD to be related to the levels of bacterial recovered using plate count methods. Although organisms show obvious evidence of detergent degradation in single culture, a better degradation process based on changes in optical density, microbial recovery, loss of important detersive properties such as foaming which is an indicator of detergent utilization were detected in the mixed culture. The test revealed *Pseudomonas* to be the best degrader of test detergents among the isolates from Otamiri River. It was followed by *Bacillus, Actinomyces, Corynebacterium* and *Staphylococcus* in that order. Statistical analysis revealed proportionality between detergent load and bacterial recovery indicative of detergent degradation.

DISCUSSION

The study has revealed that five of nine bacteria genera isolated from Otamiri River were capable of utilizing detergents as their carbon source. Similar trend in the ability of natural micro biota to degrade novel or synthetic compounds has been reported (Atlas, 1984; Green, 1994). Such parity in the utilization of novel compounds by natural micro flora is expected, since such breakdown

Time (h)	Sample	Klesiella	Enterobacter	Staphylococcus	Pseudomonas	Bacillus	Corynebacterium	Actinomyces	Serratia	E. coli	Percentage Occurrence (%)
0	А	+	+	+	+	+	+	+	-	+	88.89
	В	-	+	+	+	+	+	+	-	+	77.78
	С	-	+	+	+	+	+	+	-	+	77.78
	D	+	+	+	+	+	+	+	+	+	100
48	А	-	-	-	+	+	+	+	-	-	44.44
	В	-	-	+	+	+	+	+	-	-	55.56
	С	-	-	-	+	+	+	+	-	-	44.44
	D	+	+	+	+	+	+	+	-	-	77.78
96	А	-	-	-	+	+	+	+	-	-	44.44
	В	-	-	+	+	+	+	+	-	-	55.56
	С	-	-	-	+	+	+	+	-	-	44.44
	D	-	-	+	+	+	-	-	-	-	33.33
144	А	-	-	-	+	+	+	+	-	-	44.44
	В	-	-	-	+	+	+	+	-	-	44.44
	С	-	-	-	+	+	+	+	-	-	44.44
	D	-	-	-	+	+	-	-	-	-	22.22
192	А	-	-	-	+	+	-	+	-	-	33.33
	В	-	-	-	+	+	-	+	-	-	33.33
	С	-	-	-	+	-	+	-	-	-	22.22
	D	-	-	-	+	-	-	-	-	-	11.11

Table 2. Microbial successions of organisms during Otamiri River contact with different of detergents.

 $A = OMO^{\text{(B)}}, B = JET^{\text{(B)}}, C = PERSIL^{\text{(B)}}, D = Control.$

depends on the possession of plasmids that are not naturally present in all microbes (Lawson and Payne, 1980; Campbell, 1983). Hence, the ability to utilize xenobiotics must thus be dependent on the possession of the requisite enzymes necessary for such degradation.

There was an initial decrease in microbial count as slight progressive increase until the peak of the biodegradation process, when a decrease in population was observed. This trend corroborates the report of other workers (Swisher, 1987; Turner et al., 1983; Atlas 1984; Gibson, 1984; Okpokwasili and Olisa, 1991; Green, 1994). The initial decrease in count could be as a result of the inability of the isolates to immediately acclimatize nutritionally to the detergent solution and hence synthesize requisite enzyme(s) for the degradation of the substrate (test detergent). The slight but steady increase in microbial count observed beyond the 24 h for the test samples (A - C) justifies the possible assumption that the microbes utilized the detergents as a carbon source. Whereas the regime in microbial population observed in control sample (D) may be as a result of the inability of some allochthonous flora of the Otamiri River to survive in water for a long time in the absence of extraneous nutrient supply. Such trend has been reported by Cruickshank et al. (1982) and Bordner and Winter (1988) to be associated with the depleting nutrient levels and the obvious shock that characterizes the mere removal of organisms from its natural habitat to an artificial ecosystem.

On the exposure of these organisms to different concentrations of the detergent, floctuatory pattern of utilization was obtained, with some of the test isolates not being able to cope nutritionally. This however, could be as a result of the ability of the microbes to overcome the biochemical inertia of the detergent degradation. This trend in the microbial utilization of detergents corroborates the findings of other researchers (Oba et al., 1968; Willets and Cains, 1972; Gilbert and Watson, 1977; Green, 1994). Five out of the nine bacterial genera isolated which comprises Pseudomonas, Bacillus, Actinomyces, Corynebacterium and Staphylococcus aureus were found to degrade these three brands of detergents. The obliteration of other genera in the primary degradation test could be as a result of the toxicity of the different brands of detergents evaluated.

Isolate	0 h		24 h		48 h		72 h		96 h		120 h	
	OD ₅₇₀	log cfu/ml										
1	0.002	1.0	0.002	2.08	0.003	2.13	0.005	2.22	0.006	1.70	0.009	0.90
2	0.002	0.9	0.005	1.30	0.009	1.46	0.011	1.58	0.014	1.53	0.018	1.51
3	0.002	0.9	0.003	1.35	0.005	1.48	0.008	2.38	0.013	1.54	0.015	1.30
4	0.005	1.0	0.010	1.48	0.013	1.70	0.019	1.75	0.023	1.40	0.028	1.33
5	0.002	0.8	0.004	0.93	0.008	0.98	0.014	1.72	0.017	1.65	0.023	1.45
6	0.003	1.1	0.008	2.15	0.009	2.25	0.011	2.33	0.013	2.04	0.020	1.04
1	0.372	1.0	0.404	1.23	0.415	2.20	0.426	2.52	0.443	1.98	0.451	1.70
2	0.413	1.1	0.424	1.90	0.425	2.51	0.464	2.54	0.471	2.38	0.473	2.31
3	0.451	0.9	0.552	1.85	0.554	2.31	0.561	2.65	0.572	2.14	0.585	1.98
4	0.304	1.1	0.336	1.84	0.339	1.85	0.351	2.08	0.368	1.90	0.381	1.72
5	0.310	1.4	0.342	2.30	0.364	2.37	0.367	2.39	0.385	2.28	0.383	2.23
6	0.346	1.4	0.386	2.36	0.393	2.45	0.408	2.57	0.417	3.12	0.406	2.90
1	0.649	1.73	0.679	1.89	0.688	2.42	0.693	2.44	0.728	2.37	0.723	2.18
2	0.713	0.90	0.756	1.26	0.772	2.15	0.783	2.27	0.794	2.54	0.788	1.80
3	0.613	2.00	0.660	2.48	0.678	2.49	0.684	2.68	0.697	2.38	0.685	2.20
5	1.100	2.00	1.380	2.26	1.384	3.52	1.396	3.54	1.404	3.25	1.354	2.09
4	0.701	1.98	0.750	2.04	0.762	2.45	0.778	2.69	0.772	2.11	0.734	1.76
6	0.684	2.00	0.700	2.42	0.715	2.48	0.728	2.49	0.727	2.40	0.714	2.13

Table 3. Turbidity of the mineral salt Omo[®] broth culture.

1 = Pseudomonas, 2 = Staphylococcus, 3 = Actinomyces, 4 = Bacillus, 5 = Corynebacterium, 6 = Mixed culture of the five isolates.

Isolate	0 h		24 h		48 h		72 h		96 h		120 h	
	OD _{570 nm}	log cfu/ml										
1	0.011	2.16	0.014	2.57	0.019	2.65	0.023	2.79	0.028	2.73	0.027	2.70
2	0.016	1.73	0.020	2.05	0.024	2.48	0.031	2.50	0.035	2.21	0.034	1.82
3	0.037	1.84	0.044	2.17	0.046	2.36	0.053	2.40	0.049	1.81	0.030	1.36
4	0.026	1.91	0.038	2.30	0.041	2.53	0.049	2.60	0.051	2.45	0.048	2.25
5	0.021	1.90	0.033	2.26	0.038	2.93	0.043	2.68	0.039	2.39	0.034	2.10
6	0.009	2.10	0.013	2.50	0.019	2.56	0.024	2.60	0.021	1.90	0.020	1.86
1	1.200	2.20	1.305	2.53	1.343	2.72	1.369	2.92	1.358	2.68	1.349	2.60
2	0.510	2.08	0.656	2.30	0.683	2.48	0.721	2.58	0.748	2.48	0.750	2.12
3	0.630	2.09	0.750	2.33	0.754	2.79	0.760	2.51	0.769	2.47	0.768	1.98
4	0.540	2.13	0.658	2.40	0.668	2.55	0.675	2.57	0.683	2.40	0.686	2.38
5	0.590	2.01	0.706	2.27	0.714	2.65	0.718	2.72	0.715	2.51	0.700	2.48
6	1.000	2.33	1.095	2.65	1.135	2.82	1.165	2.91	1.156	2.77	1.149	2.53
1	1.131	2.41	1.206	2.67	1.209	2.80	1.311	2.96	1.330	2.62	1.316	2.44
2	1.000	2.01	1.010	2.27	1.014	2.72	1.018	2.80	1.020	2.60	1.026	2.46
3	1.000	2.33	1.041	2.59	1.044	2.85	1.053	2.56	1.056	2.31	1.058	2.19
4	0.890	2.26	0.964	2.42	0.966	2.83	0.971	2.91	0.973	2.78	0.979	2.76
5	0.900	2.30	1.001	2.58	1.004	2.61	1.008	2.68	1.013	2.40	1.016	2.32
6	1.190	2.54	1.315	2.83	1.343	2.97	1.377	3.08	1.366	2.34	1.368	2.14

Table 4. Turbidity of the mineral salt Jet[®] broth culture.

1 = Pseudomonas, 2 = Staphylococcus, 3 = Actinomyces, 4 = Bacillus, 5 = Corynebacterium, 6 = Mixed culture of the five isolates.

	0 h		24 h		48	48 h		2 h	9	6 h	120 h	
Isolate	OD ₅₇₀ nm	log cfu/ml	OD ₅₇₀ nm	log cfu/ml	OD _{570 nm}	log cfu/ml	OD ₅₇₀ nm	log cfu/ml	OD ₅₇₀ nm	log cfu/ml	OD ₅₇₀ nm	log cfu/ml
1	0.016	1.40	0.028	2.45	0.030	2.68	0.034	2.87	0.035	2.60	0.034	2.42
2	0.015	1.10	0.017	1.18	0.009	1.21	0.004	1.30	0.001	0.96	0.003	0.00
3	0.016	1.30	0.023	2.18	0.021	2.49	0.018	2.34	0.010	2.27	0.011	1.99
4	0.017	1.40	0.026	2.37	0.030	2.76	0.033	2.90	0.029	1.81	0.020	1.36
5	0.015	1.20	0.019	2.01	0.019	2.05	0.029	1.89	0.018	1.23	0.014	1.10
6	0.023	1.90	0.048	2.82	0.062	3.38	0.081	3.67	0.072	3.89	0.110	2.53
1	0.440	1.42	0.518	2.20	0.568	2.04	0.578	1.70	0.612	1.26	0.581	0.98
2	0.410	1.44	0.493	2.56	0.310	2.93	0.316	3.16	0.331	3.03	0.344	2.51
3	0.430	1.22	0.503	2.06	0.541	2.11	0.573	0.00	0.583	0.00	0.594	0.00
4	0.410	1.41	0.496	2.36	0.507	0.00	0.511	0.00	0.527	0.00	0.587	0.00
5	0.417	1.43	0.511	2.18	0.531	2.58	0.556	1.68	0.571	1.42	0.580	1.04
6	0.430	1.67	0.606	2.70	0.644	3.30	0.693	3.61	0.653	3.38	0.691	2.97
1	1.000	1.90	1.102	2.18	1.107	2 31	1.110	2.54	1.113	2.46	1.111	2.44
2	1.000	1.90	1.063	2.51	1.066	2.01	1.074	1.35	1.076	1.17	1.073	0.92
3	1.000	2.00	1.009	2.08	1.013	0.00	1.048	0.00	1.053	0.00	1.056	0.00
4	1.000	1.90	1.106	2.34	1.109	2.39	1.116	2.42	1.133	2.35	1.131	2.33
5	1.000	1.80	1.063	2.15	1.080	0.00	1.088	0.00	1.093	0.00	1.097	0.00
6	1.000	2.00	1.119	2.56	1.134	2.80	1.146	3.17	1.202	3.05	1.364	3.00

Table 5. Turbidity of the mineral salt persil[®] broth culture.

1 = Pseudomonas, 2 = Staphylococcus, 3 = Actinomyces, 4 = Bacillus, 5 = Corynebacterium, 6 = Mixed culture of the five isolates.

The least utilized detergent is the Persil® and this could be as a result of the fact that the organisms has never been exposed to $\text{Persil}^{\texttt{B}}$ compared with the other routinely used detergents. Also such parity in detergent utilization by isolates could be as a result of adequate level of carbon source and phosphate components, which has been reported to play a role in detergent degradation (Giddings, 1973; Okpokwasili and Olisa, 1991). The trend in Persil[®] degradation corroborates with the report of Swisher (1963, 1987), who associated it to the toxic and recalcitrant nature of products of detergent degradation. Also, since the position of the phosphate group in the detergent from the enzyme site for the oxidation of side chains has a role to play in detergent degradation, it may account for the parity in the utilization of different grades of detergent. This study has revealed that detergents are susceptible to degradation by natural aquatic bacteria flora, although the rate of utilization differs from one detergent to another. This study has also demonstrated a sound methodology for the objective evaluation of the susceptibility of detergent to natural degradation to check for the production of substandard and environmentally unfriendly detergents. It has thus, unravels the ability of natural aquatic bacterial flora in degrading detergent spill and seepages in our local streams and rivers that have been receiving detergents of unknown guality and guan-

tity over the years.

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