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DEGRADATION POTENTIALS OF TROPICAL SOIL BACTERIA ON DETERGENTS

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Abstract. Detergents are chemicals of high environmental importance owing to their production volumes. They are mainly released into the environment through wastewater pathway and it could severely affect the soil environment. In this study, we examined the degradation potentials of soil bacteria in liquid culture media induced with detergents. Using conventional enrichment methods, via contaminated soil slurry enrichment with selected alkyl-benzene sulphonates (detergents), we obtained pure bacteria species capable of using alkyl-benzene sulphonates as the sole source of carbon and energy. From the morphological and biochemical characterization and comparison with respect to the standard reference organisms, the bacteria isolates were presumably *Corynebacterium*, *Pseudomonas*, and *Bacillus* species. Different concentrations 5.0, 10.0 15.0, and 20.0 w/v of the branded detergents were prepared as sole carbon and energy and screened against our bacteria species to determine their physiological gradient fluxes after 96hours of incubation. Data obtained showed an increase in Optical density (OD) as well as increases in pH flux values. The mean OD data obtained ranged between 0.017- 0.818, with a pH of 7.47-8.95. From this study, tropical soils possess unique bacteria species capable of utilizing alkyl-benzene sulphonates (detergents).

Keywords: alkyl-benzene sulphonates, Optical density, Tropical soil, bacterial strains, pH

1. INTRODUCTION

Detergents are cleaning products derived from synthetic organic chemicals that are meant for water-based laundry or dishwashing processes [1]. It has advantages over soaps because it's readily produced from petrochemical sources; and its efficient when used in acid or hard waters. [2, 3]. Surfactants are the components mainly responsible for the cleaning action of detergents. The commercial detergents, contain between 10 and 20% surfactants, and other components which include but not limited to perfume, enzymes bleach and dyes [3]. Surfactants comprises of one or more hydrophilic and hydrophobic groups that form micelles from fatty acids [4]. Large concentrations of surfactants cause skin irritation [5]. In the developing countries, the release of detergents into the environment is rarely monitored nor regulated; thus could accumulate across the food chain or in soil or sea sediments [6].

Biodegradation is an essential mechanism for the reduction and removal of organic contaminants, including detergents from the milieu [7, 8]. The evaluation of the biodegradability of an organic pollutant is a critical parameter for environmental risk assessment [9]. Biodegradation describes how organic (carbon-containing) detergent ingredients, enzymes, and fragrances, are mineralized into carbon dioxide, water, and other compounds by the action of microorganisms such as bacteria. The bacteria utilize surfactants as substrates for energy, carbon sources or electrons. Biodegradation of surfactant in the environment are influenced by several factors. The most important influencing factors are the chemical structures and their physicochemical conditions. This study determined that our tropical bacteria are capable of the growth and utilization of local detergents as carbon sources.



2.0 MATERIALS AND METHODS

Chemicals and Reagents: The salts $(\text{NH}_4)_2\text{SO}_4$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, K_2HPO_4 , KH_2PO_4 , and NaCl are of analytical grade and obtained from Merck, Germany. The nutrient agar, nutrient broth used in this study were purchased from Micro master, India. Other media such as peptone water, Voges Proskauer medium, starch agar, urease base agar, and methyl red were procured from the Microbiology laboratory of Covenant University, Ota

Soil sample collection

Soil samples that had been contaminated with detergents from laundry wastewater were collected. The location was around the surrounding of Dorcas hall, Covenant University. The selected contaminated soil were mapped out and induced with laundry wastewater for two months. The soil samples were collected from the designated site using a hand trowel by the random sampling technique. The soil samples were collected at 6cm deep at six different spots and were mixed before putting it into the sterile container and stored at ambient temperature before the initiation of the enrichment.

Media Preparation

Chloride-free minimal salts (MS), which consisted of 0.5g $(\text{NH}_4)_2\text{SO}_4$, 0.076 g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 40 mM phosphate buffer (pH 7.25) was used in this study[10].

Preparation of Enrichment media

Five (5g) of the detergent contaminated soil were measured out and sieved. The soil sample was seeded into a conical flask containing the prepared minimal salt media. The seeded medium was homogenized by gentle stirring to allow the soil to spread in the medium evenly. Ten (10g) of detergent (Omo) was added to serve as carbon and energy source during the enrichment period. These were done in triplicates and placed in an orbital shaker for 7days at 30 °C

Bacterial cell growth

The solid MS medium was prepared by adding 1.8% Agar (Difco Laboratories, Detroit, MI USA) into the minimal salt medium. This was sterilized using an autoclave at 121°C for 15 minutes and on cooling, it was poured into labeled Petri dishes. The medium was then allowed to solidify. An aliquot of the enrichment media were added aseptically on the surface of the agar plate, afterward, streaked on the surface of the agar. The detergent was sprayed on the streaked agar surface then incubated at 30°C for 3days. After incubation, the plates were observed for growth.

The presence of growth indicate that the organisms present can use detergent as energy and carbon sources. The microorganisms that grew as colonies were subcultured onto the fresh MS-Medium. There were successive transfers on new solid MS Medium to obtain pure cultures. Morphological and microscopic identifications were carried out using the bright field microscope.

Characterization of the organisms

Distinct colonies of the bacterial strains were selected for characterization and degradation studies following their morphological classifications. The bacteria isolates were tentatively named as A, B, C, D, and characterized based on observable and physiological traits and comparison with standard reference organisms (See Table 1).

Harvesting of selected cultures

The nutrient broth was prepared, transferred into conical flasks and sterilized. The characterized and selected bacteria cells were mass-produced for further studies. This was carried out by inoculating into the nutrient broth with pure cultures of *Bacillus*, *Pseudomonas*, and *Corynebacterium* species. The conical flasks were stoppered with sterile cotton wool incubated for 5 days at 25 °C.

After incubation, the cells were harvested using a centrifuge at 25x100 rpm for 20 minutes. This speed was maintained to avoid the disruption of bacterial cells. The filtrates were decanted, and the cell pellets washed with phosphate buffer and centrifuged at 25 x100 rpm for 10 minutes. The cell pellets for each organism was collected and transferred into sterile Eppendorf tubes and stored at 4 °C.

Determination of degradative potential in the Detergents

The selected (Omo and Sunlight) detergents for this study were added to different tubes containing MS medium at a concentration of 5.0, 10.0, 15.0, and 20.0 w/v. The cultures were incubated in the stoppered tubes in an aerating shaker at a temperature of 37 °C. Growth was monitored by measuring pH and turbidity (OD) fluxes using UV spectrophotometer for a 96 hrs incubation period.

3.0 RESULTS

The result of the morphological and biochemical characterization is as shown in Table 1. All the organisms except organism C (*Pseudomonas*) were gram positive. All the organism were able to grow at 35 °C, pH 6.0, and in 5 % of NaCl. All the bacteria species were not able to ferment sucrose and lactose. The *Bacillus* and *Pseudomonas* were motile while *Corynebacterium* are non-motile.

Table 1.0: Cultural and biochemical characteristics of bacteria species capable of degrading detergent contaminated soil

Tests	A	B	C	D
Gram stain	+	+	-	+
Colony morphology	rods,with club	rods	rods	rods
Citrate	+	+	+	+
Starch hydrolysis	-	+	-	+
Indole	-	+	-	+
Motility	-	+	+	+
Catalase	+	+	+	+
Methyl red	-	-	-	-
Voges proskeur	-	+	+	+
Glucose	+	-	-	-
Sucrose	-	-	-	-
Lactose	-	-	-	-
Growth at 35°C	+	+	+	+
Growth at pH 6.0	+	+	+	+
Growth in 5% of NaCl	+	+	+	+
Most probable organism	<i>Corynebacterium</i>	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>Bacillus</i>

Negative -; Positive+

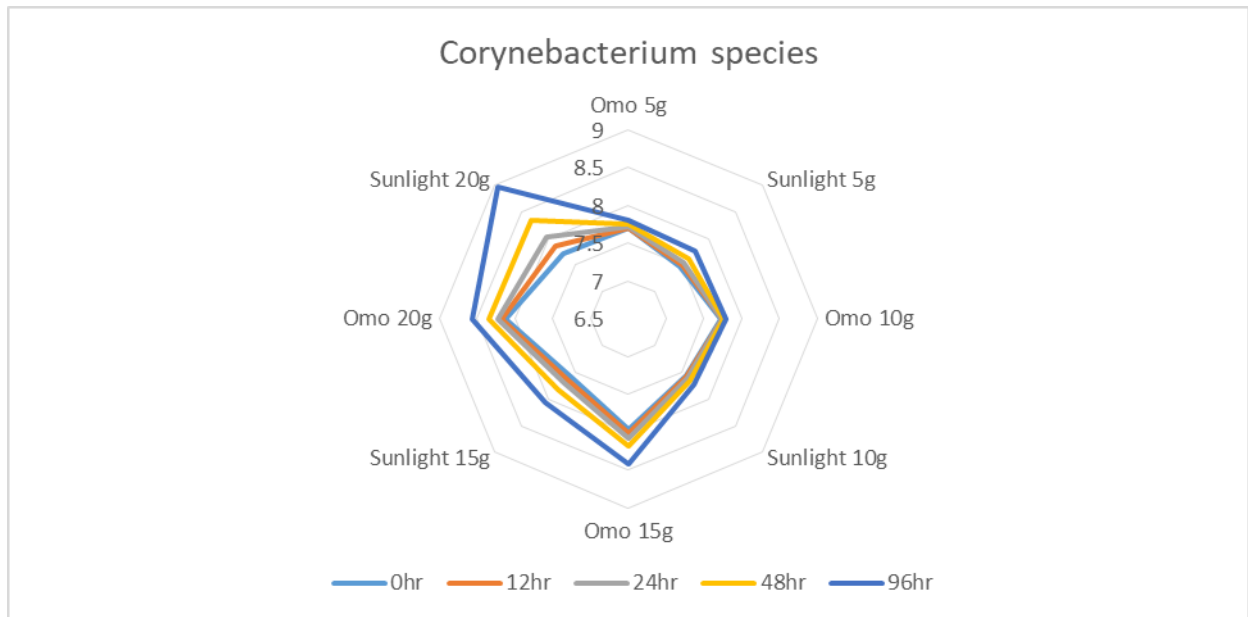


Figure 1.0a: Radar Chart of Time dependent variables of pH fluxes of Omo (detergent) and Sunlight (detergent) by *Corynebacterium* spp. The organisms were exposed at 5, 10, 15 and 20(w/v) of omo (Linear Alkyl-benzene Sulphonates) detergent.

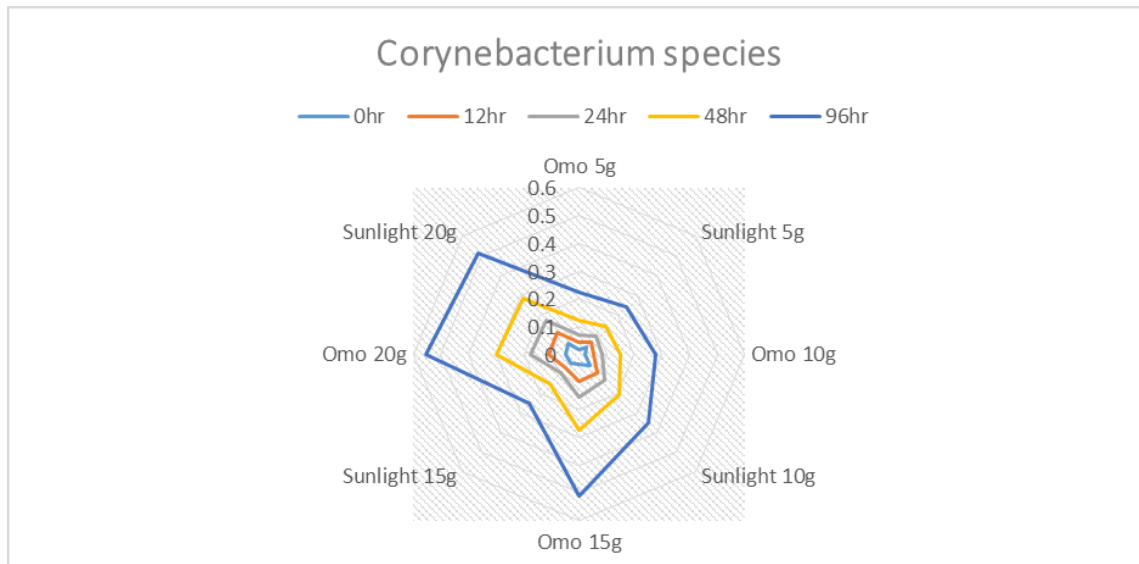


Figure 1.0b: Radar Chart of Time dependent study of Turbidity (OD) fluxes of Omo (detergent) and Sunlight (detergent) by *Corynebacterium* spp. The organisms were exposed at 5, 10, 15 and 20(w/v) of (Linear Alkyl-benzene Sulphonates) detergent.

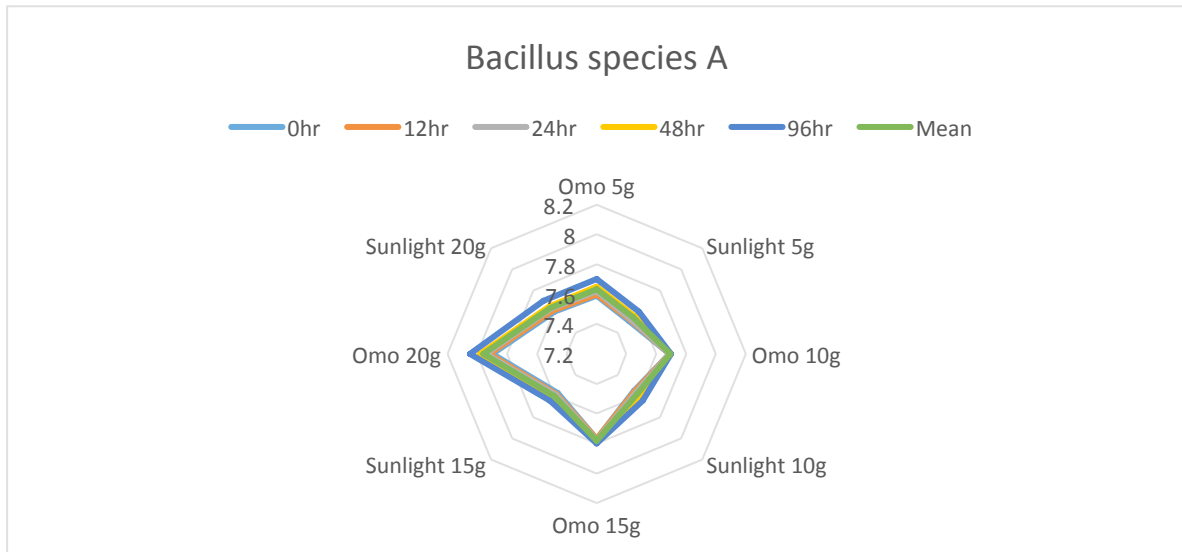


Figure 2.0a: Radar chart of Time dependent variables of pH fluxes of Omo (detergent) and Sunlight (detergent) by *Bacillus* spp A. The organisms were exposed at 5, 10, 15 and 20(w/v) of (Linear Alkyl-benzene Sulphonates) detergent.

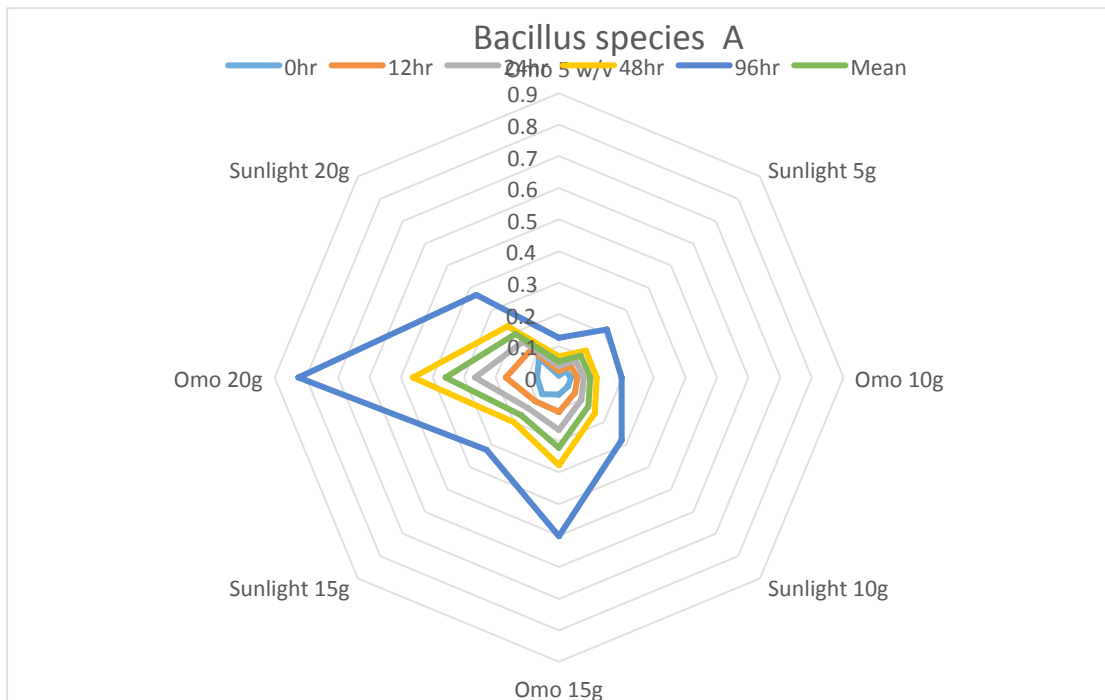


Figure 2.0b: Radar Chart of Time dependent study of Turbidity (OD) fluxes of Omo (detergent) and Sunlight (detergent) by *Bacillus* spp A. The organisms were exposed at 5, 10, 15 and 20(w/v) of omo (Linear Alkyl-benzene Sulphonates) detergent.

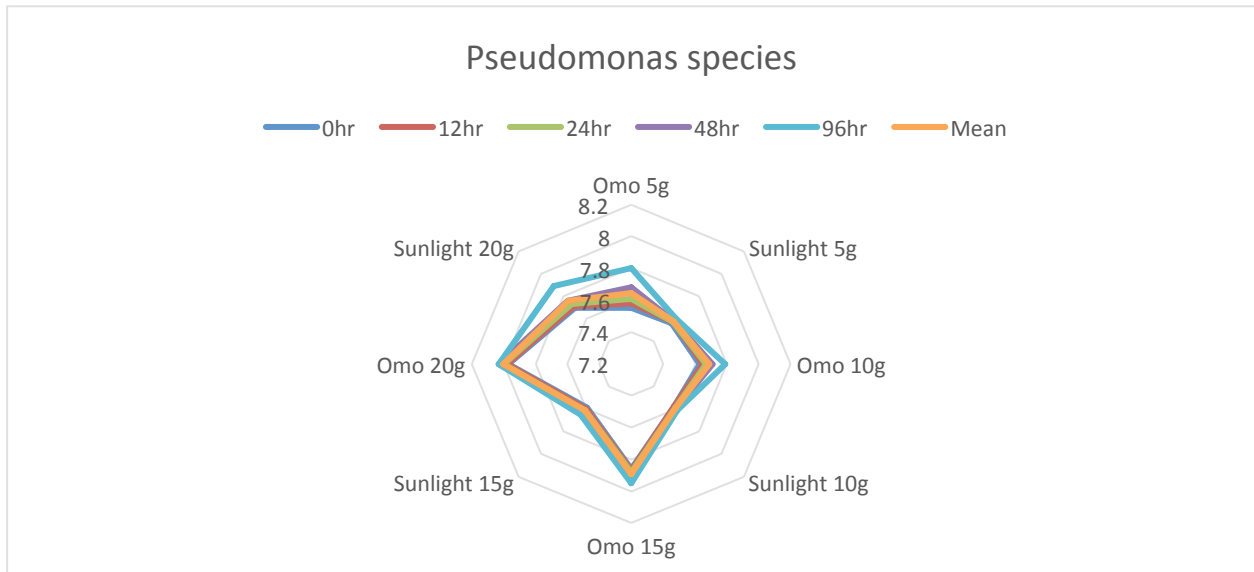


Figure 3.0a: Radar Chart of Time dependent variables of pH fluxes of Omo (detergent) and Sunlight (detergent) by *Pseudomonas* spp. The organisms were exposed at 5, 10, 15 and 20(w/v) of (Linear Alkyl-benzene Sulphonates) detergent.

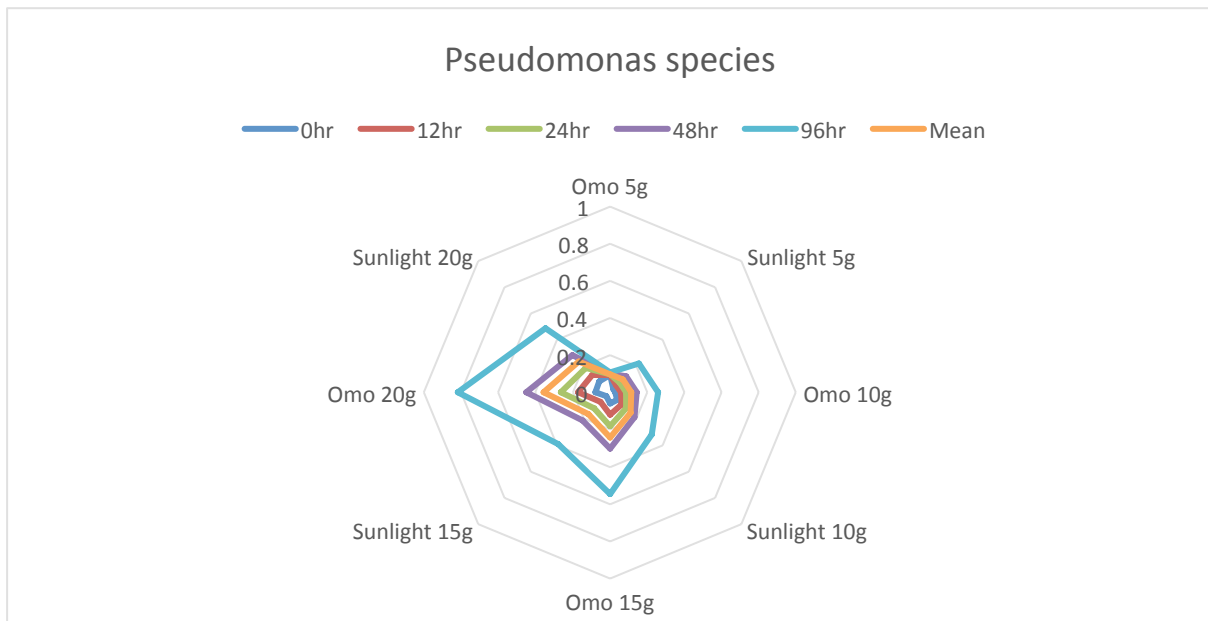


Figure 3.0b: Radar Chart of Time dependent study on Turbidity (OD) fluxes of Omo (detergent) and Sunlight (detergent) by *Pseudomonas* spp. The organisms were exposed at 5, 10, 15 and 20(w/v) of (Linear Alkyl-benzene Sulphonates) detergent.

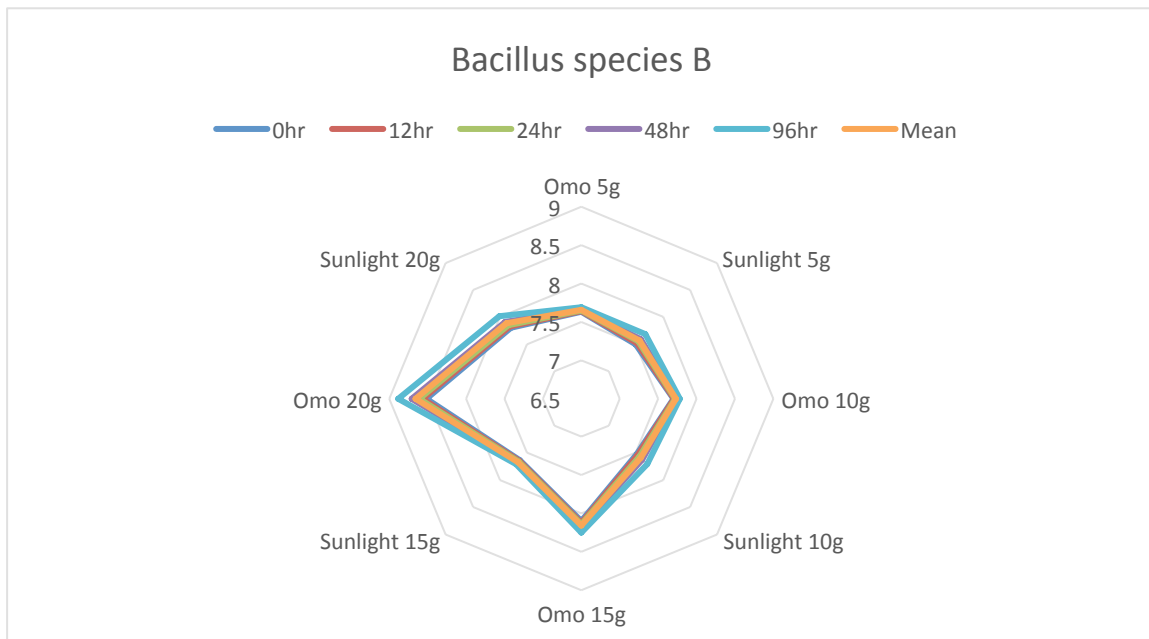


Figure 4.0a: Radar chart of Time dependent variables of pH fluxes of Omo (detergent) and Sunlight (detergent) by *Bacillus* spp B. The organisms were exposed at 5, 10, 15 and 20(w/v) of (Linear Alkyl-benzene Sulphonates) detergent.

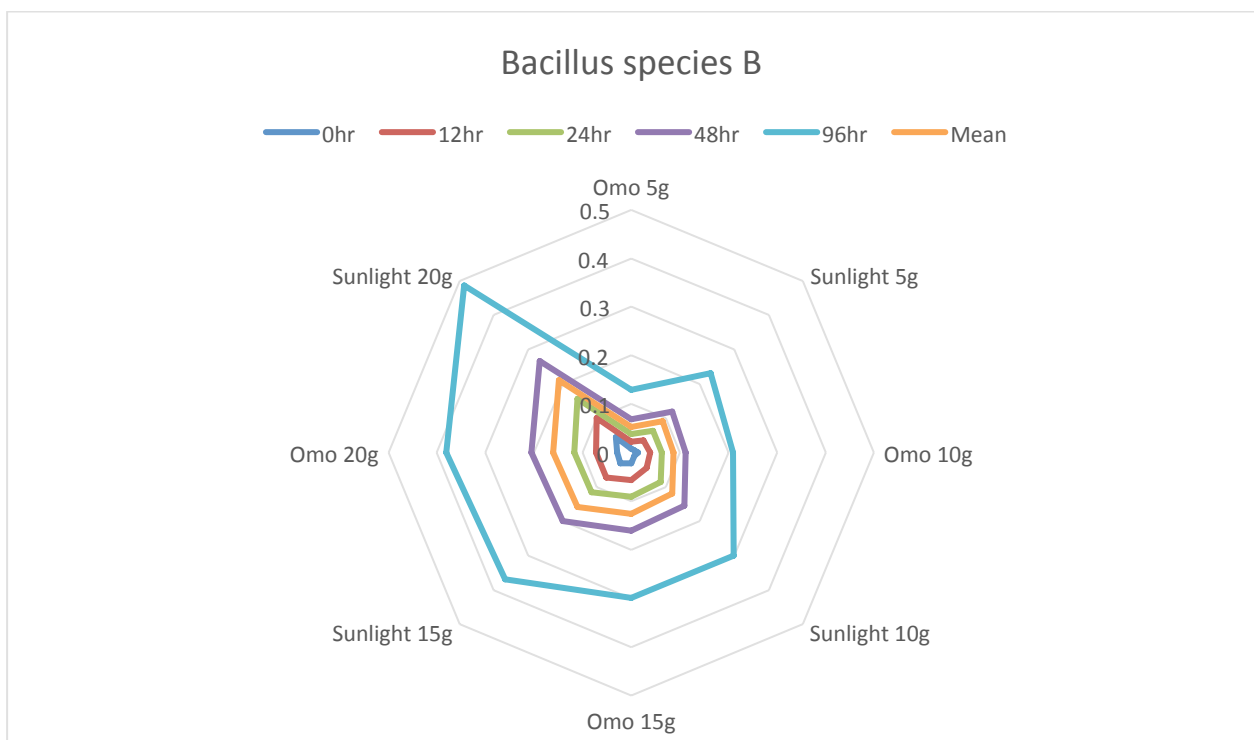


Figure 4.0b: Radar chart of Time dependent study on Turbidity (OD) fluxes of Omo (detergent) and Sunlight (detergent) by *Bacillus* species B. The organisms were exposed at 5, 10, 15 and 20(w/v) of (Linear Alkyl-benzene Sulphonates) detergent.

4.0 DISCUSSION

In this study, increase in the turbidity (Optical density OD) fluxes and pH changes, when compared with the initial values after experiment setup may be regarded as utilization of the detergents (Omo and Sunlight) as carbon and energy sources by the bacteria species. This is with the understanding that since the detergents are the only carbon/energy sources, its utilization will obviously result in multiplication of cells and increase in turbidity. As observed from Figures 1 a and b, *Corynebacterium* species was able to degrade detergent in the enrichment medium as its carbon source. The utilization rate of the bacteria isolates differed with the varying concentrations of the detergents. Within the period of incubation 0-96 hours, *Corynebacterium* species exhibited an increase in pH (7.69-8.57) as well as a corresponding increase in the turbidity OD. However, the OD readings of the sunlight detergent was less compared with the Optical density readings obtained in Omo detergent assay. The growth trend in the behavior of *Corynebacterium* species in 15 and 20 (w/v) was similar. But at lower concentrations (5, 10 w/v) the growth trends were different. In Figure 2a and b, the *Bacillus* sp. A showed an increase in pH and OD at 20 (w/v) concentration. This implies that the pathways for the utilization of the detergents is such that produces metabolites that increase the pH (7.59 - 8.05) with corresponding increase in the OD (0.006 -0.854) of the medium. However of note, is that the organism used the Omo detergent more than the Sunlight detergent at high concentrations 15, 20 (w/v) when compared with the lower concentrations 5, 10 (w/v). This increase were similar with the OD optical density trends. The sunlight detergent-MS media showed an increase in pH from 7.51 -7.70 and Optical Density (OD) from 0.028 - 0.37 during the incubation period.

In Figure 3a and b, *Pseudomonas* species showed an increase in pH (7.55 - 8.08) and the Optical Density (OD) (0.093 - 0.818) within the 96 hrs incubation period for all the concentrations of Omo detergents. In the Sunlight detergent-MS media, there was an increase in pH and OD (7.56 - 7.81) and (0.025 - 0.489) within the period of incubation. In all, *Pseudomonas* species effectively utilized all the concentrations of the selected detergents. *Pseudomonas* species are renowned for their abilities to degrade compounds which are highly recalcitrant to other organisms. These recalcitrant compounds include but not limited to aliphatic and aromatic hydrocarbons, and fatty acids. *Pseudomonas* could grow well in media with some organic matter at neutral pH, mesophilic temperatures and without added growth factors. They are known to exhibit respiratory behavior in terms of growth.

In Figure 4 a and b, *Bacillus* spp. showed an increase in pH and Optical density (OD) readings (pH 7.63 - 8.89) and Optical Density (0.008 - 0.381) for the varying concentrations of Omo detergent. In the Sunlight detergent-MS media there were increases in the pH (7.50 - 8.01) and Optical Density from (0.007-0.487) within the incubation period. In all, the *Bacillus* species used the high concentrations of omo detergent (15 and 20 w/v) when compared to the lower concentrations of other detergents.

In this study, the varying difference in the OD and pH readings were probably due to the difference in the growth rate of each bacteria. Some grew faster than others possibly due to better adaptation to the detergent environment or low synthesis of enzymes. Similar organisms isolated in this study, had been implicated in detergent utilization by [11]. The ability of the bacteria species to grow in these detergents (Omo and Sunlight detergent) as a sole carbon and energy sources is an indication that the bacteria species possess the necessary enzymes/metabolic pathways for the utilization of the detergents. The better utilization of Omo detergent may be due to its initial use during the enrichment stage. Thus, it may have induced selective pressure and expression of the genes for the utilization of the Omo detergents. In the report of [11], *Bacillus* was not able to grow at higher concentration of Omo detergent due to the presence of biocides in detergents as reported by [12]. Sunlight detergent was more resistant to biodegradation compared to that of Omo detergent. In this study, the significant growth/utilization of the detergent was exhibited by *Pseudomonas* spp. This outcome is in line with the report of [13] that inferred *Pseudomonas* as fast growing bacteria species with capacity to use wide variety of organic compound.

In conclusion, the isolated Bacteria species (*Corynebacterium*, *Bacillus* and *Pseudomonas* species) have the capacity to utilize waste detergents when released into the environment.

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