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Detergent Disposal into Our Environmentand Its Impact on Marine Microbes

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Detergent Disposal into Our Environmentand Its Impact on Marine Microbes

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Abstract. Detergents figure in an extensive array of industrial and home cleaning applications, released into the flow of wastewater coming from the home, can far-reaching environmental impacts. Microorganisms are crucial to nutrient recycling in ecosystems as they act as decomposers, pathogen, antibiotic producer, biodegradation of pollutants etc. The research is aimed to examine effect detergent disposal to bacterial population growth in marine environment both *in vitro* and *in situ* condition. Seawater samples were collected from Sungai Kayu Ara Village, and Dumai River estuary, Siak Regency and Dumai City, Riau Province. Experimental method with complete randomized design (RAL) 2 (two) factors; a detergent brand (a1: ATTACK, a2; RINSO and a3; SURF) and b concentration of detergent concentration with 5 (five) concentration level, b1 (0%) as control, b2 (0.3%), b3 (0.6%), b4 (0.9%) and b5 (1.2%) wass applied. The study showed that there was an effect of detergent addition, periode of exposure, and doses to the growth of bacterial population both *in vitro* and *in situ* conditions. The higher levels of detergent in the water column and the longer contamination duration, causing more and more depressed bacterial populations. It is suggested to run a further research on identification, and growth optimatioan of the species capable of degrading detergent.

***Keywords* :** Deteregent, pollution, seawater, biodegradation, and environment.

1. Introduction

Detergents has been part of of our life nowadays, commonly available as powders or concentrated solutions. The chemical figure in an extensive array of industrial and home cleaning applications, including laundry and dishwasher detergents. Released into the flow of wastewater coming from the home, these detergents can have far-reaching environmental impacts.

Detergents work because they are amphiphilic: partly hydrophilic (polar) and partly hydrophobic (non-polar). Their dual nature facilitates the mixture of hydrophobic compounds (like oil and grease) with water. Because air is not hydrophilic, detergents are also foaming agents to varying degrees. The main component of the detergent is surface active agents or surfactants. The most commonly used surfactant contains LAS or linear alkylbenzen sulfonate. LAS is an anionic detergent that is classified as hard detergent. This becomes an important issue because the concentration of surfactants in detergents entering a waters can be toxic, affecting the life of the organism in the ocean waters.

Microorganisms or microbes are very diverse and include all bacteria, archaea and most protozoa. This group also contains some fungi, algae, and some micro-animals such as rotifers. Microorganisms are

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crucial to nutrient recycling in ecosystems as they act as decomposers. As some microorganisms can fix nitrogen, they are a vital part of the nitrogen cycle, and recent studies indicate that airborne microorganisms may play a role in precipitation and weather. There is growing interest in the role of marine microorganisms in biogeochemical processes, biotechnology, pollution and health. In recent years, many authors have focused on the great potential of marine microbes for use as prolific producers of bioactive substances, and they have exploited the vast marine microbial treasures for utilization as sources of drugs, antimicrobial agents, biodegradation of pollutants etc. This research was carried out to examine effect detergent disposal to bacterial population growth in marine environment both *in vitro* and *in situ* condition.

2. Method

This research was carried out from February to April 2017. Seawater samples were collected from the seawaters of Sungai Kayu Ara Village, Sungai Apit Subdistrict, Siak Regency and Dumai River estuary, Dumai City, Riau Province. The sample analysis was conducted at the Laboratory of Microbiology and Marine Chemistry Laboratory, University of Riau.

Experimental method with complete randomized design (RAL) 2 (two) factors; a detergent brand (a1: ATTACK, a2; RINSO and a3; SURF) and b concentration of detergent concentration with 5 (five) concentration level, b1 (0%) as control, b2 (0.3%), b3 (0.6%), b4 (0.9%) and b5 (1.2%) is applied. Each treatment was given 3 (three) repeated treatments and measured with 3 (three) replication measurements. While the survay method was done to see the correlation between detergent concentration with the population of heterophic bacteria in *in situ* condition in seawater. Water quality in ie. temperature, pH, salinity and dissolved oxygen with a view to directly observing the condition of the waters to support the growth of microorganisms and to avoid extreme conditions at the time of sampling.

In this study the effect of detergent brand with various concentrations was observed *in vitro* in the microcosms. The microcosms used is a 500 ml erlenmeyer flask filled with 250 ml of sea water. Each flask was poured with detergent with 0% concentration (control) 0,3, 0,6, 0,9 and 1,2% for each detergent brand. Each flask was wrapped with aluminum foil to minimize the effects of light on microbial growth. All microcosms are placed in a cold room with a temperature range of 5-10 ^{o}C. Seawater samples were taken aseptically by using a pipette with sampling time at 0, 5, 10, 15 and 20 day intervals.

Population growth of heterophic bacteria in seawater is counted by using a spreader plate method. The seawater (1 ml) sample was taken aseptically, diluted with physiologic solution (NaCl 0.9%) with dilution levels of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Voleme of 0.1 ml from each dilution was then taken and poured into a petri dish that has contained nutrient agar medium. The sample was then immediately spreaded using a glass spreader. The inoculated petri dishes were then incubated at 18-22 °C for 48 hours. The number of bacterial cells is calculated based on the number of colony forming units in which the colony count sample is taken from a petri dish with a range of 30-300 colonies. The correlation between the concentration of detergent dissolved in seawater with the population of heterotrophic bacteria *in situ* conditions in seawater was obtained by taking samples of seawater at the estuary of the Dumai River. The samples were then processed the same as the previous procedures.

The calculation of dissolved surfactant (detergent) content in the waters of the Dumai River estuary is obtained through the following procedure. Seawater sampling is carried out under tidal conditions. Samples are taken as much as 300 ml on surface water with a depth of 30 cm. The sample is poured into the sample bottle and labeled, stored in an ice box, given ice and taken to the Marine Chemical Laboratory for calculation of dissolved surfactant levels. Surfactant concentration is measured according to SNI procedure (1992). The stages includes; making a 1000 ppm mains solution, preparing a standard solution of 10 ppm LAS, providing sash solution calibration curve configuration and surfactant value analysis. The surfactant content was obtained by using a simple linear regression with the equation Y = a + bx, where Y is the detergent concentration, x is the absorbance value and a and b are the constants.

The data obtained were analyzed statistically and presented in the form of tables and drawings, using SPSS and MS applications, Excel, which is used in the advanced test of Anova, (analysis of variance) and regression analysis.

3. Result

In this study, water quality parameters at the Sungai Kayuara Village are as follows; salinity (24 ppt), acidity (pH 5-6), temperature (28-30 ^{0}C) and dissolved oxygen (6.28 mg/ l). While at the the Dumai River estuary, water quality are as follows; temperature (30-32 ^{0}C), salinity (14-28 ppt), brightness (45-88 cm), current velocity (0.6 m / sec), and pH (7). From the data collected from both research sites, it can be seen that the condition of the waters is in normal condition and can support the growth of the local organism so that it can be used as sample for the research interest.

There was an effect of the addition of detergent (RINSO, SURF and ATTACK) with different doses to the growth of bacterial population in the sea water column. The pressure on the growth of heterotrophic bacteria population was observed from the beginning of observation (0 days), second observation (5 days) to the last observation on day 20. The most prominent effect on population growth resulted from the addition of SURF detergent with a concentration of 1.2%. Then followed by RINSO and ATTACK detergents at the same concentration. The higher the concentration of detergent added, the stronger the impact of the suppression of bacterial population growth resulted. The duration of contamination also affected growth pattern of bacterial population in seawater. In general, the longer the contamination occurs, the lower the total number of viable count (TVC) shown for each microscosm. More detailed data are presented in the following Table 1. The effects of adding detergents with different concentrations and length of contamination will be more clearly illustrated in the following Figure 1, 2 and 3.

Table 1.
Effect of concentration and length of contamination on the growth of sea water bacteria (Log TVC)

	Detergent concentration (%)				
Detergent Brand/ Day of Sampling	**0**	**0,3**	**0,6**	**0,9**	**1,2**
RINSO 0 day	6,85	6,83	6,82	6,83	6,82
RINSO 5 days	6,83	6,62	6,51	6,41	6,10
RINSO 10 days	6,81	6,41	6,39	6,11	5,76
RINSO 15 days	6,79	6,29	6,03	5,83	5,31
RINSO 20 days	6,55	6,06	5,74	5,30	5,06
SURF 0 day	7,99	7,44	7,27	7,12	7,26
SURF 5 days	7,77	7,52	6,12	6,11	6,11
SURF 10 days	7,73	7,04	6,04	6,05	6,01
SURF 15 days	6,73	6,78	6,10	5,78	6,00
SURF 20 days	6,51	6,21	5,36	5,27	5,03
ATTACK 0 day	7.72	7.77	7.48	7.81	7.82
ATTACK 5 days	7.54	7.47	7.22	7.15	6.98
ATTACK 10 days	6.44	6.25	6.19	6.17	6.08
ATTACK 15 days	6.34	6.26	6.23	6.11	5.99
ATTACK 20 days	6.36	6.31	6.23	6.18	5.46

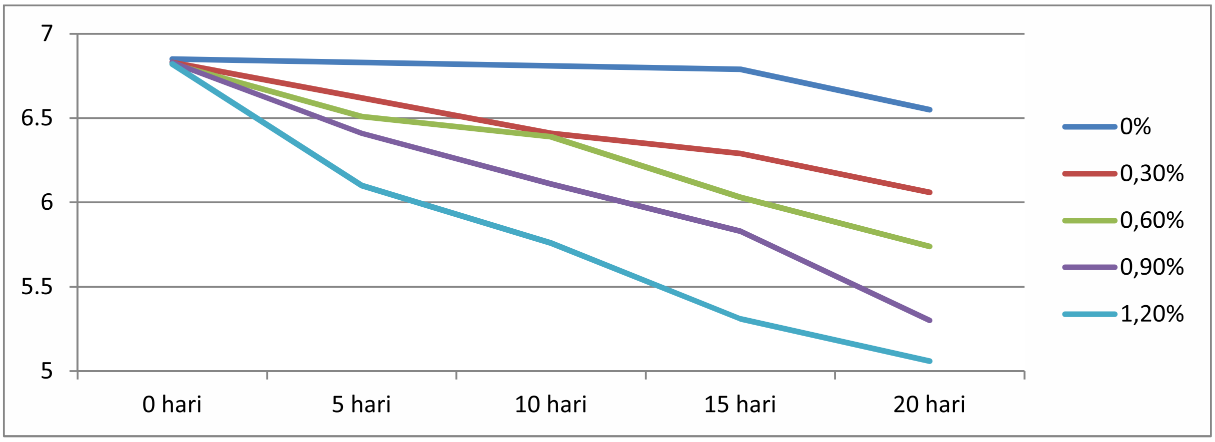

Figure 1. Effect of RINSO detergent on population growth of heterophic bacteria in seawater.

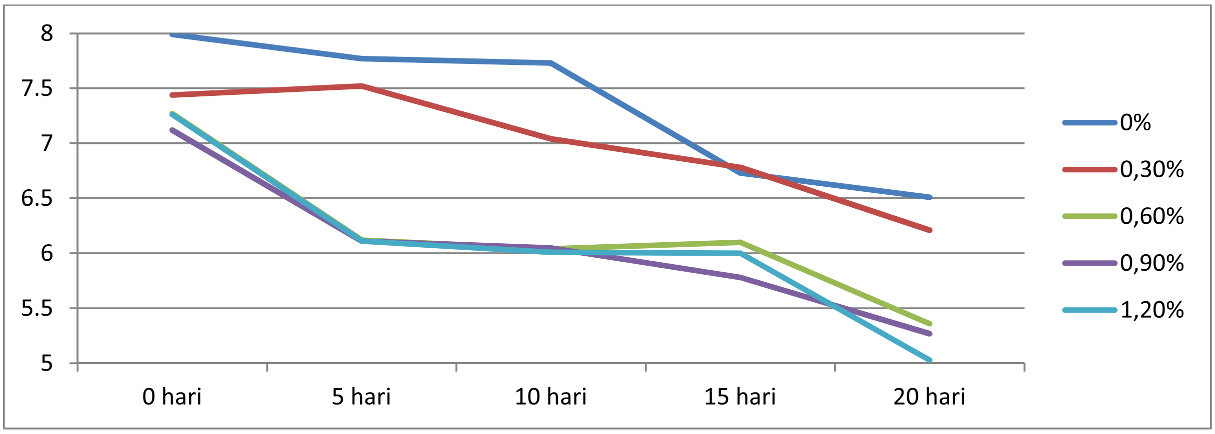

Figure 2. Effect of SURF detergent on population growth of heterophic bacteria in seawater.

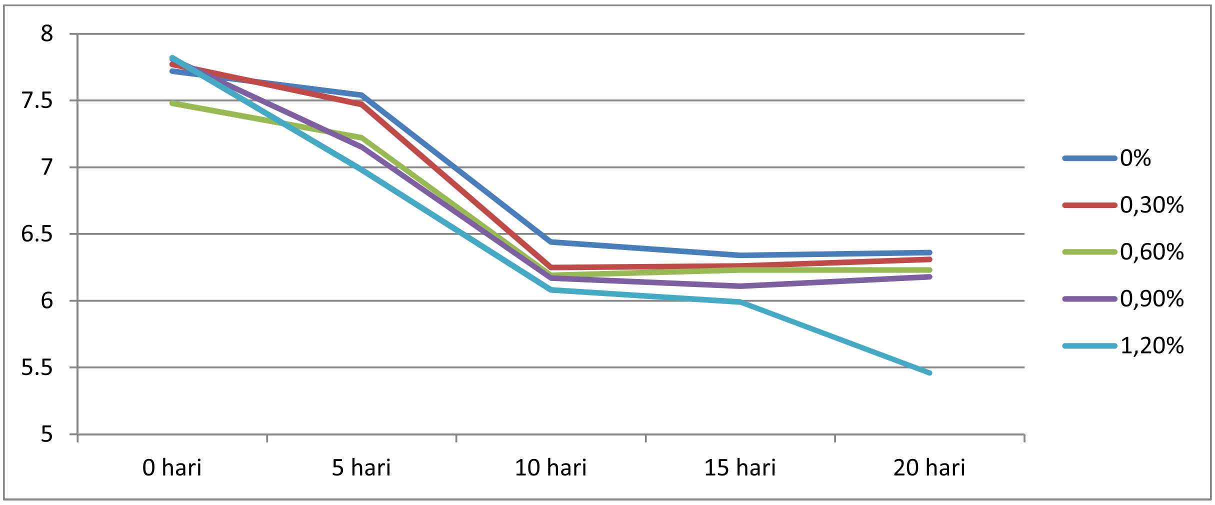

Figure 3. Effect of ATTACK detergent on population growth of heterophic bacteria in seawater.

Table 2.
Number of heterotrophic bacterial cells in seawater at each observation station.

No.	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Sample 1	6,26	6,15	6,13	6,67	6,71	6,62
Sample 2	6,08	6,06	6,18	6,76	6,68	6,69
Sample 3	6,21	6,19	6,12	6,74	6,58	6,67
Rata-rata	6,19	6,13	6,15	6,73	6,66	6,66

Table 3.
Concentration of surfactant in seawater at each observation station.

No.	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
Sample 1	0,1987	0,3091	0,3043	0,7829	0,8599	0,7829
Sample 2	0,2078	0,3353	0,3711	0,8019	0,8723	0,8111
Sample 3	0,2011	0,2989	0,3794	0,8103	0,8291	0,8673
Rata-rata	0,2025	0,3144	0,3516	0,7984	0,8538	0,8204

The correlation analysis showed that the concentration of surfactant has a significant effect on the population of heterotrophic bacteria. These two factors have negatif correlation, the higher surfactant levels in each station cause the decrease bacterial cell number in sea water column (Figure 4).

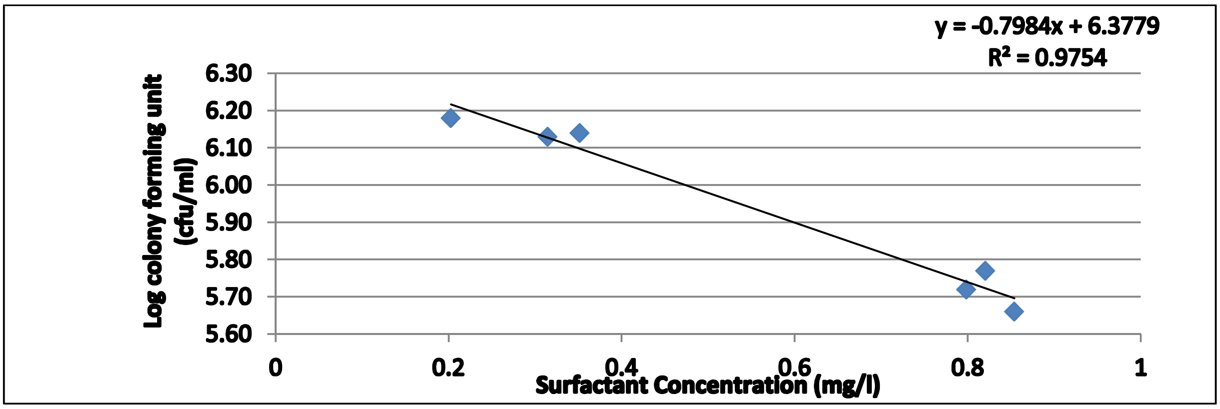

Figure 4. Correlation between concentration of surfactant with bacterial cell population in sea water.

4.Discussion

The results of this study showed that there was an effect of detergent addition (RINSO, SURF and ATTACK) with different brand, periode of exposure, and doses to the growth of bacterial population in the sea water column. Similarly, *in situ* conditions there was a negative correlation between surfactant concentration and bacterial populations in the waters of the Dumai River estuary. Negative effects of detergent or surfactant have been widely reported by researchers around the world. Scott, J. M. and M. Njones (2000) mentioned that detergent or surfactants which pass into the environment and the potential problems which can arise as a consequence. The chemicals can have poisonous effects in all types of aquatic life if they are present in sufficient quantities, and this includes the biodegradable detergents. All detergents destroy the external mucus layers that protect the fish from bacteria and parasites; plus they can cause severe damage to the gills. Most fish died when detergent concentrations approach 15 parts per million. Detergent concentrations as low as 5 ppm killed fish eggs. Surfactant detergents are implicated in decreasing the breeding ability of aquatic organisms.

Detergents are molecules that are amphiphilic in nature with a hydrophobic hydrocarbon tail and a hydrophilic head group. It is the hydrophile on the head group that defines the detergent as anionic, cationic, nonionic or amphoteric, all of which may be used in detergent formulations. Cationic in characteristic and cause damage to the outer membrane and promote their own intracellular uptake and entry (Russell 2002 and Holah *et al,* 2002). There are two kinds of detergents with different characteristics: phosphate detergents and surfactant detergents. Detergents that contain phosphates are highly caustic, and surfactant detergents are very toxic. The differences are that surfactant detergents are used to enhance the wetting, foaming, dispersing and emulsifying properties of detergents. Phosphate detergents are used in detergents to soften hard water and help suspend dirt in water (Siposova *et al*, 2017).

Sudiana (2004) explained that an increase in LAS concentrations that go beyond cell capacity (lysosomes) will cause disruption of cell metabolism. This lead to a decrease in LAS degradation and may even lead to cell death. Furthermore, the side effects of LAS and ABS presence that exceed the natural degradation capacity are polluted environments that can cause damage to ecosystem components (Bitton, 1983), and microbes (Niven et al., 1988). Further, Russell (2001) explained that surfactant as a detergent component can cause environmental problems. The stable foam formation in the river is highly undesirable because it blocks the transformation of the oxygen-mass from air to water. Hydrophilic constituents of toxic surfactants will endanger the survival of aquatic animals and bacteria in water. According to KEP / 51 / MENLH / 2004, the concentration of surfactants in waters is 1 mg / l. In this study the highest concentration of surfactant was located at station 5 (0.8538 mg / l) followed by station 6 (0.8204 mg / l) and 4 (0.7984 mg / l). Based on this, it can be seen that the waters of Dumai River estuary not yet contaminated seriously by surfactant so that it can still support the life of aquatic organisms.

Detergents also add another problem for aquatic life by lowering the surface tension of the water. Organic chemicals such as pesticides and phenols are then much more easily absorbed by the fish. A detergent concentration of only 2 ppm can cause fish to absorb double the amount of chemicals they would normally absorb, although that concentration itself is not high enough to affect fish directly. Phosphates in detergents can lead to freshwater algal blooms that releases toxins and deplete oxygen in waterways. When the algae decompose, they use up the oxygen available for aquatic life (Cohen and Keiser, 2017).

The main contributors to the toxicity of detergents were the sodium silicate solution and the surfactants- with the remainder of the components contributing very little to detergent toxicity (Lisa *et al*, 2017). The potential for acute aquatic toxic effects due to the release of secondary or tertiary sewage effluents containing the breakdown products of laundry detergents may frequently be low. However, untreated or primary treated effluents containing detergents may pose a problem. Bhat *et al* (2011) reported that 1% soap showed higher colony growth of *E. coli, Pseudomonas* and *Staphylococcus,* whereas 3% soap inhibited the growth of all bacteria except *E. coil* and *Micrococcus*. It was seen clearly that Gram positive bacteria were killed at low concentration of soaps than Gram negative bacteria. It is also reported that as the concentration of detergent or soap increases the intensity of inhibition also increases. Similar results were obtained in the present study (Bhat *et al*, 2011). The minimum inhibitory concentrations of detergents for *E. coli* are 3% and 2% respectively and for other bacteria minimum inhibition was observed at 2%.Surfactants are the most important ingredient in detergent products (up to 15-40% of total detergent formulations). This substance can activate the surface, because it tends to concentrate on the surface (interface), or substances that can raise and lower the surface tension. With surfactant there may be changes in surface tension accompanying the wetting process, stable foam power, stable emulsifying power (Scheibel, 2004).

Actually, the surfactants themselves show little toxicity their breakdown products, principally nonyl and octal phenols adsorb readily to suspended solids and are known to exhibit estrogen-like properties, possibly linked to a decreasing male sperm count and carcinogenic effects. While there is little serious risk to the environment from commonly used anionic surfactants, cationic surfactants are known to be much more toxic and at present there is a lack of data on the degradation of cationic and their fate in the environment (Cai and Hakkinen, 2014).

Detergent, however, nowadays has been well designed and in nature can be degraded. In this study we only looked at bacterial colonies in general, regardless of species. It is expected that the bacterial cells that survive inside the microcosms are bacterial species capable of decomposing the detergent. Number of 5-7 types of colony were noted in the research. Some microorganisms have a blessing of energy sources for their growth in the marine environment. Sources of nutrients derived from detergent compounds in a medium that can be utilized by heterotrophic bacteria for growth, where bacterial populations are able to utilize some compounds in the media as a source of carbon and energy for its growth (Chaturvedi and Kumar, 2011). Harijati (1994), reported that within 11 days certain bacteria can reduce the detergent level by 0.10%. It is expecpected the bacteria have used detergent composition in their foodstuff and the release of sulphonate group which then oxidized to sulfate. Heterotrophic bacteria themselves play a role in the waters as decomposers so that the growth of these bacteria in the water will affect the fertility of the waters in the marine environment, this is in accordance with Kurnia *et al*., (2016) stated that heterotrophic bacteria play an important role in aquatic systems due to the ability of metabolism activities.

Goodnow and Harrison (1972) studied for surfactant degradation among aerobic bacteria in peptone medium where such a degradation, if it occurs, will be gratuitous. Tallow-alkyl-sulfate (TAS), alkyl-ethoxylate-sulfate (AES), and linear-alkyl-benzene-sulfonate (LAS) were used. Fortyfive strains of 34 species in 19 genera degrade one or more of these detergent compounds. With some species, the surfactant inhibits degradation without inhibiting growth, whereas with one species slight degradation took place even at a toxic concentration of surfactant. Wide differences in ability to degrade surfactants may occur within a particular genus. For example, all three surfactants (TAS, AES and LAS) were degraded within 24 hr by *Acetobacter suboxydans*, whereas *A. peroxydans* had degraded no TAS in 72 hr and had degraded only 41 and 42%, respectively, of the available AES and LAS. Several species of *Azotobacter* were strong degraders, whereas *A. beijerinckii* was unable to withstand or appreciably degrade the three surfactants (Okpokwasili and Olisa (1991).

Schleheck *et al* (2000) found the bacterial community of the Proteobacteria class to dominate the bacterial community capable of degrading detergents from the marine water ecosystem. The ability of microbes, especially bacteria in using detergents as the main carbon source, indicates that bacteria play an important role in environmental bioremediation process of contaminated detergents waters (Kertesz et al., 1994). Several studies have shown that *Pseudomonas, Clostridium, Corynebacterium, Alcaligenes, Achromobacter, Bacillus* are able to degrade LAS. Amund *et al* (1997) isolated and identified SDS-degrading bacteria from different detergent contaminated ponds situated in Varanasi city, UP, India. Some selected isolates were identified on the basis of 16S rDNA sequencing. It was found that these isolates belonged to *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, *Pseudomonas stutzeri*, *Pseudomonas alcaligenes, Pseudomonas pseudoalcaligenes, Pseudomonas putida* and *Pseudomonas otitidis* respectively (Chaturvedi and A. Kumar, 2011). Among these isolates *P. aeruginosa, P. putida and P. otitidis* have been previously shown to degrade and metabolize SDS.

The detergent-utilizing bacteria identified were mainly Gram-negative and of the following genera: *Vibrio, Klebsiella, Flavobacterium, Pseudomonas, Escherichia, Enterobacter, Proteus, Shigella and Citrobacter. Vibrio* was the most frequently encountered organism while *Proteus* was the rarest. The cyclohexanol degrading bacteria were identified as species of *Pseudomonas. Acinetobacter, Vibrio, Micrococcus and Flavobacterium. Pseudomonas* sp. had the highest growth potential on cyclohexanol. These organisms play important roles in reducing the pollutant loads (London *et al*, 2016).

5.Conclusion
The results of this study indicated that detergents, namely RINSO, SURF and ATTACK have an impact on the growth of bacterial population in the sea water. The higher levels of detergent in the water column and the longer contamination duration, causing more and more depressed bacterial populations, both *in vitro* and *in situ* conditions. This study only studied the number of bacterial cells globally. It is suggested further research on the species of microbes involved and their ability to degrade detergent or surfactant.

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