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Ultimate Biodegradability potential of Trichloroethylene (TCE) used as Degreaser in marine, brackish and fresh water

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Abstract: *Ultimate biodegradability of Trichloroethylene used as degreaser (Rigwash and Aquabreak) was investigated in the laboratory using marine, brackish and fresh water systems. Total heterotrophic bacteria count decreases from day 1 to day 20 with the Rigwash contaminated marine sample having the highest count ($9.6x10^6$, $9.3x10^5$, $6.8x10^6$, $3.0x10^5$, $2.1x10^5$) followed by Aquabreak ($6.6x10^6$, $1.36x10^6$, $1.10x10^6$, $2.2x10^5$, $1.7x10^5$) and then control ($1.47x10^5$, $7.0x10^5$, $6.9x10^5$, $2.0x10^5$, $1.6x10^5$). The following dreaser degrading bacterial genera were isolated; Pseudomonas, Bacillus, Micrococcus and Enterobacter, with Pseudomonas occurring most with the frequency of 35.7%, followed by Bacillus with the frequency of 30.7%, Micrococcus 15.4% and Enterobacter 15.4%. The study also revealed that overall degreaser utilizing bacteria value was high with Rigwash having 5.94 ±0.52 log10 cfu/ml while Aquabreak is 2.77±0.17 log10 cfu/ml. Evaluation of the ultimate biodegradation shows Rigwash to be more biodegradable than Aquabreak. At day 28, the percentage (%) ultimate biodegradation were as follows; Rigwash- brackish water (96.6%) > Aquabreak – brackish water (96.2%) > Aquabreak – marine water (67.5%) > Rigwash – marine water (53.4%) > Rigwash – fresh water (48.3%) > Aquabreak – fresh water (34.6%). Summarily, the study revealed that brackish water degrades Trichloroethylene used as degreaser faster, followed by marine water; then least in fresh water. Concequently, discharge of degreaser contaminated effluent into fresh water environment should be discouraged as it poses great threat to human life.*
Keyword: *Trichloroethylene (TCE), Degreaser, Ultimate biodegradability*

I. Introduction

Degreasers are chemicals that are used to clean metal by washing dirt, grease and oil from auto engine parts. A degreaser can either be oil-based or water-based. Oil-based degreasers are usually toxic and flammable. Even small amounts entering surface or groundwater can result in serious pollution. Many oil-based degreasers readily evaporate and contribute to smog or ground level ozone. Water based cleaners are generally safer for the user and the environment. They are less toxic than oil based degreasers and small amounts can be broken down in sewage treatment facilities. (Department of Energy and Environmental Protection, 2013).

Trichloroethylene **(**TCE**)** Is one of the compounds that is mainly used a degreaser. Chlorinated aliphatic hydrocarbons (CAH_S) may enter into water bodies and contaminate water sources and affect human health in a direct or indirect manner. Many efforts have reported in removal of organic material from aqueous solutions (Mesdaghinia et al., 2007; Naghizadeh et al., 2008).

Trichloroethylene is one of (CAH_S) chlorinated aliphatic hydrocarbons that has been used as a degreaser (Watts, 1998). It is a volatile, colourless and nonflammable liquid and has different uses in many industries such as electronic and electrical, fabricated metal products and transport operations (ATSDR, 1995).

Because of improper handling and disposal practices, TCE has been frequently detected in groundwater. TCE is considered as a probable carcinogenic chemical (Group B_2) to human (Watenberg et al., 2000) it has also many other adverse effects on human and animals, (CEPA, 1993; Gist and Burg, 1995; Wartenberg et al., 2000). Due to its serious health effects, U.S Environmental protection Agency (U.S EPA) has set the maximal contaminant level (MCL) and maximum contaminant level goal (MCLG) for TCE as 0.005mg/L and zero respectively. Most conventional treatment processes such as coagulation, sedimentation, precipitate softening, filtration and chlorination are not efficient in removal of TCE (Russell et al., 1992).

Air stripping can induce secondary air pollution because of the phase shift of TCE. Although membrane processes and granular activated carbon are used most often, but they are expensive and transfer the contaminant to another phase (Nutt et al., 2006). TCE is used mainly for vapour degreasing of fabricated metal parts in the automotive and metal industries.

This study was designed to assess the effect of industrial degreasers on aquatic microorganism in marine, brackish and fresh water.

II. Materials And Methods

Collection of water samples and degreasers

Marine water samples were collected from Bonny sea, near Bonny oil terminal, Brackish water samples were collected from Trans-Amadi Industrial Layout River near Port Harcourt while fresh water samples were collected from Asarama Stream, Asarama, Andoni all in Rivers State, Nigeria. The water samples were collected with sterile plastic ten (10) litre containers The containers were rinsed three times with the respective water samples to be collected at the site before collection was made.

Trichloroethylene (Degreaser) used in the study were KLYNE 025A - Rigwash and Aquabreak PX from Wilhelmsen Chemicala AS, P.O.Box 15, N-3141 KjØpmanskjær NORWAY. Tel: +473335100, Fax: +4733351505; e-mail: chemicals@wilhelmsen.com

Biodegradation monitoring

Biodegradation monitoring was set up for each TCE type, 300ml of the marine water sample collected from Bonny sear (marine), Trans-Amadi River (brackish), Asarama Stream (Fresh) were dispensed into three 500ml Erlenmeyer flask. After that, 1% (3ml) of Teepol (T.pol) Liquid detergent was dispensed into the first flask and 1% (3ml) of Gamazyme (BTC) liquid detergent was dispensed into the second flask. The third flask was not contaminated with any detergent and was used as a control. The flasks were perforatedly pluged to allow for aeration, and were kept at ambient temperature (28 ± 2^0C) for 20 days.

Samples were taken at day 0, 5, 10, 15, and 20 from the Erlenmeyer flasks containing marine water contaminated with the test detergents. This was to determine the Hydrogen ion concentration (pH), Total Dissolved Solids (TDS), Total Heterotrophic Bacterial Counts, Total Fungal Counts, and Total Detergent Utilizing Bacteria. (Okpokwasili and Olisa, 1991).

Microbiological Analysis

Isolation and enumeration of total heterotrophic bacteria

Total heterotrophic bacteria for each biodegradation set up were enumerated by spread plate method. 0.1ml aliquot of the 10^{-1} to 10^{-4} was transferred unto well-dried nutrient agar plates and incubated at 370C for 24 to 48 h. after incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plates using the streak plate technique. Discrete colonies on the plates were aseptically transferred into agar slants, properly labeled and stored as stock cultures for preservation and identification (Odokuma and Ibor, 2002).

Isolation and enumeration of total fungal count

The total fungi population in the biodegradation set up (Habitat water sample and TCE) were enumerated and isolated by inoculating 0.1ml aliquot of the mixture unto well-dried potato dextrose agar containing antibiotics (Tetracycline, Penicillin and Ampicillin) to inhibit bacterial growth. Pure cultures of the fungi isolates were enumerated and transferred unto potato dextrose agar slants as stock cultures for preservation and identification (Odokuma and Okpokwasili, 1992).

Isolation and enumeration of Trichloroethylene (TCE) utilizing bacteria

Enumeration of Trichloroethylene (TCE) utilizing bacteria was performed by inoculating 0.1ml aliquot of the dilutions unto mineral salt agar plates containing the TCE (Odokuma and Okpokwasili, 1992). Colonies were counted after 48 to 72 h incubation at ambient temperature. The bacterial colonies on the plates after incubation were counted and sub-cultured onto fresh mineral salt agar plate.

Identification Of Bacterial And Fungal Isolates

The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology (1994). The morphological and biochemical test include; gram staining, motility, catalase, oxidase, citrate utilization, hydrogen sulphide production, indole production, methyl red and voges proskauer tests.

The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics as well as cultural characteristics were used in the identification of the fungal isolates of the biodegradation flask set up (Cheesbrough, 2006).

Physico-chemical analysis

The physico-chemical parameters analyzed were; pH using electrometric pH meter (Jenway 3015 method), dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined by modified winkler method (APHA, 1998), chemical oxygen demand (COD) was determined by permanganate oxidation method from the biodegradation set-up on days 0, 5, 10, 15 and 20.

Ultimate biodegradation monitoring using the percentage ratio of BOD to COD

The biochemical oxygen demand (BOD) of each biodegradation test set up was monitored (APHA, 1998) at 0, 5, 10, 15, 20 days. The chemical oxygen demand was determined at day 0. The ultimate biodegradability (Swisher, 1987) also referred to as the percentage of carbon in the material that is potentially mineralizable was calculated from the percentage of the ratio of BOD (for day 0, 5, 10, 15, 20) to COD at day 0. The percentage of mineralizable carbon in the test compounds that was actually mineralized was derived from this formula,

$$\frac{P}{I} \times 100$$

$$100 - M = N$$

P = percentage of potentially mineralizable carbon in the test compound
I = percentage of potentially mineralizable carbon in the test compound at day 0
N = percentage of potentially mineralizable carbon in test compound that was actually mineralized.

III. Results And Discussion

The value of some physico-chemical properties of the three aquatic environments used for the study were as follows; the general appearance of the water were clear, odour of fresh water is unobjectionable while Brackish and marine water have objectionable sensory evaluation. From fresh to marine water; colour decreases from 20.0 to 1.0 Hazen, pH increased/ranges from 7.67 to 8.21, Electrical conductivity (EC) ranges from 20 to 9200µS/cm. Turbidity ranges from <1.0 to 2.0 NTU with marine water having the lowest and brackish water the highest. Total hardness ranges from 30.70 to 3456.00 mg/L, Total alkalinity (22.0 – 142.95mg/L), Chloride (28.0 – 3488.0 mg/L). Total Dissolved Solids followed a similar pattern ranging from 10.0 to 7400.0 mg/L, Total Solids ranged from 18.5 to 7435.0 mg/L but Total Suspended Solids ranged from 8.5 to 44.5 mg/L with brackish water having the highest value, fresh water the lowest.

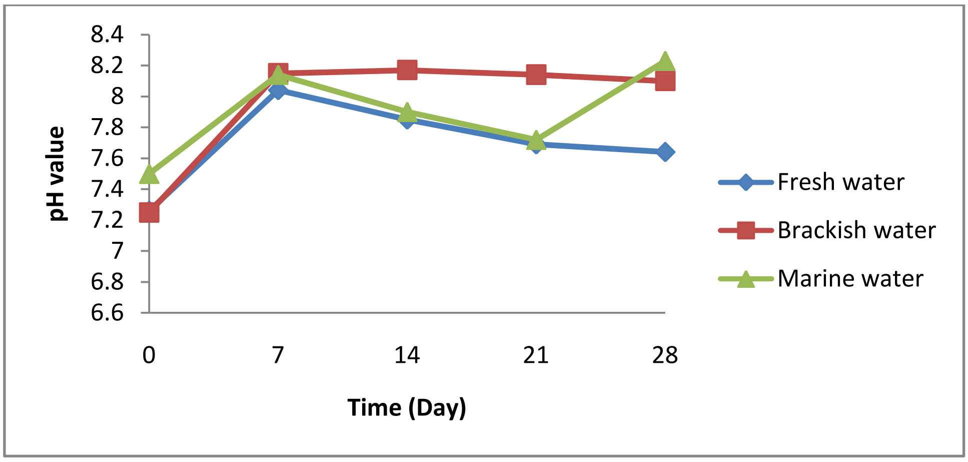

Fig.1: Hydrogen ion concentration (pH) of Rigwash in the tri-aquatic systems

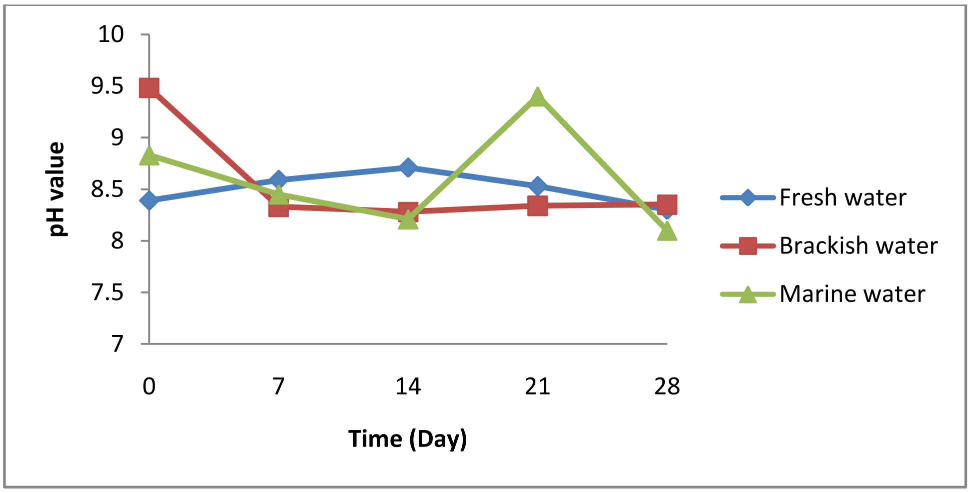

Fig.2: Hydrogen ion concentration (pH) of Aquabreak in the tri-aquatic systems

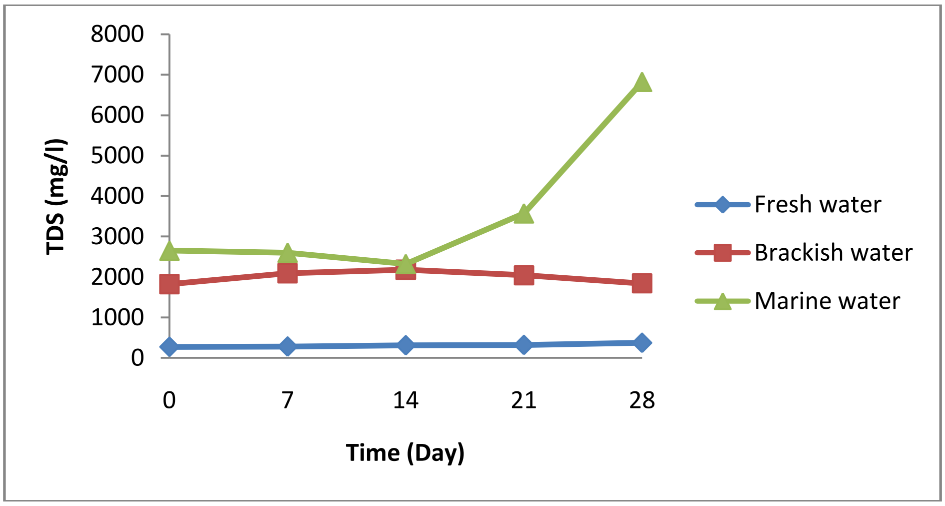

Fig.3: Total Dissolved Solids (TDS – mg/l) of Rigwash in the tri-aquatic systems

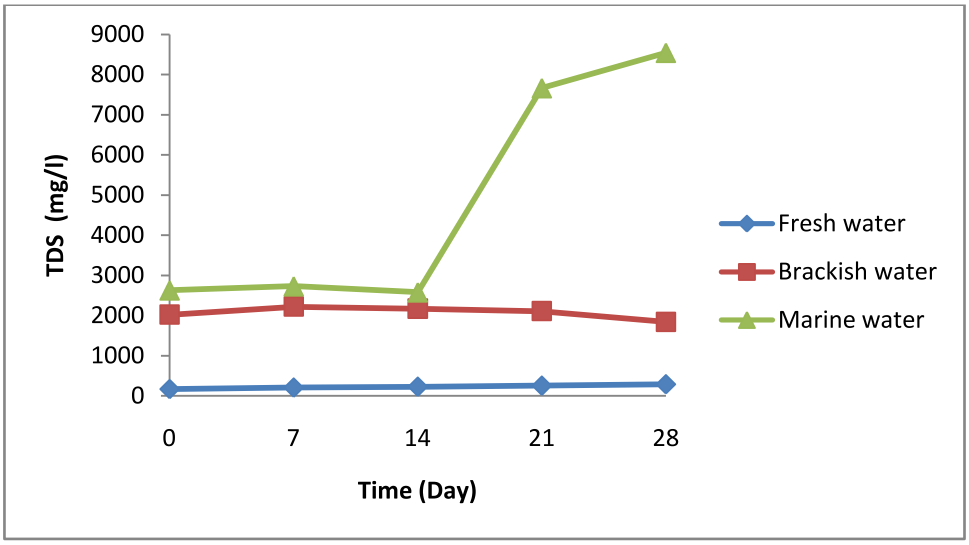

Fig.4: Total Dissolved Solids (TDS – mg/l) of Aquabreak in the tri-aquatic systems

The value of other parameters in fresh, brackish and marine water were as follows: Nitrate (2.30, 1.65 and 1.90 mg/L), Sulphate (9.80, 2.50 and 998.50 mg/L), Calcium (7.75, 384.00 and 1152.00 mg/L), Magnesium (1.40, 234.00 and 140.50 mg/L). BOD and COD did not reflect this pattern. The results showed that the differences in pH, organic carbon (biodegradable and chemically oxidizable) content of the three aquatic systems (fresh, brackish and marine) were not statistically significant at 95% probability levels. Some physic-chemical parameters (pH, TDS, DO, BOD and COD) monitored at day 0, 7, 14, 21 and 28 during the experiment were represented in fig. 1-10.

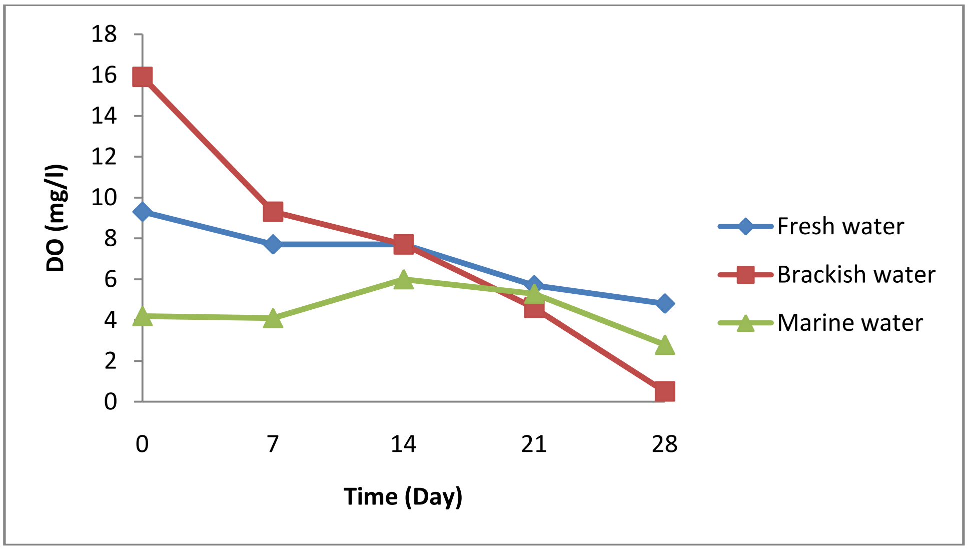

Fig.5: Dissolved Oxygen (DO – mg/l) of Rigwash in the tri-aquatic systems

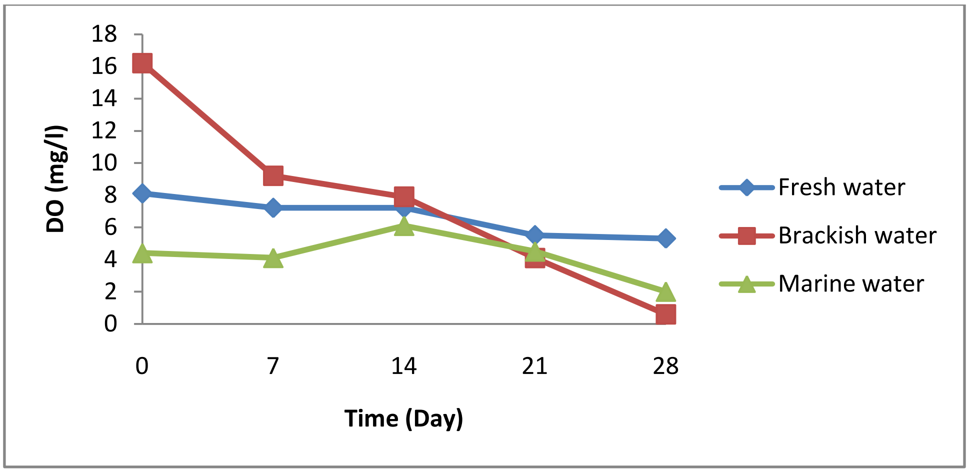

Fig.6: Dissolved Oxygen (DO – mg/l) of Aquabreak in the tri-aquatic systems

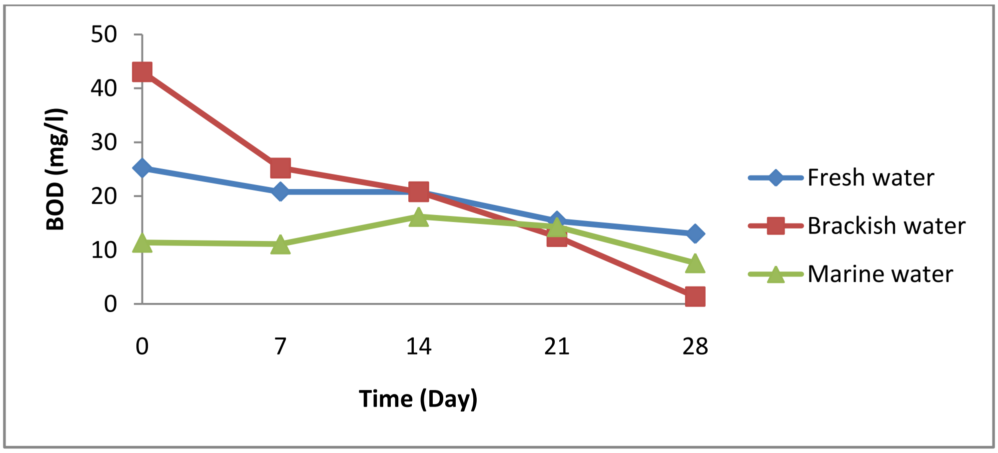

Fig.7: Biochemical Oxygen Demand (BOD – mg/l) of Rigwash in the tri-aquatic systems

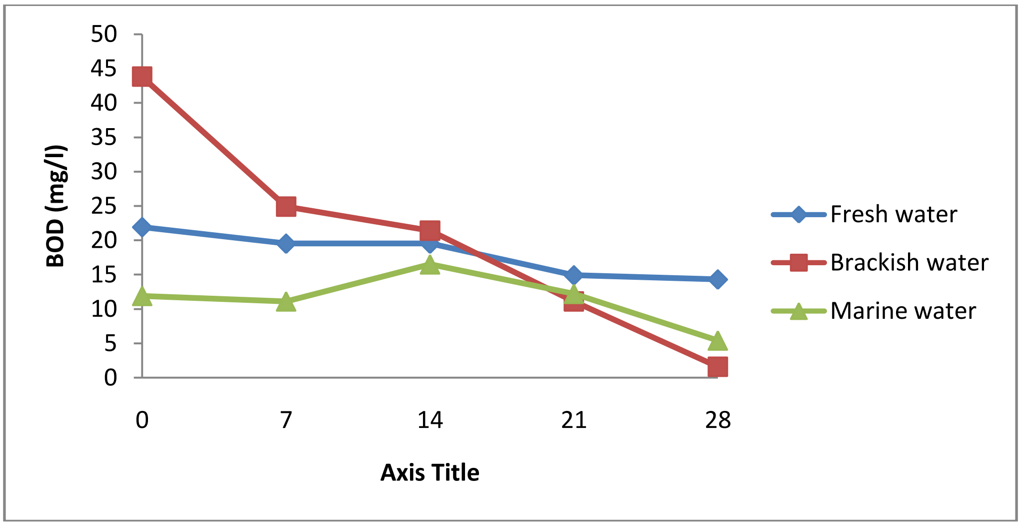

Fig.8: Biochemical Oxygen Demand (BOD – mg/l) of Aquabreak in the tri-aquatic systems

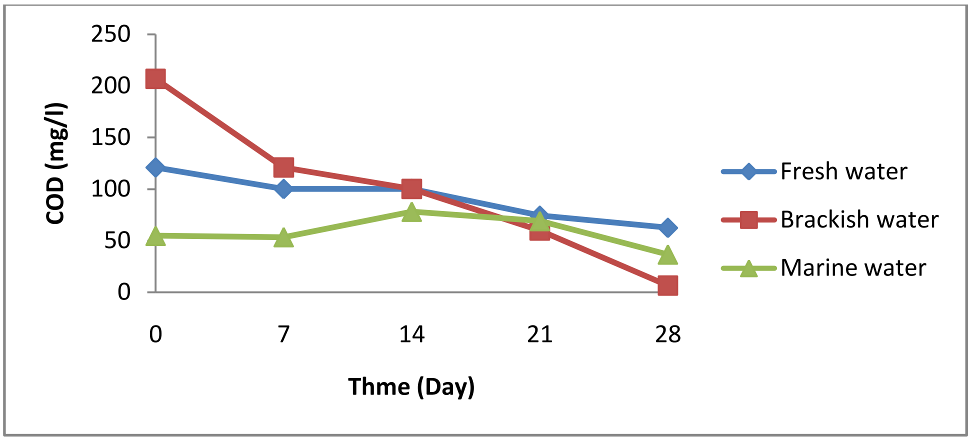

Fig.9: Chemical Oxygen Demand (COD – mg/l) of Rigwash in the tri-aquatic systems

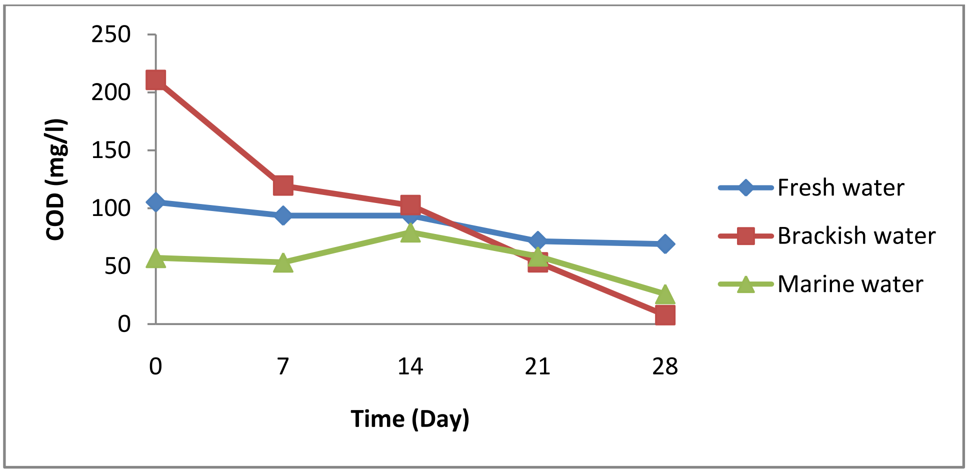

Fig.10: Chemical Oxygen Demand (COD –mg/l) of Aquabreak in the tri-aquatic systems

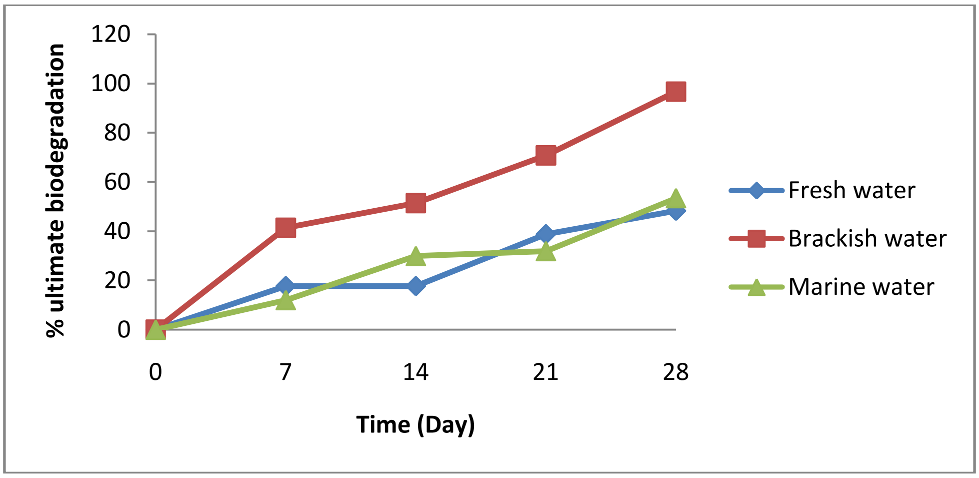

Fig. 11: Percentage (%) Utimate biodegradation of Rigwash in the tri-aquatic systems

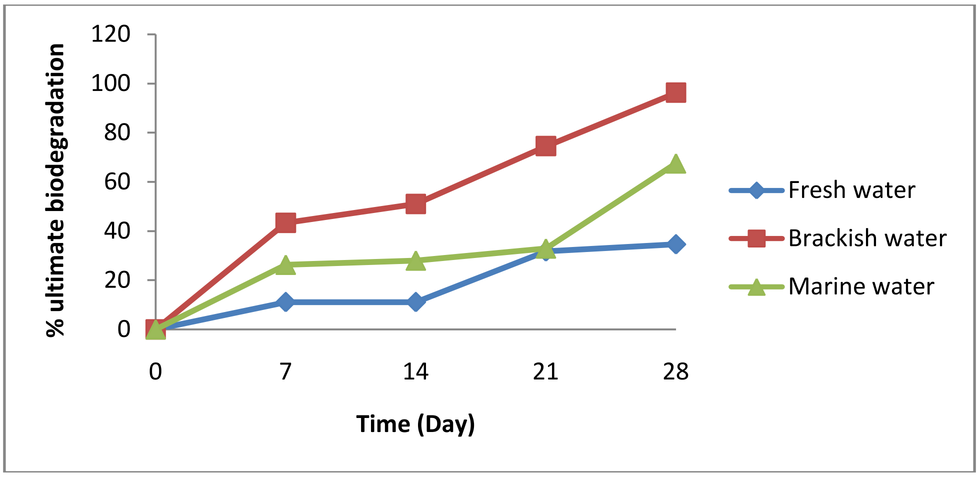

Fig. 12: Percentage (%) Utimate biodegradation of Rigwash in the tri-aquatic systems

Ultimate biodegradation of the trichloroethylene used as degreaser – KLYNE 025A –Rigwash and AQUABREAK PX in the three aquatic environment types is presented in fig. 11-12. The results showed that primary biodegradation of KLYNE 025A –Rigwash were greatest in marine water. Primary biodegradation of AQUABREAK PX occurred at the same extent in both fresh water and brackish water. This possible biodegradation of degreaser (Trichloroethylene)-Rigwash and Aquabreak agrees with the findings of Henson et al., 1988; that a mixed consortia of bacteria can effectively mineralize TCE. This involves co-metabolism of TCE (epoxidation) by bacteria that oxidize gaseous hydrocarbons such as methane, propane and butane, followed by hydrolysis of the TCE epoxide. The hydrolysis products are then utilized by other naturally occurring bacteria. Wackett et al. (1989) surveyed a number of propane oxidizing bacteria for their ability to degrade TCE. While TCE oxidation was not common among the bacteria surveyed, unique members could oxidize TCE. High concentrations (>15% v/v) of the gaseous hydrocarbons was found to inhibit co-metabolism of TCE. Oxygen concentrations also could be limiting in aqueous treatment systems since oxygen is required by the gaseous, alkane- utilizing and heterotrophic population.

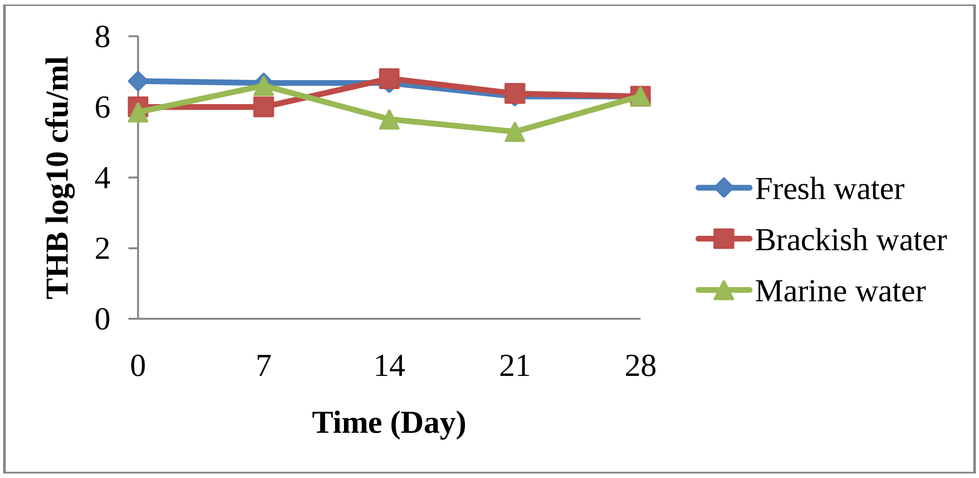

Fig. 13: Total Heterotrophic Bacteria (THB – log10 cfu/ml) of Rigwash in the tri-aquatic systems

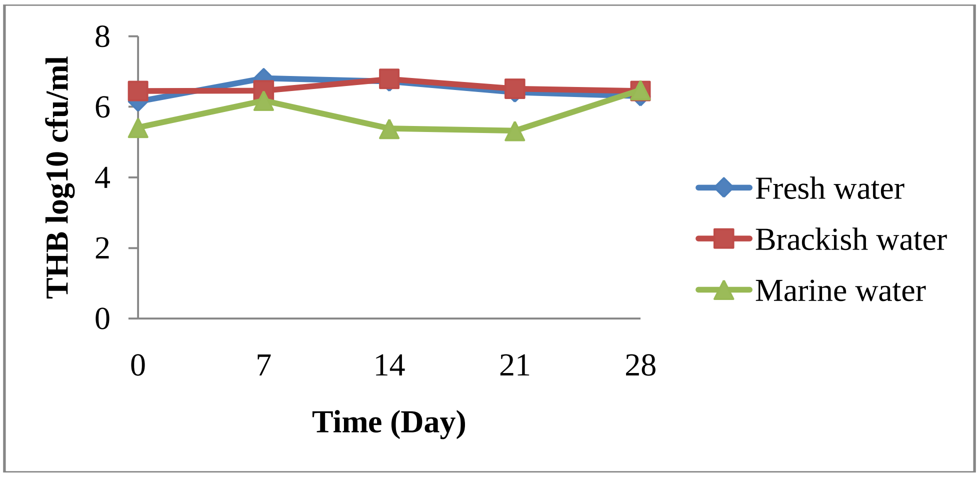

Fig.14: Total Heterotrophic Bacteria (THB - log10 cfu/ml) of Aquabreak in the tri-aquatic systems

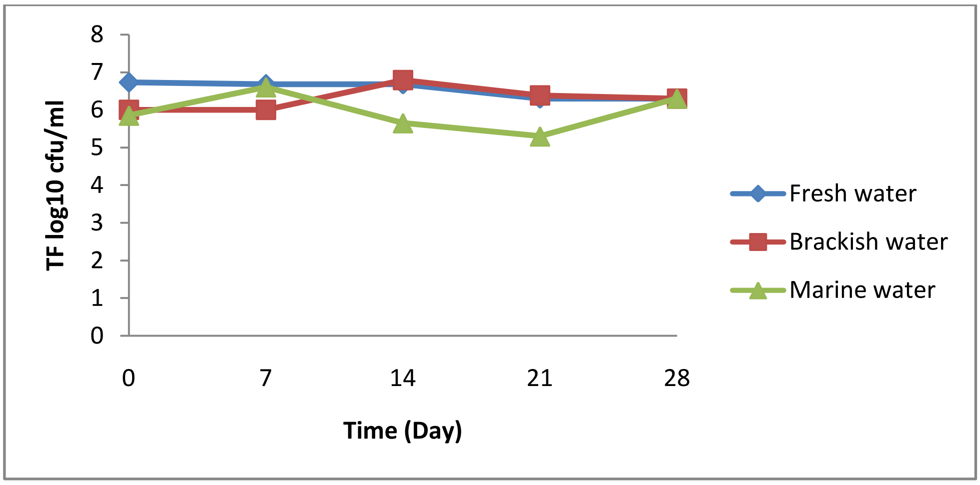

Fig.15: Total Fungi (TF - log10 cfu/ml) of Rigwash in the tri-aquatic systems

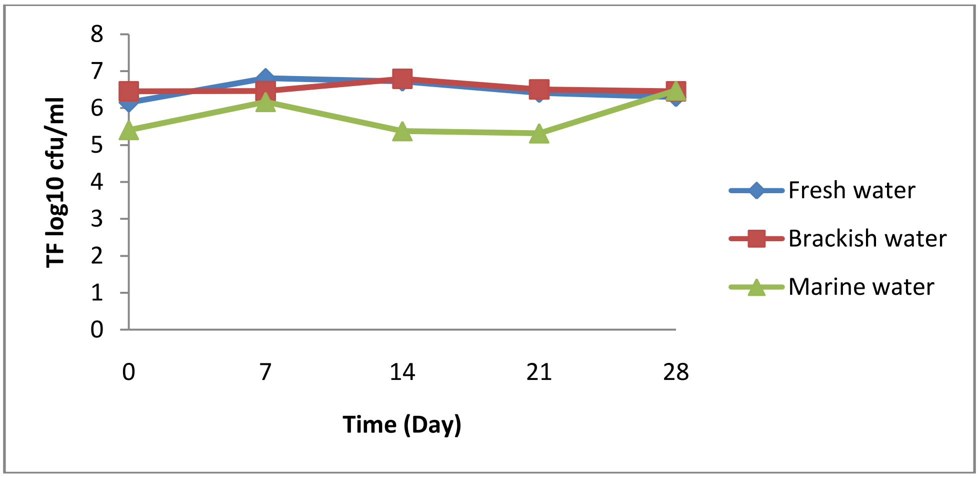

Fig. 16: Total Fungi (TF - log10 cfu/ml) of Aquabreak in the tri-aquatic systems

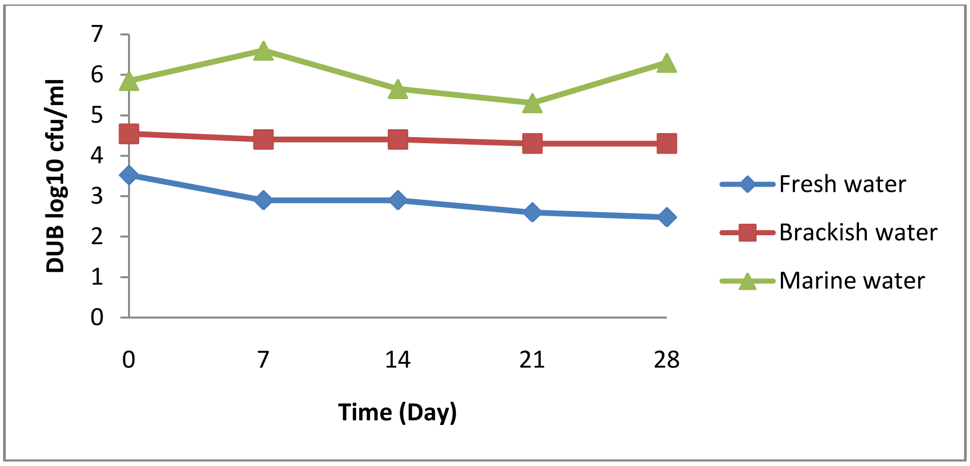

Fig.17: Degreaser Utilizing Bacteria (DUB - log10 cfu/ml) of Rigwash in the tri-aquatic systems

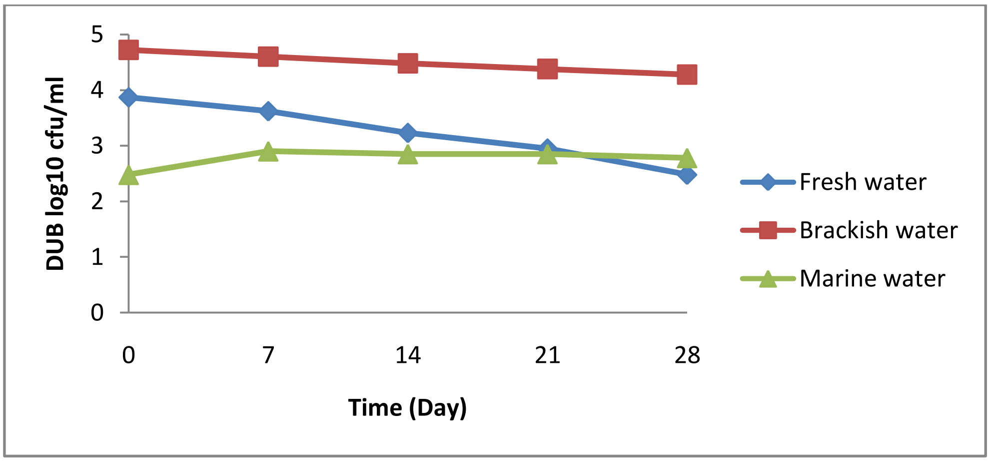

Fig.18: Degreaser Utilizing Bacteria (DUB - log10 cfu/ml) of Aquabreak in the tri-aquatic systems

In fig. 13-14 results suggested that the brackish water and marine water systems supported a higher total heterotrophic bacterial count (THB) than the fresh water system during the biodegradation tests., though the initial THB counts at day 0 for all water types were of the same magnitude (x10^6cfu/ml).

There was an initial slight rise in pH from day 0 to day 7, which was then followed by a slight decline from day 14 to day 28 in all the biodegradation test systems (Fig 1-2). This pattern was exhibited in all water types. Under these conditions, there is no buildup of vinyl chloride, and complete mineralization is possible. The enzyme methane monooxygenase (MMO) produced by methylotrophic bacteria growing in the presence of oxygen at the expense of methane has a wide range of growth substrates and pseudosubstrates; one of which is TCE. This enzyme epoxidates TCE. The resulting chemical complex is unstable and quickly hydrolyzes to various products dependent on the pH of the menstruum. TCE epoxide in phosphate buffer at pH 7.7 has a half-life of 12 seconds (Miller and Guengerich, 1982).

IV. Conclusion

It has long been thought that TCE is resistant to degradation under aerobic conditions due to its already oxidized state. Recently, a number of monooxygenases produced under aerobic conditions have been shown to degrade TCE (Harker and Young, 1990). This study in line with the above findings have prove beyond doubt that microbial degradation/mix microbial consortia is the primary alternative now for removal of TCE from contaminated aquatic systems even soil contaminated environment.Biodegradation, though unproven at this point, could destroy TCE completely. More research is required, however, before full-scale implementation of such technology.

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