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Degradation of Anionic Surfactants by *Bacillus subtilis* and *Bacillus cereus*

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***Abstract**: Surface active agents (Surfactants) are chemical compounds which are largely used as raw material in detergent production and their introduction into the environment in large concentrations causes harm to the aquatic bodies. Bacillus subtilis and Bacillus cereus were analysed for their capacity to degrade surfactants in laundry and dish washing detergents. Bacteria were isolated from soil at the outlet of these detergents and identified by biochemical tests. Methylene Blue Photometric Assay and Methylene Blue Active Substance Test were used to determine the amount of degradation by the bacteria. Bacillus subtilis showed better degradation for both dish and cloth washing detergent. Degradation was highest during the first 24 hours of incubation. Increase in surfactant concentration after 24 hours is attributed to the production of biosurfactant by both bacteria.*

***Keywords**: Bacillus cereus, Bacillus subtilis, Biodegradation, Methylene Blue Active Substance, Surfactant*

I. Introduction

Detergents are substances used for the purpose of cleaning laundry or dishes. The main component in detergents is a surfactant or soap which aids in the cleaning process. [1]. They are most widely used in different forms, both solid and liquid, in industries, households, laundries and cosmetics. Surfactants are organic substances which enhance the cleaning, rinsing and/or fabric softening process due to their surface-active properties and are discharged into the environment by the wastewater pathway, either after treatment in a wastewater treatment plant or directly where no treatment system is available. [2][3]. They can thus act on biological wastewater treatment processes and hinder aeration and treatment facilities due to their high foaming, low oxygenation capacity[4]. Bioaccumulation factors of surfactants were found in aquatic fish to be around 300. Laboratory and field studies into the bioaccumulation of alkylphenol ethoxylate surfactants indicate a moderate tendency to bioaccumulate although metabolism and depuration rates are often rapid [5].

Large concentrations of surfactants cause skin irritation [6]. The threshold value that can impair aquatic life is 3-12 mg/l [7]. Bacterial detergent-degraders such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus majodoratus*, *Klebsiella liquefasciens*, *Enterobacter liquefasciens*, *Klebsiella aerogenes*, *Enterobacter agglomerans*, *Staphylococcus albus*, *Proteus* sp., *Klebsiella oxytoca* and *Brevibacterium* sp., were isolated and tested them for degradation by Methylene Blue Active Substance (MBAS) Assay and were found to be positive for degradation [8]. Biodegradation can be performed by soil or aquatic microorganisms leading to generation of water and carbon dioxide gas [9]. Rate of biodegradation is dependent on temperature and oxygen such that aerobic conditions and a high temperature are beneficial for the process [10].The objective of the current study was to isolate and characterize surfactant degrading bacteria from detergent contaminated soil and to determine the extent of biodegradation.

II. Materials

2.1. Source of bacterial sample and collection: Soil from the outlet of dish washing water and laundry washing water from a residential area in Udupi, Karnataka, India was collected in sterile containers, stored at 4˚C till processing and serially diluted. This served as the source of detergent-degrading bacteria. The bacteria were isolated on nutrient agar and screened for the degradative capacity on trypticase soy broth supplemented with detergent.

2.2. Detergents Used:

Laundry Washing Detergent – Tide, Ariel and Surf.

Dish Washing Detergent – Dish Drops, Laboline and Vim.

2.3. Media Used:

Trypticase Soy Broth (TSB) - 4.5g Tryptic Animal Peptone, 1.5g Phytone and 1.5g of Sodium Chloride.

Trypticase Soy Agar (TSA) - 4.5g Tryptic Animal Peptone, 1.5g Phytone, 1.5g of Sodium Chloride. 0.3% Glucose and 1.6% Agar

Mineral Media - 0.0125g of $FeCl_3.6H_2O$, 1.375g anhydrous $CaCl_2$: 1.125g $MgSO_4.7H_2O$, 0.425g KH_2PO_4, 1.087g K_2HPO_4, 0.885g NaH_2PO_4, 0.085g NH_4Cl.

III. Methods

3.1. Methylene Blue Photometric Assay For Degradation of Surfactant [11]

Cultures were first maintained on TSA medium. Bacteria from TSA are inoculated into TSB and incubated on rotary shaker at 31˚C until bacterial count reached 10^9-10^{10}cells/ml and determined by measuring the absorbance at 540nm. This was used as inoculum for 3 conical flasks of TSB to test for surfactant degradation. The first flask was used as a blank and did not contain surfactant but contained 1ml inoculum and 99ml TSB. The second flask contained 99ml TSB containing 1% surfactant and 1 ml of inoculum and served as the experimental flask. The third flask contained 100ml TSB with 1% surfactant and did not contain inoculum, thus serving as the standard for the test. The flasks were incubated at 31˚c in the shaker for 24, 48 and 72 hours. 3 ml aliquots from each flask were taken at intervals of 24hrs, 3ml of chloroform and 3ml of methylene blue were added and the flasks were shaken for 30minutes. Samples were assayed at the end of 24, 48 and 72 hours for surfactant degradation (SD) using Methylene Blue Photometric Assay at 652nm. Methylene blue is a cationic dye. Anionic detergents bind methylene blue and dye partitions in chloroform with surfactant; nonbinding of the dye to the surfactant indicates degradation. Degraded surfactant fails to bind methylene blue so the dye remains in the aqueous phase. Quantity of dye in the chloroform layer indicates the amount of surfactant degradation [12]. Percentage degradation was then calculated using the formula (1)

$$\text{Percentage deg radation} = 100 - \left[\frac{A_{652}\,\text{exp} - A_{652}\,\text{blank}}{A_{652}\,\text{std}} \right] \times 100 \text{ ------- (1)}$$

3.2 Methylene Blue Active Substance [13]

50ml of autoclaved mineral media was taken in 12 sterile conical flasks. 2% detergent was added along with a loop full of inoculum and incubated in rotary shaker at 31˚C for 60rpm. At the end of 24 hours, 4ml of this sample, 4ml of chloroform and 4ml methylene blue was mixed well and allowed to settle. The absorbance was measured at 625nm for up to 10 days. Absorbance obtained is a direct indication of the amount of residual surfactant present in the solution.

IV. Tables And Figures

Table 1: Micromorphology and Biochemical Characterization of Bacterial Detergent–Degraders [14]

Test	Result	Result
Gram Stain	+	+
Gelatin Liquefaction	+	+
Starch Hydrolysis	+	+
Lactose Fermentation	-	-
Sucrose Fermentation	+	+
Nitrate Reduction	+	+
Indole Production	-	-
Methyl Red Test	-	-
Voges Proskauer Test	+	-
Citrate Utilization Test	-	-
Urease Test	-	+
Catalase Test	-	+
Oxidase Test	+	-
Organism	*Bacillus cereus*	*Bacillus subtilis*

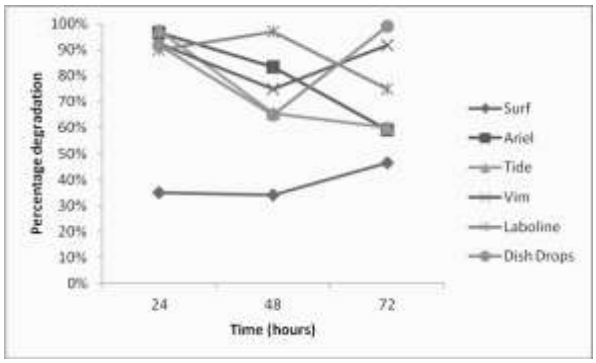

Figure 1: Methylene Blue Photometric Assay Showing Percentage Degradation by *Bacillus cereus*

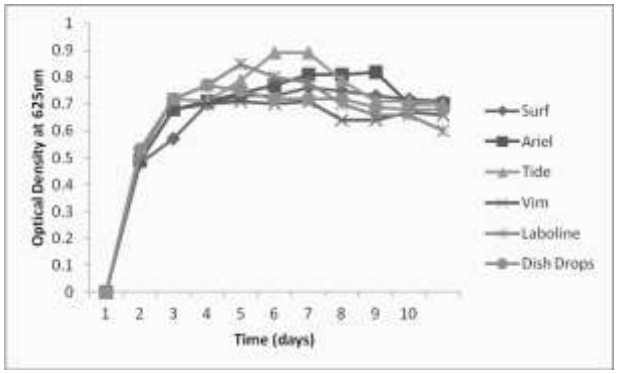

Figure 2: Methylene Blue Photometric Assay Showing Degradation of Surfactant by *Bacillus subtilis*

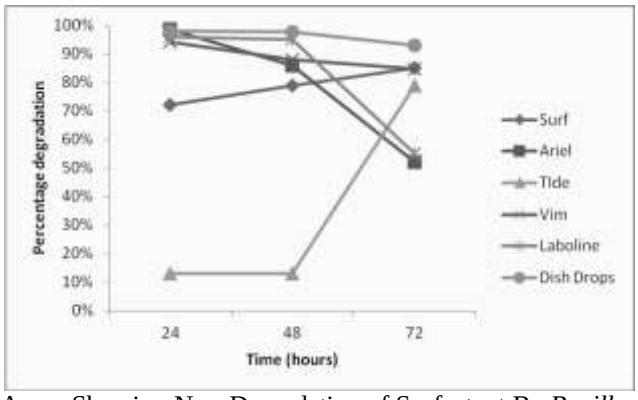

Figure 3: MBAS Assay Showing Non-Degradation of Surfactant By *Bacillus cereus*

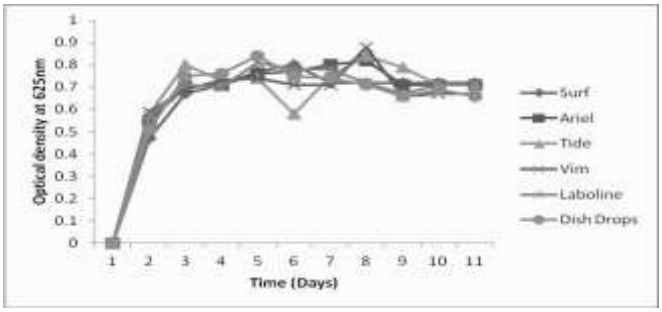

Figure 4: MBAS Assay Showing Non-Degradation OF Surfactant by *Bacillus subtilis*

IV. Results And Discussion

Both *Bacillus subtilis* and *Bacillus cereus* show maximum degradation within 24 hours of incubation, this indicates that the bacteria are capable of degrading the surfactant during the early stages of the growth (Fig 1 and 2). *Bacillus subtilis* was found to be a better degrader as compared to *Bacillus cereus. Bacillus subtilis* showed best degradation for Tide (97.6%) and Vim (94.3%), while *Bacillus cereus* showed best results for Ariel

(96.6%) and Dish Drops (99%). Among all the detergents used in the study, Dish Drops is the most biodegradable

MBAS assay shows the increase in the amount of residual surfactant which correlates with the Methylene Blue Photometric Assay. The amount of surfactant increases after 24 hours in the medium hypothesized to be because of the production of biosurfactants by the bacteria as secondary metabolites as indicated in earlier studies [15]. *Bacillus subtilis* and *Bacillus cereus* were isolated from water and soil respectively and were found to possess biosurfactant producing capabilities thus supporting the present study. [16]

V. Conclusion

Degradation of surfactant detergents was successfully carried out and *Bacillus sps* were found to be efficient degraders. Although biosurfactants produced by the bacteria are secondary metabolites they seemingly provide nutrient for the growth of the organisms thus enhancing growth of the organism and thereby degradation. *Bacillus sps* are soil inhabitants and therefore can carry out the process of degradation in nature.

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