



Biodegradation of Cetyltrimethylammonium Bromide and Methylparaben in Shampoo and Hair Dressing Salon Waste Using Bacteria Isolated from Sewage Treatment Sludge

P. U. Onuche^{1*}, F. G. Okibe¹ and V. O. Ajibola¹

¹Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors VOA and FGO designed and supervised the research work. Author PUO performed the experimental, analyzed the data and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present study investigated the biodegradation of Cetyltrimethylammonium bromide (CTAB) and Methyl paraben (MP) both in Shampoo and hair dressing salon waste using bacteria isolated from Sewage treatment sludge. The biodegradation was carried out according to the OECD Guideline for ready biodegradability and was monitored by Standard Spectrophotometric methods. The results obtained indicated that these compounds were degraded biotically by simple bacteria identified using a Microgen Kit for bacteria characterization.: *Bacillus cereus* and *Pseudomonas fluorescens* degraded 98.3% of the initial CTAB level in the Standard CTAB solution on 13 days of incubation; *Pseudomonas fluorescens* and *Actinobacillus hominis* degraded 96.7% of the initial CTAB level on 10 days of incubation and 92.0% of the original MP on 13 days of incubation in the Shampoo solution; *Pseudomonas aeurogonisa* and *Klebsiella planticola* degraded 95.3% of the initial CTAB level on 5 days of incubation and 94.7% of the original MP level on 4 days of incubation in the washing solution; *Pseudomonas aeuroginosa* and *Salmonella typhi* were found present in the Inoculum control. In conclusion, the results of this study suggested that the bacteria obtained from

*Corresponding author: E-mail: onucheugbedeajo@yahoo.com;

the sewage sludge can be used as a cost effective and environmentally friendly agent for the biodegradation of surfactants in sewage treatment processes.

Keywords: Cetyltrimethylammonium bromide; methyl paraben; spectrophotometric; biodegradation; shampoo; hair dressing salon.

1. INTRODUCTION

Surfactants are amphipathic compounds consisting of both a hydrophobic region (alkyl chains of various length, e.g. alkyl phenyl ethers, alkyl benzenes, etc.) and a hydrophilic region (e.g. carboxyl, sulphate, sulphonates, phosphates etc.) [1]. Surfactants have also been defined as compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. They may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants [2]. They have long been known to reduce surface tension in water and allow aqueous solutions to spread and penetrate more easily [3,4]. Many different types of these compounds have been synthesized, although they can be classified into three main groups according to their charge: Anionic, Non-ionic, and Cationic; the first and second groups accounting for the highest production volumes. Cetyltrimethylammonium bromide (CTAB) also known as Cetrimonium bromide or hexadecyltrimethylammonium bromide with the chemical formula $(C_{16}H_{33})N(CH_3)_3Br$ (Fig. 1) is an amine based cationic quaternary surfactant. It is one of the components of the topical antiseptic cetrimide [5]. It is also widely used in Hair conditioning products.

Methylparaben (MP) is an anti-fungal agent often used as a preservative in a variety of cosmetics and personal-care products such as Shampoo. The chemical formula of Methylparaben (Fig. 2) is $CH_3(C_6H_4(OH)COO)$. Any environmental compartment (surface waters, sediment,) is susceptible to be contaminated by Surfactants and/or their degradation metabolites [8,9].

Quaternary Ammonium Compounds have been measured at levels ranging from $<2 \mu g/l$ in surface water [10] to $>100 mg/kg$ in sediments [11,12]. These surfactants have been reported to have adverse effects on the biotic and abiotic components of the environment. Their harmful effects on the environment have been well characterized, and include remobilization of organic pollutants and inhibition of enzyme activity such as microbial dehydrogenase and algae nitrogenase [13]. Eichhorn et al. [14] reported that Surfactants and their derivatives released into aquatic and / or terrestrial environments act on biological waste water treatment processes and cause problems in sewage aeration and treatment facilities due to their high foaming, lower oxygenation potentials and causing death of waterborne organism. Ndu also reported that the surface tension reduction property of Surfactants affects aquatic life adversely; for example, altering the properties of a fish's gill. Such alteration consequently changes the fish's normal uptake of ions from the water [15]. Hrenovic and Ivankovic investigated the potential toxicity of two surfactants; sodium dodecyl sulfate (SDS) and hexadecyltrimethylammonium bromide (HDTMA) in a pure culture of *Acinetobacter junii*, a phosphate (P)-accumulating bacterium. Their results showed a high acute toxicity of these surfactants against the bacterium. The negative effects of these toxicants could greatly decrease populations of P-accumulating bacteria, as well as eukaryotic organisms, inhabiting activated sludge systems, which in turn could result in the decrease of the system efficiency [16]. Chude and Ekpo found out that Hair dressing salon effluents which also contain surfactants

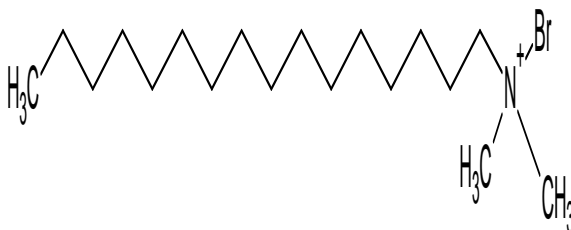


Fig. 1. Chemical structure of CTAB [6]

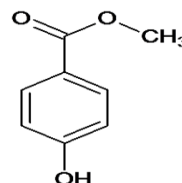


Fig. 2. Chemical structure of MP [7]

adversely affected fingerling [17]. Because of their widespread use, surfactants are released in abundance into the environment, particularly in wastewaters. As a result, surfactants represent potential toxicants to organisms inhabiting activated sludge systems [16]. As a consequent of these problems among others, the removal of surfactants from the environment has been of concern to Environmentalists all over the World and not just the removal but in an environmentally friendly way such as biodegradation. Biodegradation has been defined as the chemical dissolution of materials by bacteria, fungi, or other biological means [18]. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process [19]. Surfactants can be biodegraded exclusively by bacteria [20,21] which use organic compounds as their source of carbon and are referred to as heterotrophic bacteria [22]. This Carbon utilizing bacteria can be isolated from activated sludge culture [23-25] preferably from the same environment because of environmental factors and adaptation. For instance Hosseini et al. [26] isolated bacteria from activated sludge from Tehran municipal activated sludge there in Iran and others like [27-30] and so on.

Several studies have been reported on the biodegradation of various surfactants [26-30] and so on. So far, there is no report on the biodegradation of surfactants in shampoo and Hair Salon waste water using bacteria from Sewage treatment sludge. Hair dressing Salon waste either solid or liquid have become a very common pollution of the environment especially in sub-Saharan Africa as a result of the modernization and development of Africans responsible for the unending desires for European body care fashion in the name of beautification which has led to the proliferation of Hair Salon. Hence the need to find a cost effective and environmentally friendly means of eliminating these surfactants which was the focus of this work.

2. MATERIALS AND METHODS

2.1 Sample Collection

2.1.1 Collection of shampoo and washings

20 ml of Shampoo and its Hair washing were collected from Hair Salon into sterile plastic containers and transported to the laboratory for analysis.

2.1.2 Collection and pretreatment of inoculum from activated sludge

Fresh samples of Activated sludge were collected from the Aeration tank of the Sewage Treatment Unit. Coarse particles were removed by filtration through a fine sieve and the sludge kept aerobic thereafter [31].

2.2 Experimental Procedure

2.2.1 Determination of cetyltrimethylammonium bromide

5 mL of CHCl_3 , 1 mL of CTAB and 1 mL of 1×10^{-3} M bromophenol blue were transferred into a 15 mL glass stoppered and stirred in a magnetic stirrer for 30 minutes. It was then transferred into a separatory funnel and allowed to stand for 10 minutes and the organic phase was extracted and the absorbance read with a spectrophotometer at 603 nm against the blank solution of chloroform [32].

2.2.2 Determination of methylparaben

0.5 ml of Methyl paraben standard solution 100 $\mu\text{g}\cdot\text{ml}^{-1}$ and 0.5 ml of 1 M sodium hydroxide solutions were added to 0.5 ml of ortho-aminobenzoic acid and 0.5 ml of 1% sodium nitrite and 0.5 ml of 1 M HCl were mixed together and made up to mark with distilled water into a 10 ml volumetric flask and stirred. The resulting solution was then cooled in ice bath for 2 minutes until an orange color developed and the absorbance measurement was carried out at a wavelength at 442 nm, against a blank solution prepared in the same method but without Methyl paraben [7].

2.2.3 Preparation of mineral medium

10 ml of solution **A** was mixed with 800 ml water, then 1 ml of solutions **B**, **C** and **D** were added and made up to 1 litre with distilled water. Preparation of A, B, C and D is stated [31] also.

2.2.4 Preparation of biodegradation assays

Appropriate volume in each case of the Stock Surfactant solution was added to 800 ml portions of mineral medium in 2-litre conical flasks and made up to 1 L and then mix. After mixing, a sample from each flask was taken to determine the initial concentration of the test substance which should give the intended initial concentration and the pH checked and adjusted

to 7.0. Then the flask was inoculated with 0.5 ml of water from a Secondary treatment of the Sewage Treatment Plant (STP) of Ahmadu Bello University, Zaria that operates with active sludge. The openings of the Erlenmeyer flask flasks was covered with aluminum foil, in such a way as to allow free exchange of air between the flask and the surrounding atmosphere. To start the test, the vessels were inserted into the shaking machine and then left in darkness in a thermostatically controlled chamber at 25°C. The constant rocking of the Shaker (125 sweep/min) provided the necessary aeration [31].

2.2.5 Preparation of control

2.2.5.1 Preparation of shampoo and washing control

To check whether the test substance is degraded abiotically, a flask was setup containing a sterilized uninoculated solution of the test Substances. Two Flasks one containing Shampoo and the other Washing were sterilized using Dettol antiseptic liquid and without inoculation and left undisturbed from the same time the biodegradation of the samples until its completion.

2.2.5.2 Preparation of inoculum control

0.5ml of the Sludge (inoculum) was transferred into a flask containing the mineral medium as was done for the Samples but without test or reference substance.

2.2.6 Culturing of bacteria

With a sterile pipette, 1 ml of sample was taken from the setup in 9 ml of distilled water from which series of dilutions are made until reaching a dilution of microorganisms of between 30 and 380 viable cells per ml of test solution [31]. Then 1 ml of each dilution was taken and added to the appropriate amount nutrient in a culturing plate and kept in an incubator incubated aerobically at 37°C for 18 – 24 hours.

2.2.7 Isolation and sub-culturing of the bacteria

All inoculated plates were incubated aerobically at 37°C for 18 – 24 hours. The cultures were examined for bacterial colonies showing typical characteristic of the target organism being selected by the various media. Such typical colonies were picked and streaked on nutrient agar (NA) slants and incubated aerobically at

37°C for 24 hours to obtain pure isolates of the target pathogens. The pure isolates were stored in the refrigerator at 5°C for characterization [33].

2.2.8 Characterization and identification of the bacteria

The bacteria were identified using biochemical test alone [25] initial identification Schemes were performed by the Mango Park Method using the Conventional Biochemical tests [33] as used by Hosseini et al following the suggestion of the Bergeys Manual of Systematic Bacteriology. The final identification was carried out using the Microgen Kit.

3. RESULTS AND DISCUSSION

3.1 Biodegradation of CTAB in Standard, Shampoo and Washing Solutions

Figs. 3.1, 3.2 and 3.3 presented the biodegradation profile of CTAB in Standard, Shampoo and washing Solutions respectively. The plots gave sigmoidal curves which showed that the % degradation of the surfactant increased as the incubation period increased which pointed out that the bacteria utilized the CTAB in the Solutions. The profiles also showed that 98.3%, 96.7% and 95.3% degradation of CTAB in the Standard, Shampoo and Washing solutions was achieved on an incubation period of thirteen, ten and five days respectively. These results agreed with that of the degradation of benzalkonium chloride (BAC) carried out by [30].

3.2 Comparison of the Degradation of CTAB in the Standard, Shampoo and Washing Solutions

Presented in Table 3.3 is the Comparism of the degradation of CTAB and MP in the Standard, Shampoo and Washing Solution. The result showed that the CTAB and MP in the Standard degraded more than that for the Shampoo and Washing at a degradation rate of approximately 1.7 µg per mL per day and 0.667 µg per mL per day respectively. This is due to the presence of CTAB and MP as the only carbon source in the Standard CTAB solutions whereas the Shampoo and Washing Solutions contained other source of carbon. The Shampoo with a degradation rate of approximately 1.48 µg per mL per day and 0.647 µg per mL per day respectively degraded more than the Washing with a degradation rate of approximately 0.71 µg per mL per day and

0.457 μg per mL per day respectively. This is probably as a result of the presence of some amount of other carbon containing substances present in the Washing as dirt from the hair, hair cream and so on available to the inoculated bacteria.

3.3 Biodegradation of the CTAB in Control

Presented in Table 3.1 is the result for the biodegradation of the Control containing Cetyltrimethylammonium bromide and the

Inoculum control. It was observed that the concentration of CTAB in the Washing ($2.20 \mu\text{g mL}^{-1}$), Shampoo ($9.00 \mu\text{g mL}^{-1}$) and Standard ($5.00 \mu\text{g mL}^{-1}$) Solutions remained unchanged as no degradation was observed when kept for thirteen days after which the concentration of the analyte was made. This result showed that no Abiotic degradation of the analyte occurred as stated by [31]. No determination was made within the thirteenth day as represented by ND and the Inoculum control was does not contain the analyte and so no determination was made as represented by NIL.

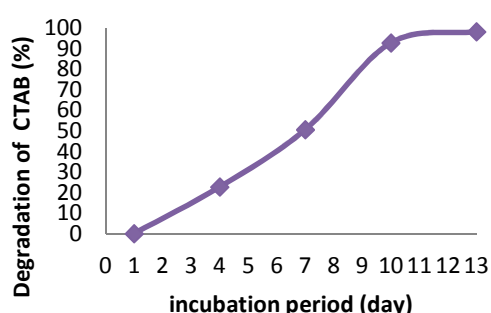


Fig. 3.1. Biodegradation profile of standard CTAB solution

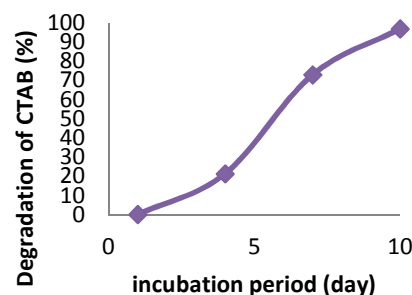


Fig. 3.2. Biodegradation profile of shampoo solution

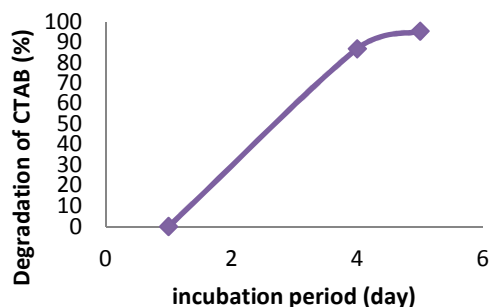


Fig. 3.3. Biodegradation profile of washing solution

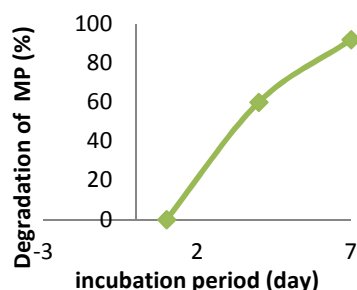


Fig. 3.4. Biodegradation profile of standard MP solution

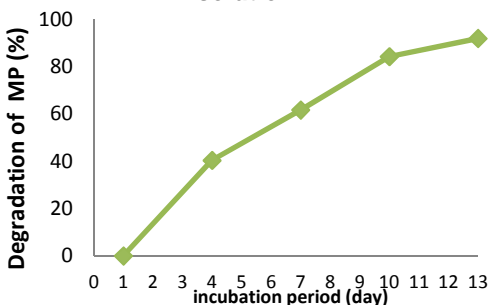


Fig. 3.5. Biodegradation profile of MP in shampoo solution

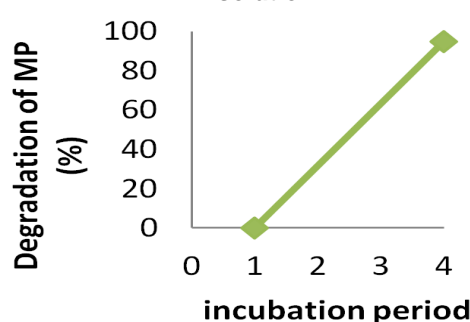


Fig. 3.6. Biodegradation profile of MP in washing solution

Table 3.1. Result of biodegradation of CTAB in control

Incubation period (days)	Washing (control) CTAB level (μgml^{-1})	Shampoo (control) CTAB level (μgml^{-1})	Standard (control) CTAB level (μgml^{-1})	Inoculum (control)
1	3.75	15.31	22.60	NIL
4	ND	ND	ND	NIL
7	3.75	ND	ND	NIL
10	ND	15.31	ND	NIL
13	ND	ND	22.60	NIL

ND= Concentrations of analyte not determined, NIL = no analyte was added

Table 3.2. Result of biodegradation of MP in control

Incubation period (days)	Washing (control) MP level (μgml^{-1})	Shampoo (control) MP level (μgml^{-1})	Standard (control) MP level (μgml^{-1})	Inoculum (control)
1	1.9	9.0	5.0	NIL
4	1.9	ND	ND	NIL
7	ND	ND	5.0	NIL
10	ND	ND	ND	NIL
13	ND	9.0	ND	NIL

ND= Concentrations of analyte not determined, NIL = no analyte was added

Table 3.3. Comparison of the degradation of CTAB and MP in the standard, shampoo and washing solutions

Compound	Degradation rate in standard solution ($\mu\text{g per mL per day}$)	Degradation rate in shampoo solution ($\mu\text{g per mL per day}$)	Degradation rate in washing solution ($\mu\text{g per mL per day}$)
CTAB	1.70	1.48	0.71
MP	0.66	0.64	0.45

Table 3.4. Biochemical test result for the bacteria isolates

Isolate number	TSI	Citrate	Urease	Motility	MR	VP	Indole	Inference bacteria
11	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
12	K/A	+	-	+	-	+	-	<i>Bacillus spp</i>
13	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
14	A/A	+	+	-	-	+	-	<i>Actinobacillus</i>
15	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
16	A/A,G	+	+	-	-	+	-	<i>Klebsiella</i>
17	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
18	K/A	-	-	+	+	-	-	<i>Salmonella typhi</i>

Table 3.5. Results of identification by microgen GNA and Bacillus test kits

11	<i>Pseudomonas fluorescens</i>
12	<i>Bacillus cereus</i>
13	<i>Pseudomonas fluorescens</i>
14	<i>Actinobacillus hominis</i>
15	<i>Pseudomonas aeruginosa</i>
16	<i>Klebsiella planticola</i>
17	<i>Pseudomonas aeruginosa</i>
18	<i>Salmonella typhi</i>

3.4 Biodegradation of Methyl Paraben

Figs. 3.4, 3.5 and 3.6 presented the biodegradation profile of MP in the Standard, Shampoo and Washing Solutions respectively. The plots gave sigmoidal curves which showed that the % degradation of the MP increased as the incubation period increased which pointed out that the bacteria utilized the MP. The profile also showed that 92.0%, 92.0% and 94.7% degradation of the MP was achieved on

incubation periods of seven, thirteen and four days respectively. Other Studies have shown that Methyl paraben are biodegradable [34].

3.5 Biodegradation of the Methyl Paraben in Controls

Presented in Table 3.2 are the results for the biodegradation of the Controls containing Methyl paraben and the Inoculum control. It was observed that the concentration of MP in the Washing ($1.90 \mu\text{g mL}^{-1}$) Shampoo ($9.00 \mu\text{g mL}^{-1}$) and Standard ($5.00 \mu\text{g mL}^{-1}$) Control Solutions remained unchanged as no degradation was observed when kept for thirteen days. This result showed that no abiotic degradation of the analyte occurred as stated by [31]. No determination was made within the thirteen day as represented by ND and the Inoculum control does not contain the analyte and so no determination was made as represented by NIL.

4. CONCLUSION

From the results of the various studies carried out in this investigation, we hereby conclude that:

- (i) The growth of simple bacteria in hair dressing salon, household and industrial sewage can be cost effective and environmentally friendly method for the elimination of Surfactants.
- (ii) The bacterial degradation of Surfactants is faster in their Standard solutions than in the presence of other organic compounds.

5. RECOMMENDATIONS

We recommend that further works be done on other surfactants as well as other major sources in our surroundings such as Ternary, laundry shops, and Pharmaceutical companies effluents, household sewage as well as other sources. We also recommend that the identified bacteria be applied individually for biodegradation of surfactants so as to compare their biodegradation capacity and rate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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