



# Biodegradation of Sodium Dodecyl Sulphate and Methyl Paraben in Shampoo and Hair Dressing Salon Waste by Bacteria from Sewage Treatment Sludge

P. U. Onuche<sup>1\*</sup>, F. G. Okibe<sup>1</sup> and V. O. Ajibola<sup>1</sup>

<sup>1</sup>Department 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.

## Article Information

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## ABSTRACT

The present study investigated the biodegradation of Sodium dodecyl sulphate (SDS) and Methyl paraben (MP) both in Shampoo and Hair dressing salon wastewater 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*, *Escherichia coli* and *Klebsiella pneumoniae* degraded 98.3% of the original SDS level in the Standard SDS solution on 7 days of incubation; *Klebsiella planticola* and *Proteus vulgaris* degraded 98.9% of the original SDS level for 10 days of incubation and 94.4% of MP on 13 days of incubation in the Shampoo solution; *Vibrio cholera*, *Pseudomonas beteli* and *Escherichia coli* degraded 98.1% of the initial SDS level on 5 days of incubation and 90.5% of the initial MP level on 4 days of incubation; *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* degraded 92.0% of the MP level in the

\*Corresponding author: E-mail: [onucheugbedeajo@yahoo.com](mailto:onucheugbedeajo@yahoo.com);

standard MP solution on 7 days of incubation while the inoculum control was found to contain *Pseudomonas aeuroginosa* *Salmonella typhi*. In conclusion, the results of this study suggested that this method of biodegradation of surfactant is cost effective and using bacteria as a biodegradation agent is environmentally friendly.

**Keywords:** Sodium dodecyl sulphate; methylparaben; spectrophotometric; biodegradation; shampoo; hair salon.

## 1. INTRODUCTION

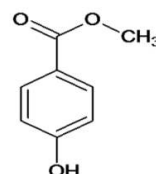
Surfactants are amphipathic compounds consisting of both a hydrophobic region (alkyl chains of various length, e.g. alkyl phenyl ethers, alkyl benzenes, etc.) and a hydrophilic region (e.g. carboxyl, sulphate, sulphonates, phosphates etc.) [1]. Surfactants have also been defined as compounds that lower 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. Sodium dodecyl sulphate (SDS) is an anionic surfactant that is used in household products such as toothpastes, shampoos, shaving foams and bubble baths [5]. Sodium dodecyl sulphate (Fig. 1), sodium laurel sulphate or sodium lauryl sulphate (SLS) is an organic compound with the formula  $\text{CH}_3(\text{CH}_2)_{11}\text{SO}_4\text{Na}$ . The salt is of an organosulphate consisting of a 12-carbon tail attached to a sulphate group, giving the material the amphiphilic properties required of a detergent.



**Fig. 1. Chemical structure of SDS**

Source: [6]

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  $\text{CH}_3(\text{C}_6\text{H}_4(\text{OH})\text{COO})$ .



**Fig. 2. Chemical structure of MP**

Source: [7]

Any environmental compartment (surface waters, sediment,) is susceptible to be contaminated by Surfactants and/or their degradation metabolites [8,9]. 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 [10]. Eichhorn et al 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 [11]. 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 [12]. 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 [13]. Chude and Ekpo found out that Hair dressing salon effluents which also contain surfactants

adversely affected fingerlings [14]. 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 [13]. As a consequent of these problems among others, the removal of surfactants from the environment has been of concern to Environmentalist 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 [15]. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process [16]. Surfactants can be biodegraded exclusively by bacteria [17,18] which use organic compounds as their source of carbon and are referred to as heterotrophic bacteria [19]. This Carbon utilizing bacteria can be isolated from activated sludge culture [20,21,22] preferably from the same environment because of environmental factors and adaptation. For instance Hosseini et al. [23] isolated bacteria from activated sludge from Tehran municipal activated sludge there in Iran and others like Marchesi et al. [24] Muayad et al. [25], Jurado et al. [26] Ivankovic [27] and so on.

Several studies have been reported on the biodegradation of various surfactants [23,24,25,26,27] and so on. So far, there is no report on the biodegradation of surfactants in shampoo and 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 as lead to the proliferation of Hair dressing salon. Hence there is a need to find a cost effective and an 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 washing**

20 ml of Shampoo and 20 ml of waste water collected immediately after hair washing were

collected from Hair dressing 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 of Ahmadu Bello University Zaria, Nigeria into a sterile plastic. Coarse particles were removed by filtration through a fine sieve and the sludge then transferred into another sterile container which has been cut open to allow aeration of the sludge [28].

## **2.2 Experimental Procedure**

#### **2.2.1 Determination of Sodium dodecyl sulfate (SDS)**

2.0 mL of ethanol and 1.5 mL of 0.01M Toluidine blue were added to 16.5 mL aqueous sample solution containing SDS (pH = 2.5), in a 50mL separatory funnel after which 7.0mL of dichloromethane was added as extracting organic solvent. The Separatory funnel was shaken mechanically for 5 min and allowed to stand for 10 minutes for complete phase separation. The dark blue extracted organic phase was transferred to a 1 cm quartz cell and its absorbance measured at 579 nm against dichloromethane as blank [29].

#### **2.2.2 Determination of methylparaben**

0.5 ml of Methyl paraben standard solution 100 µg·ml<sup>-1</sup> and 0.5 ml of 1M 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 1M HCl were mixed together and made up to mark with distilled water into a 10 ml volumetric flask and stirred. It was then cooled in an 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 make up to 1 litre with distilled water. Preparation of A, B, C and D is stated in [28] also.

#### **2.2.4 Preparation of biodegradation assays**

Specific volume in each case of the stock surfactant solution was added to 800 ml portions of mineral medium in 2 litre conical flasks and make up to 1 L and then mixed. After mixing, a sample from each flask was taken to determine the initial concentration of the surfactant which should give the intended initial concentration. The pH of the solution was adjusted to 7.0 using a pH meter. The flask was then inoculated with 0.5 ml of sludge from a Secondary treatment of the Sewage Treatment Plant (STP) of Ahmadu Bello University, Zaria. The Erlenmeyer flask was covered with aluminum foil, so as to allow free exchange of air between the flask and the surrounding. 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 at 125 sweeps per minute provided the necessary aeration. The biodegradation profiles were determined by measuring the concentration of the surfactants during the biodegradation process [28].

#### **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 [28].

##### *2.2.5.2 Preparation of inoculum control*

0.5 ml 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. 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 24 hours.

#### **2.2.7 Isolation and sub-culturing of the bacteria**

All inoculated plates were incubated aerobically at 37°C for 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 [30].

#### **2.2.8 Characterization and identification of the bacteria**

The bacteria were identified using biochemical test alone [21]. Initial identification Schemes were performed by the Mango Park Method using the Conventional Biochemical tests [30] as used by Hossemi et al. [23] following the suggestion of the Bergeys Manual of Systematic Bacteriology. The final identification was carried out using the Microgen Kit.

### **3. RESULTS AND DISCUSSION**

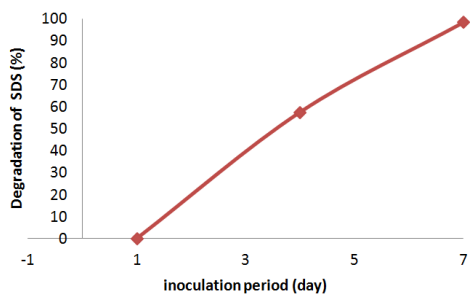
#### **3.1 Biodegradation of Sodium Dodecyl Sulphate (SDS) in the SDS Solution**

Fig. 3 showed the biodegradation profile of SDS which is a plot of degradation in percentage against the incubation period in days. The plot gave a sigmoidal curve and showed that the % degradation of the surfactant increased as the incubation period increased which pointed out that the bacteria utilized the SDS. The profile also showed that 98.3% degradation of the SDS in the Standard solution was achieved within an incubation period of 7days. This result agreed with that of other studies with different levels of surfactant utilization in closed cultures such as reported by of Hossemi et al. [23] and Schlehek et al. [31].

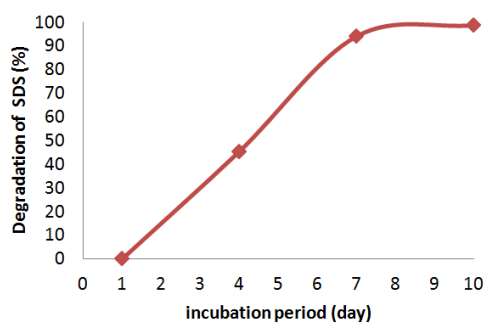
#### **3.2 Biodegradation of SDS in the Shampoo Solution**

Fig. 4 which showed the biodegradation profile of SDS in the shampoo solution gave a sigmoidal curve which indicated that the % degradation of the surfactant increased as the incubation period

increased which pointed out that the bacteria are depleting the level of the SDS in the Setup. The profile also showed that the SDS in the Shampoo solution with an initial concentration of  $4.58 \mu\text{g mL}^{-1}$  was degraded by 98.9% within an incubation period of ten days. This result agreed with that reported in other studies with different levels of surfactant utilization in closed cultures [23] and [31].



**Fig. 3. Biodegradation profile of standard SDS solution**



**Fig. 4. Biodegradation profile of shampoo solution**

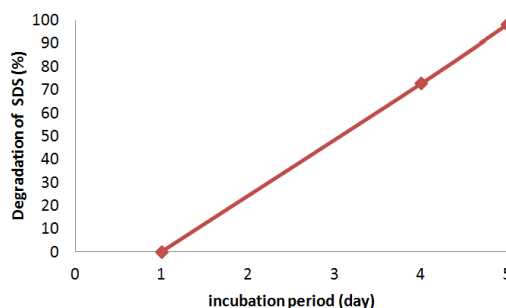
### 3.3 Biodegradation of SDS in the Washing Solution

Fig. 5 which represented the biodegradation profile gave a linear curve showed that the % degradation of the surfactant increased linearly as the incubation period increased which pointed out that the bacteria are depleting the level of the SDS. The profile also showed that 98.1% degradation of the SDS in the washing solution was achieved within an incubation period of five days. This result agreed with that reported for the biodegradation of SDS by bacteria from Tehran sewage sludge as reported by [31].

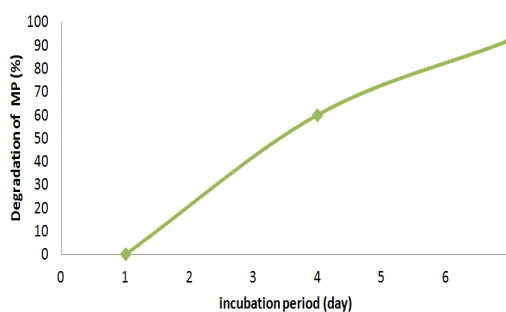
### 3.4 Biodegradation of Methyl Paraben (MP) in the Standard MP Solution

Fig. 6 presented the biodegradation profile of MP. The plot gave a sigmoidal curve which

showed that the % degradation of the surfactant increased as the incubation period increased which pointed out that the bacteria utilized the MP. The profile also showed that 92% degradation of the MP was achieved within an incubation period of seven days. Microbial degradation of parabens in a short period of time as this has also been reported [32] and these microbes have been shown to be effective in paraben degradation.



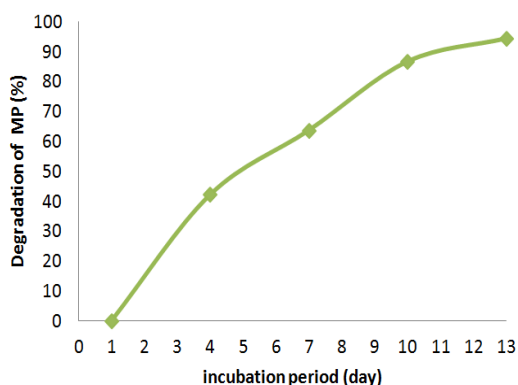
**Fig. 5. Biodegradation profile of washing solution**



**Fig. 6. Biodegradation profile of standard MP solution**

### 3.5 Biodegradation of MP in the Shampoo Solution

Fig. 7 presented the biodegradation profile of MP. The plot gave a sigmoidal curve which showed that the % degradation of the surfactant increased as the incubation period increased which pointed out that the bacteria utilized the MP. The profile also showed that 94.4% degradation of the MP was achieved within an incubation period of thirteen days. Microbial degradation of parabens in a short period of time as this has also been reported [32] and these microbes have been shown to be effective in paraben degradation.

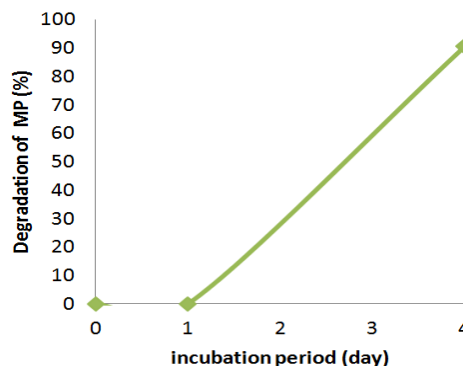


**Fig. 7. Biodegradation profile of MP in shampoo solution**

### 3.6 Biodegradation of MP in the Washing Solution

Fig. 8 presented the biodegradation profile of MP in the washing solution. The plot gave a straight line which showed that the % degradation of the surfactant increased as the incubation period increased which pointed out that the bacteria utilized MP. Only two points were observed on the line as only two sample collections were

made before the MP degradation was complete which was as a result of the very low MP concentration and hence faster degradation of the MP. The profile also showed that 90.5% degradation of the MP was achieved within an incubation period of four days. The effective of microbes in paraben degradation in a short period of time as this has also been reported [32].



**Fig. 8. Biodegradation profile of MP in washing solution**

**Table 1. Result of biodegradation of SDS in control**

Incubation period (days)	Washing (control) SDS level ( $\mu\text{gml}^{-1}$ )	Shampoo (control) SDS level ( $\mu\text{gml}^{-1}$ )	Standard (control) SDS level ( $\mu\text{gml}^{-1}$ )	Inoculum (control)
1	2.86	4.58	4.00	NIL
4	ND	ND	ND	NIL
7	2.86	4.58	4.00	NIL

*ND = Concentrations of analyte not determined, NIL = No analyte was added*

**Table 2. Result of biodegradation of MP in control**

Incubation period (days)	Washing (control) MP level ( $\mu\text{gml}^{-1}$ )	Shampoo (control) MP level ( $\mu\text{gml}^{-1}$ )	Standard (control) MP level ( $\mu\text{gml}^{-1}$ )	Inoculum (control)
1	2.2	9.0	5.0	NIL
4	2.2	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. Comparison of the degradation of SDS in the standard, shampoo and washing**

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 ( $\mu\text{g per mL per day}$ )
SDS	0.56	0.45	0.36
MP	0.66	0.65	0.50

**Table 4. Biochemical test result for the bacteria isolates**

Isolate number	TSI	Citrate	Urease	Motility	MR	VP	Indole	Inference bacteria
1a	A/A	+	-	+	-	-	+	<i>Vibro cholera</i>
1b	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
2	A/A,G	-	-	+	+	-	+	<i>Escherichia coli</i>
3a	A/A,G	-	-	+	+	-	+	<i>Escherichia coli</i>
4	A/A,G	+	+	-	-	+	-	<i>Klebsiella</i>
5b	A/A,G	-	-	+	+	-	+	<i>Escherichia coli</i>
6	A/A,G	+	+	-	-	+	-	<i>Klebsiella</i>
7	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
8	K/A	-	-	+	+	-	-	<i>Salmonella typhi</i>
9	K/NC	+	-	+	-	-	-	<i>Pseudomonas</i>
10	K/A,G	+	+	+	+	-	+	<i>Proteus vulgaris</i>
3b	NA	NA	+	NA	+	+	-	<i>Staphylococcus</i>
5a	K/A	+	-	+	-	+	-	<i>Bacillus spp</i>

**Key:** + = Positive; - = Negative; NC= No change, NA= Not applicable, A= Acid, K= Alkaline, G= Gas, Numbers = Isolates number, a = Colony a, b = Colony b

**Table 5. Results of identification by MICROGEN GNA, BACILLUS and STAPH test kits**

Isolate number	Identified bacteria
1a	<i>Vibro cholera</i>
1b	<i>Pseudomonas beteli</i>
2	<i>Escherichia coli</i>
3a	<i>Escherichia coli</i>
3b	<i>Staphylococcus aureus</i>
4	<i>Klebsiella planticola</i>
5a	<i>Bacillus cereus</i>
5b	<i>Escherichia coli</i>
6	<i>Klebsiella pneumoniae</i>
7	<i>Pseudomonas aeruginosa</i>
8	<i>Salmonella typhi</i>
9	<i>Pseudomonas aeruginosa</i>
10	<i>Proteus vulgaris</i>

### 3.7 Biodegradation of the SDS and MP in Control

Tables 1 and 2 presented the result for the biodegradation of the control solutions containing SDS, MP and the inoculum control. It was observed that the concentration of SDS in the washing ( $2.86 \mu\text{g mL}^{-1}$ ), shampoo ( $4.58 \mu\text{g mL}^{-1}$ ) and standard ( $4.00 \mu\text{g mL}^{-1}$ ) and MP 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 seven days after which the concentration of the analyte was made. This result showed that no Abiotic degradation of the analyte occurred as stated by [28]. No determination was made on the fourth 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.

### 3.8 Comparison of the Degradation of SDS and MP in the Standard, Shampoo and Washing Solutions

A comparison of the degradation of the standard, shampoo and washing solutions is as presented in Table 3. The result showed that the SDS and MP in the standard degraded at a rate of approximately  $0.56 \mu\text{g per mL per day}$  and  $0.66 \mu\text{g per mL per day}$  respectively which is higher than approximately  $0.45 \mu\text{g per mL per day}$  and  $0.65 \mu\text{g per mL per day}$  of the shampoo. This is due to the presence of SDS and MP as the only carbon source in the standard SDS solution whereas the shampoo solution contained other source of carbon. The washing degraded at a

degradation rate of approximately 0.56 µg per mL per day for the SDS which is same as that of the standard solution and 0.65 µg per mL per day for the MP. This is probably as a result of low amount of some other organic substances which might be present in the washing as dirt from the hair, hair cream and others which are other sources of carbon for the inoculated bacteria.

#### 4. CONCLUSION

From the results obtained from this investigation, we hereby conclude that the bacteria obtained from the sewage sludge can be used for the biodegradation of salon waste for surfactant. The bacteria can also be used as cost effective and environmentally friendly agent for the biodegradation of surfactants in the environment. We also conclude that the bacterial degradation of surfactants is faster in the standard solutions than in hair dressing salon.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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