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# Alkylsulphatase Activity of Fungi Isolated from Rivers Contaminated with Surfactants in Akure, Nigeria

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### Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

### Article Information

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## ABSTRACT

**Aims:** To isolate, characterize and identify surfactant degrading fungi from selected rivers in Akure, Nigeria and also to compare and quantify the biodegrading potentials of each of the fungal isolates.

**Place and Duration of Study:** Akure metropolis, Ondo state, Nigeria, between March and September, 2014.

**Methodology:** Surfactant degrading fungi were isolated from the water samples by supplementing culture media with test surfactant. The fungi isolated were later subjected to the alkylsulphatase enzyme assay to quantify their various enzyme production/activity.

**Results:** The total fungi count of the water samples was within the range of  $5.00 \pm 1.15 \times 10^2$  sfu/ml to  $11.00 \pm 1.73 \times 10^2$  sfu/ml. Surfactant degrading fungi count was within the range of  $6.0 \pm 0.11 \times 10^1$  sfu/ml to  $1.20 \pm 0.05 \times 10^2$  sfu/ml. *Penicillium italicum* and *Trichoderma viridae* were able to produce more of the alkylsulphatase enzyme amongst the isolated surfactant degrading fungi.

**Conclusion:** It can be concluded that the set of fungi isolated from the selected aquatic environments are capable of carrying out biodegradation of surfactants and that they are abundant

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in the selected environments. *Penicillium italicum* and *Trichoderma viridae* have higher biodegrading potentials and they can be exploited in the bioremediation of water bodies polluted with surfactants.

*Keywords: Bioremediation; surfactant; enzymes; alkylsulphatase; biodegradation; water pollution.*

## 1. INTRODUCTION

Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater). Water pollution occurs when pollutants are directly or indirectly discharged into water bodies without adequate treatment to remove harmful compounds [1]. It affects plants and organisms living in these bodies of water. In most cases, the effect is damaging not only to individual species and populations, but also to the natural biological communities [2]. The specific contaminants leading to pollution in water include a wide spectrum of chemicals, pathogens and physical or sensory changes. While many of the chemicals and substances that are regulated may be naturally occurring (calcium, sodium, iron and manganese), the concentration is often the key in determining the natural component of water and a contaminant [3]. Human activities, such as carrying out laundry close to the river bed can directly introduce surfactants such as detergents to surface waters impairing aquatic life and the water capacity for its usage as a pollutant [4,5].

Detergent refers to mixtures of chemical compounds including alkylbenzenesulfonates, the core components of detergent and cosmetic product formulations [6]. It contributes significantly to the pollution profile of sewage and wastewaters of all kinds. The ever-increasing demand of detergents since the middle of this century is causing great concern about its role in environmental pollution. Biodegradation processes and adsorption on sludge particles remove these chemicals from wastewater to a greater or lesser extent [7]. Biodegradation of surfactants is being performed by soil or aquatic microorganisms leading to the generation of water, biomass, salts and carbon (iv) oxide gas [8]. The alkylsulphatase enzyme produced by some microorganism is involved in the biodegradation of detergents, which hydrolyses inorganic sulphate from its ester linkage with alcohols, the later being readily assimilated through normal metabolic pathways [9]. This research therefore, examines the biodegrading capabilities of some fungi species isolated from selected aquatic environments on surfactants, in

Akure, Nigeria by comparing the alkylsulphatase activities of each fungal isolate.

## 2. METHODOLOGY

### 2.1 Collection of Samples

Water samples were collected from rivers contaminated with surfactants. The samples were collected using sterile containers. The containers with the collected samples were labelled accordingly and transported to the laboratory for Analysis.

### 2.2 Isolation of Fungal Surfactants Degraders

Isolation of fungi was done by collecting the water samples in sterile containers; they were labelled and transported to the laboratory; serial dilutions were carried out on the water samples. The diluted sample was inoculated onto potato dextrose agar supplemented with test surfactant. The plates were incubated at room temperature for 5 days and observations were made for fungal identification and characterization [10]. Isolated fungi were characterised by macroscopic (physical appearance of agar plates) and microscopic techniques (under light microscope) [10,11].

### 2.3 Alkylsulphatase Enzyme Production

#### 2.3.1 Enzyme extraction

Broth culture containing the fungal isolates and potato dextrose broth supplemented with SDS at 0.01% was incubated in an orbital shaker at 150 rpm. Some certain quantity (Fifty millilitres) of the broth culture was collected at the end of specific time intervals (twelve hours) and it was centrifuged for 15 minutes at 4°C. The supernatant was decanted off [12]. Cell pellets were collected with one millilitre (1ml) of tris buffer and it was subjected to homogenization for a period of 15 minutes. The homogenized pellets were collected and centrifuged for 15 minutes at 4°C. The supernatant was collected and kept for the enzyme assay [12].

### 2.3.2 Alkylsulphatase enzyme assay

A mixture containing four hundred and fifty micro litres (450  $\mu$ l) of fifty millimolar (50 mM) Tris-hydrochloric acid (pH 7.5) and five hundred micro litres (500  $\mu$ l) of one hundred millimolar (100 mM) SDS was pipette into a container containing fifty micro litres (50  $\mu$ l) of extracted enzyme. The mixture was incubated for 15 minutes. One hundred micro litres (100  $\mu$ l) of the mixture, 9.9 ml of distilled water, two and a half millilitres (2.5 ml) of methylene blue solution and one millilitre (1 ml) of chloroform was pipette into a separating funnel and shaken vigorously for 40 seconds. The chloroform layer formed was carefully collected and the absorbance which indicates the quantity of enzyme produced was read at 652 nm [12].

### 2.3.3 Concentration of protein in enzyme extract

Some reagents were prepared to determine the concentration of protein in the enzyme extract. The first reagent (A) was prepared by mixing 2 g of sodium carbonate in 0.1 M of sodium hydroxide. The second reagent (B) was prepared by mixing 2% sodium potassium tatarate and 1% copper sulphate. The third reagent was prepared by mixing one millilitre (1ml) of reagent B and fifty millilitres (50 ml) of reagent A. The fourth reagent (D) was folin solution [12]. Fifty micro litres (50  $\mu$ l) of the enzyme extract was then collected in a container, then four hundred and fifty micro litres (450  $\mu$ l) of reagent B and two and half micro litres (2.5  $\mu$ l) of reagent C were then added to the enzyme extract and it was left to stand for 10 minutes. Then a quarter micro litres (0.25  $\mu$ l) of reagent D was then added and left to stand for 30 minutes. The absorbance of the mixture which indicates the concentration of protein in the extract was read on a Colorimeter, at a wave length of 660 nm [12].

### 2.4 Analysis of Data

The data obtained were subjected to descriptive one way analysis of variance, using SPSS version 16 Microsoft windows 7 and means were separated with Duncan's Multiple Range Test.

## 3. RESULTS AND DISCUSSION

Table 1 represents the fungal population of the selected rivers; the total fungal count was within the range of  $5.00 \pm 1.15 \times 10^2$  and  $11.00 \pm 1.73 \times 10^2$ , while the surfactant degrading fungal count was within the range of  $6.0 \pm 0.11 \times 10^1$  and  $1.20$

$\pm 0.05 \times 10^2$ . The range of the surfactant degrading fungal count was less than the total fungal count because of the toxicity of the surfactant on the fungi population [10]. The fungal detergent degraders isolated from the river water samples are *Varicosporum elodeae*, *Gonatobotryum apiculatum*, *Aspergillus flavus*, *Trichoderma viridae*, *Articulospora inflata*, *Penicillium italicum* and *Aspergillus saprophyticus*. Some of which were isolated in other related research [10]. The adaptability of native microbial population in water to surfactant component would be the reason for their success at mineralizing surfactant component in an aquatic system where the physicochemical properties of the water ecosystem were supportive of the survival of these microorganisms.

**Table 1. Total fungal count and surfactant degrading fungal count of the river water samples**

Sample	TFC (sfu/ml)	SDFC (sfu/ml)
A	$5.67 \pm 2.01 \times 10^{2b}$	$7.3 \pm 0.08 \times 10^{1a}$
B	$5.00 \pm 1.15 \times 10^{2a}$	$8.7 \pm 0.17 \times 10^{1a}$
C	$11.00 \pm 1.73 \times 10^{2e}$	$1.20 \pm 0.05 \times 10^{2b}$
D	$7.00 \pm 1.73 \times 10^{2c}$	$6.0 \pm 0.11 \times 10^{1a}$
E	$7.67 \pm 0.88 \times 10^{2d}$	$7.3 \pm 0.12 \times 10^{1a}$

Values of means  $\pm$  Standard error, values with dissimilar alphabets are significantly different from each other at  $P \leq .05$  and vice versa

Key: A = River Ala; B = River Majo; C = River Ero; D = River Otere and E = River Owena; TFC = total fungal count; SDFC = surfactant degrading fungal count

Fig. 1 depicts the enzyme activity of *Articulospora inflata*. *Articulospora inflata* was able to produce an enzyme activity of 0.02 mM/min/ml with protein concentration of 11.31 mg/ml. From Fig. 2, the highest enzyme activity of *Penicillium italicum* was 0.09 mM/min/ml at the end of the 48 hrs incubation period. This value was maintained till the end of the 72 hrs incubation period. The protein concentration got to its peak (11.31 mg/ml) at the end of the 36 hrs incubation period.

Fig. 3 illustrates the enzyme activity of *Aspergillus saprophyticus*. The highest enzyme activity of *Aspergillus saprophyticus* was 0.06 mM/min/ml. This was detected at the end of the 60 hrs incubation period, the protein concentration at this point was 17.38 mg/ml. From Fig. 4, *Varicosporum elodeae* was able to produce the highest enzyme activity of 0.09 mM/min/ml; this was detected with the protein concentration of 10.76 mg/ml. Fig. 5 depicts the enzyme activity of *Gonatobotryum apiculatum*. The highest enzyme activity of *Gonatobotryum*

*apiculatum* (0.05 mM/min/ml) was detected at the end of the 36 hrs incubation period, with protein concentration of 9.10 mg/ml. Fig. 6 illustrates the enzyme activity of *Aspergillus flavus*. The highest enzyme activity of *Aspergillus flavus* (0.03 mM/min/ml) was detected at the end of the 72 hrs incubation period. The highest protein concentration produced by *Aspergillus flavus*

(12.14 mg/ml) was detected at the end of the 60 hrs incubation period. Fig. 7 shows the enzyme activity for *Trichoderma viridae*. The highest enzyme activity of *Trichoderma viridae* (0.10 mM/min/ml) was detected at the end of 72 hrs. The highest protein concentration produced by *Trichoderma viridae* (16.0 mg/ml) was detected at the end of the 72 hrs incubation period.

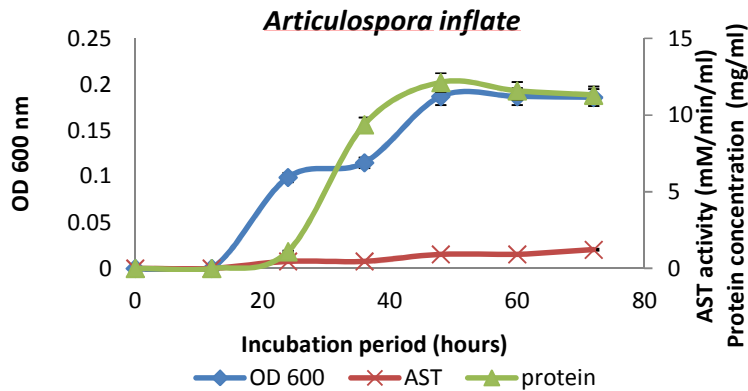


Fig. 1. Alkylsulphatase (AST) activity and protein concentration of *Articulospora inflata*

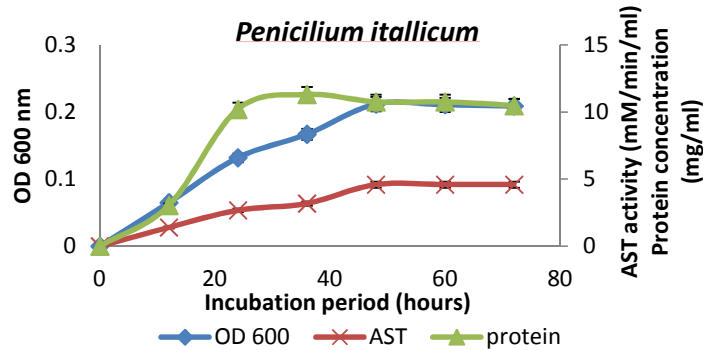


Fig. 2. Alkylsulphatase (AST) activity and protein concentration of *Penicillium italicum*

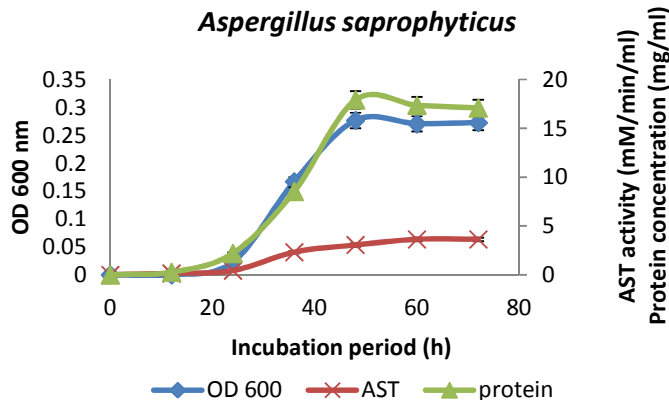


Fig. 3. Alkylsulphatase (AST) activity and protein concentration of *Aspergillus saprophyticus*.

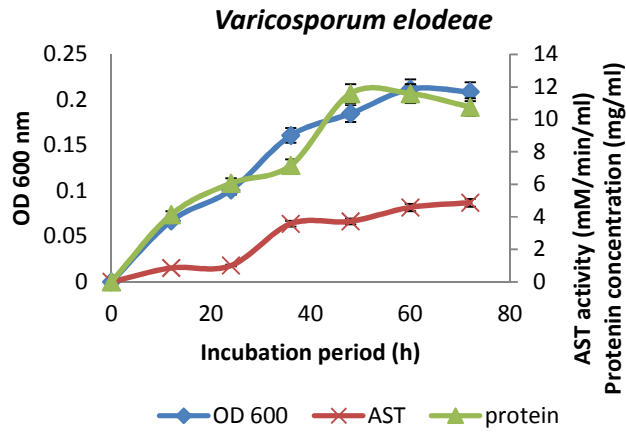


Fig. 4. Alkylsulphatase (AST) activity and protein concentration of *Varicosporum elodeae*.

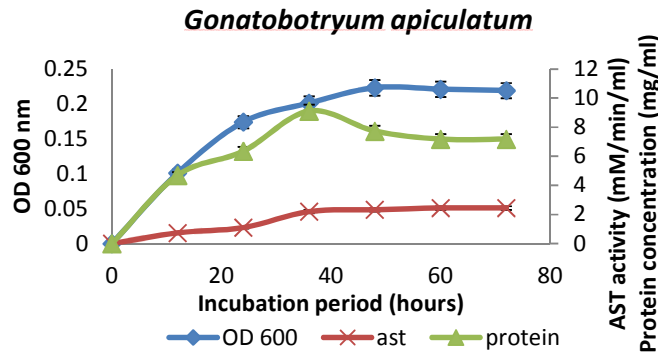


Fig. 5. Alkylsulphatase (AST) activity and protein concentration of *Gonatobotryum apiculatum*

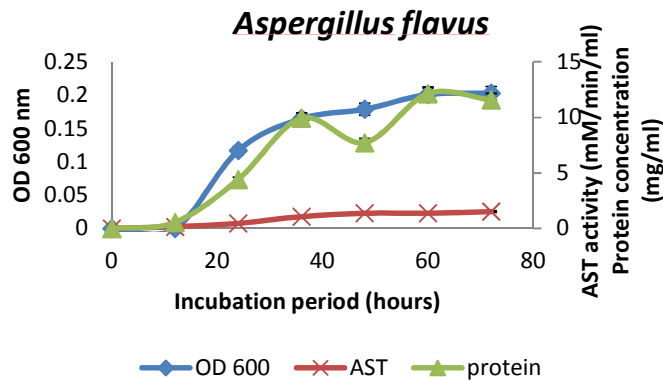
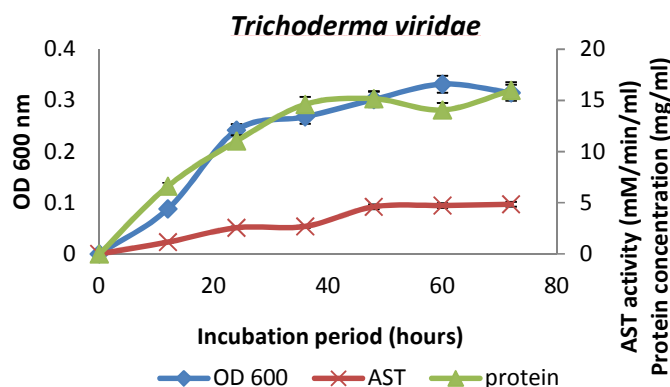


Fig. 6. Alkylsulphatase (AST) activity and protein concentration of *Aspergillus flavus*



**Fig. 7. Alkylsulphatase (AST) activity and protein concentration of *Trichoderma viridae***

The fungal isolates were able to produce the alkylsulphatase enzyme as presented in the enzyme assay results. Fungi can act as potential degraders by excreting enzymes from vesicles on top of their hyphae to their environment to break down pollutants to simpler forms, which can be used for their growth and biomass accumulation [13]. There were variations in the quantity of alkylsulphatase enzymes produced by the fungal isolates. Some showed better biodegrading potentials, and this could be related to the genetic makeup of the fungal isolates [11]. The present study results suggest that bioremediation by the fungal isolates are promising for the biodegradation of surfactants as pollutants in aquatic systems.

#### 4. CONCLUSION

The study was able to illustrate the pattern of enzyme production and activity of the various isolates with respect to time, microbial growth and protein concentration of each isolates. The study indicates an array of fungi that could be employed in the remediation of aquatic environment contaminated with surfactants. The study indicates that enzyme activity increases with time, microbial growth and protein concentration. It could be concluded that the set of fungi isolated can be exploited in the bioremediation of aquatic environments polluted with surfactants.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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