

## Antifouling strategy for marine transport efficiency in Nigeria: The isothiazolone option

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### Abstract

**The sustainability of Nigerian maritime industry is threatened by drag and deterioration caused by biofilms on painted marine structures. The bacterial population of scrapings from twenty boat hulls on Ojo waterside shores, Lagos, Nigeria and the antibacterial activity of in-can isothiazolone biocide against the isolated biofilm bacteria were investigated. The disc diffusion technique using Mueller Hinton agar (MHA) media and sterile filter paper discs immersed into the biocides was employed. Bacterial population density of the scrapings ranged from 1.3 to 2.2 x 10<sup>5</sup>CFU/g. They were identified as *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Staphylococcus aureus* using the analytical profile index (API). All the isolates were susceptible to isothiazolone biocide with the highest inhibition zone of 24.75mm (*P. mirabilis*) and the lowest inhibition zone of 4.63mm (*S. aureus*). The MIC was observed as 15µl/ml (*S. aureus*), 5µl/ml (*P. aeruginosa*) and 5µl/ml (*P. mirabilis*) while MBC was 20µl/ml (*S. aureus*), 5 µl/ml (*P. aeruginosa*) and 15µl/ml (*P. mirabilis*). Results showed that isothiazolone biocide has broad spectrum antibacterial activity against bacterial biofilms on marine structures.**

**Keywords:** Biofouling; boat; isothiazolone biocides; maritime industry; Nigeria

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### Introduction

Maritime transport play significant role in Nigeria's economy. In Nigeria, maritime transportation of goods and services facilitates trade and commerce, recreation, employment and job creation, revenue generation, tourism, industrial growth and development, defense and security, improved international relations as well as socio-political harmony (Igbokwe, 2001). However, according to (Lazarus and Ukpere, 2011), Nigeria loses as much as \$4 billion US dollars to foreign ship owners yearly owing to lack of indigenous capacity in the local maritime transportation. Unknown to many, biofouling contributes to this loss by preventing the smooth

and cost-effective running of marine transport. Much attention has been given to the provision of equipment and implementation of tariff structure, manual examination of goods, transport logistics, ports congestion, custom clearance for containers and security of cargo in transit (Igbokwe, 2001; Ogunsanya and Olawepo, 2008; Ogunsiji and Ogunsiji, 2010; Ogbonnaya et al., 2011; Eniola et al., 2014). However, there is paucity of information on the resilient activities of microorganisms in depleting the economic potentials of the maritime industry by slowing down boat and ship movement. The Nigerian ports system has been unproductive, static and malfunctioning leading to ports

congestion and disruption of economic activities. Over time, the accumulation of organisms that form biofouling on hulls have caused decrease in the hydrodynamic performance of vessels and the frictional effects has led to increased drag of up to 60% resulting in 10% reduction in speed (Yebra, 2004). An estimate by Liu et al. (1997) indicates that on average, fuel consumption increases by 6% for every 100  $\mu\text{m}$  increase in the average hull roughness caused by fouling organisms.

Boat hulls are susceptible to micro and macro fouling irrespective of the material from which they are constructed. The fouling, if allowed to continue proliferating, causes increased drag on the hull, leading to increased fuel consumption, and can eventually cause significant damage to the boat structure depending on the extent of infestation (Darbyson et al., 2009). A heavily fouled vessel may also lose maneuverability. It is therefore, necessary to apply some form of coating which will protect the hull against infestation. These coatings are generally known as antifouling paints and they are applied to the hull at regular intervals because they wash off with time (Briane et al., 2013). They are also applied to reduce the risk of spreading of invasive species (Ashton et al., 2006). Studies have been performed investigating the ability of capsaicin and zosteric acid as paint additives to prevent biofouling (Xu et al., 2005). There have also been reports of nanoparticle-based coatings to prevent fouling (Anyagou et al., 2008). All these have their various drawbacks. Isothiazolone biocides on the other hand, are safe and have been widely used in industrial water treatment applications to control microbial growth and biofouling (Williams, 2007). Isothiazolone biocides such as Kathon 5287 have been found to be highly effective, broad-spectrum and licensed for use as active antifouling agents in the UK and other countries. They were the first organic booster biocide to be registered for use by the USAEPA (Voulvoulis, 2006). The killing mechanism of isothiazolone biocides include inhibition of dehydrogenase enzymes such as pyruvate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase, lactate dehydrogenase and alcohol dehydrogenase (Chapman and Diehl, 1995). Isothiazolones have also been shown to inhibit ATP, which will

ultimately lead to growth inhibition, lack of cell division and irreparable damage (Nwachukwu and Akpata, 2003). Although, isothiazolone biocides have been used in the UK, USA and other countries, there is paucity of information on its use as antifouling agent on boats in the shores of Nigeria. It is therefore, the aim of this study to evaluate and report its efficacy in reducing antifouling of boats at the Ojo water side in Lagos, Nigeria. This is particularly necessary because biofouling contributes to making Nigerian ports user-unfriendly and unattractive to some liners, shippers and importers who now prefer berthing at neighboring ports especially Cotonou port to berthing in Nigerian ports (Igbokwe, 2001). This is expected because in Nigeria, cargo throughput has a turnaround time of 5.25 days on the average (Eniola et al., 2014) which is still on the high side compared to the International Maritime Organization's stipulation of 48 h. The prevention of marine biofouling on our maritime infrastructure using antifouling paints fortified with isothiazolone biocides could help achieve this feat.

## Materials and methods

### *Sampling Locations and Procedures*

Scrapings from painted boat hulls were aseptically collected in duplicates with a scalpel on the 10<sup>th</sup> of April, 2015 from twenty speed boats at Ojo water side, Lagos after issued permission from the water side authorities. The scraping pieces of about 1-5  $\text{cm}^2$  which were firmly attached or partly detached were collected to a depth of about 1-1.5 mm. They were subsequently placed in sterile, properly labeled petri dishes which were carefully sealed and taken to the laboratory for analyses.

### *Microbial Isolation and Preparation of Inoculum*

Bacteria were isolated from the paint scrapings in the laboratory using spread plate technique (Nwachukwu and Akpata, 2003). Following maceration and serial dilution, aliquots (0.1ml) from selected dilutions ( $10^{-3}$  and  $10^{-5}$ ) were inoculated on nutrient agar (NA) plates. The plates were incubated aerobically at 37°C for 24 h. After incubation, developed colonies were counted, purified by subculturing and

preserved on NA slants at 4°C. A loop full of pure overnight cultures from the labeled stock culture bottles was picked and introduced into sterile peptone water and incubated at 37°C for 24 h to obtain growth. Inoculum was then standardized by diluting bacterial suspension in each test tube with sterile peptone water to match the turbidity of 0.5 McFarland standard ( $10^8$  CFU/ml).

#### *Antibacterial Activity of Isothiazolone Biocide*

##### *Preparation of Antimicrobial Disks*

The modified disk diffusion method was used to evaluate the antimicrobial activity of isothiazolone biocide against the test bacterial isolates. This method was carried out using Mueller Hinton agar (MHA) media. Sterile filter paper discs were immersed into the biocides in a sterile glass Petri dish in order to embed the biocides onto the perforated paper discs (Shirley et al., 2010).

##### *Susceptibility Tests*

To carry out the susceptibility test, aliquots (0.1ml) of the standardized inoculum was seeded uniformly onto the petri dish MHA and was spread over the surface of the agar with a sterile hockey stick. With a sterile forceps, the impregnated disks were carefully placed in the middle of the MHA petri dishes. Control plates had no biocide. The plates were incubated aerobically at 37°C for 24 h. The zones of inhibition surrounding the disks were observed after the incubation period and measured in millimeter.

##### *Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)*

In order to determine the bacteriostatic and bactericidal effects of the isothiazolone biocide on the isolated bacteria, a loopful each of overnight cultures of the test isolates on NA was inoculated into sterile peptone water and incubated aerobically at 37°C for 24 h to achieve an exponential phase. Subsequently, bacterial concentration was determined by diluting with peptone water to give a cell concentration of approximately  $10^8$  colony forming units (CFU/mL) corresponding to the 0.5 McFarland standard. The MIC of the isothiazolone biocide for individual isolates was performed by the modification of the broth dilution method based

on the National Committee for Clinical Laboratory Standards (NCCLS) in test tubes. The tubes were supplemented with varying concentrations of the biocide (5, 10, 15, 20, 40, 60, 80 and 100µl/ml). The well labeled tubes were incubated at 37°C for 24 h. At the end of incubation period, the tubes that showed no visible microbial growth were recorded as the MIC of the biocide against the particular tested isolates (Qi et al., 2004). Subsequently, aliquots (0.1 ml) of cell suspension from the test tubes that showed no visible growth of microorganisms were plated out on NA to investigate microbial growth. After 24h incubation, the MBC was defined as the lowest concentration killing > 99.9% of the isolate.

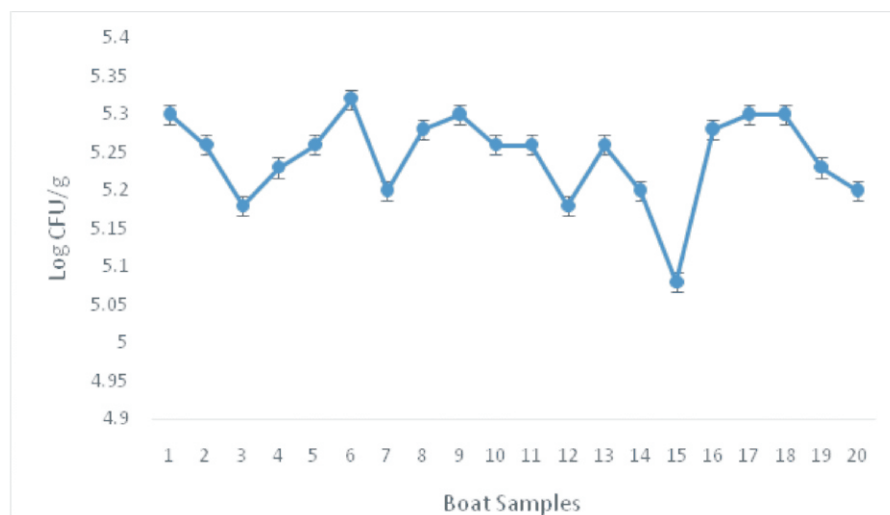
## **RESULTS**

### *Isolation of Marine Fouling Bacteria from Scrapings of Boat Hulls*

Following isolation and 24 h aerobic incubation at 37°C, bacterial growths were observed and counted by the number of visible colonies using a colony counter. The bacterial population density ranged from  $1.2 \times 10^5$  CFU/g to  $2.2 \times 10^5$  CFU/g. The mean bacterial population density observed is displayed in Fig. 1.

### *Antibacterial Effect of Isothiazolone on Cell Viability*

The biocides were tested against *S. aureus*, *P. mirabilis* and *P. aeruginosa* for the antibacterial efficacy which resulted in formation of varying zones of inhibition when compared with the control plates. The diameter of inhibition zone of biocide (24.75 mm) against *P. mirabilis* showed significant potency as compared to *S. aureus* (4.64 mm) and (12.75) *P. aeruginosa* (Table 1). There was no inhibition zone observed in the control plates. The isothiazolone biocide exhibited antibacterial properties against all the tested isolates in the order: *P. mirabilis* > *P. aeruginosa* > *S. aureus*. This study therefore demonstrates that isothiazolone biocide can be very effective and potent against common bacterial isolates occurring on boat hulls. The MIC and MBC values ranged between 5 and 15 µl/ml and between 15 and 20 µl/ml for *P. mirabilis*, *P. aeruginosa* and *S. aureus* respectively (Table 2).



**Fig. 1.** Mean Bacterial Population Density of Scrapings from Twenty Boat Samples

**Table 1:** Zone of inhibition (mm) of isothiazolone biocide against isolated bacterial strains

Microorganism	Zone of Inhibition (mm)
<i>P. aeruginosa</i>	12.75
<i>S. aureus</i>	4.64
<i>P. mirabilis</i>	24.75

**Table 2:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of isothiazolone biocide

Microorganism	MIC ( $\mu\text{l/ml}$ )	MBC ( $\mu\text{l/ml}$ )
<i>P. aeruginosa</i>	5	15
<i>S. aureus</i>	15	20
<i>P. mirabilis</i>	5	15

### Discussion

The result of the antibacterial activity of isothiazolone-based biocide determined by zone of inhibition, MIC and MBC provide sufficient evidence to support the incorporation of these biocides in paints used in recreational boats. The results is similar to findings of Williams, (2007) who reported that all isothiazolone biocide tested were highly efficient, at levels less than  $1 \text{ mg/kg}^{-1}$ . Furthermore, Diehl and Chapman, (1999) reported the rapid onset of toxic effects at suprainhibitory 5-chloro-2-methyl-isothiazol-3-one (CMI) on cells of *Pseudomonas aeruginosa* and *P. fluorescens* within 5-10 min exposure. This effective killing was attributed either to the fact that the proton motive force is involved directly in CMI association in an active transport process or that an intact PMF is needed for some facet of the cell metabolism which maintains the cells as a sink

for CMI. Similarly, the biocidal studies carried out in this study showed isothiazolone-based biocides inhibited *P. mirabilis*, *P. aeruginosa* and *S. aureus* which was evident from the MIC and MBC values that ranged between 5 and 15  $\mu\text{l/ml}$  and between 15 and 20  $\mu\text{l/ml}$  for *P. mirabilis*, *P. aeruginosa* and *S. aureus* respectively. Although the mechanisms of inhibition was not determined in the present study, Isothiazolone-based biocides have been reported to make use of a two-step mechanism which involves a rapid inhibition of growth and metabolism (Williams, 2007). Microbial cells are inhibited by a disruption of metabolic pathways involving critical enzymes like the dehydrogenase enzymes. This is followed by a disruption of critical physiological functions such as growth, reproduction and energy generation. All these leads to an irreversible cell damage resulting in loss of viability and cell death after

some hours (Williams, 2007). The implication of shutting down respiration on bacterial cells involves preventing oxygen consumption for energy production which is needed for growth and metabolism. Therefore, all aerobic processes are terminated and cell death becomes inevitable.

### Conclusion

Isothiazolone biocide at a concentration of 5-15 µl/ml has an impressive antibacterial impact in the inhibition of bacterial biofilms which are the primary precursors of macrofouling that leads to reduced energy efficiency on boat and ship hulls.

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