The Antibacterial Activity of ZrO_2 Nanoparticles on Biocide Resistant Bacilli in Paints

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Abstract: The antibacterial activity of four biocides against members of the class Bacilli isolated from paint and paint scrapings was investigated using the disc diffusion technique. *Bacillus* species were selected for this study amongst other organisms isolated from paint and paint scrapings because they were regularly isolated in highest populations when compared with other isolates detected. The biocides were made up of benzimidazole carbamate/2-n-octyl-4-isothiazolin -3-one (OIT)/Urea derivative), (5-Chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (CIT/MCIT); (Carbendazim octylisothiazolone and Diuron) and (Chloromethyl and methylisothiazolone). *Bacillus cereus* and *B. coagulans* had 0% inhibition of all the biocides tested. Similar experiments with ZrO_2 nanoparticles yielded 65% inhibition of *Bacillus sphaericus* and 80% of *Brevibacillus choshinensis*. The result of this study indicates the need for more sophisticated antimicrobials and underscores the superior efficacy of nano-structured antimicrobials over conventional biocides used in the paint industry. These findings will enhance efforts geared towards combating biocide-resistant microorganisms in the paint industry.

Keywords: Antibacterial activity, Bacilli, Biocides, Biodeteriorated paints, Nanoparticles.

1. INTRODUCTION

The human society is constantly interacting with the world of microbes. The consequences of microbial contamination and related deterioration of paints include a drastic reduction in paint quality and its shelf life. Biodeteriorated paint is presently a source of concern to manufacturers and now constitutes a major challenge bewildering the paint industry. Previous researches have shown that isolates from paints are potentially dangerous to any economy as they result in continual spoilage, loss of viscosity which affects quality and finally, complete degradation of paints [1, 2]. Generally, in-can paints may be colonized by a wide range of Gram-negative and Gram-positive bacteria, particularly the spore formers such as *Bacillus* spp. The most serious concern that has received the greatest attention in recent times is viscosity loss [3] since viscosity is a major consideration in paint application. The most common thickeners used for paints to achieve high quality with the right viscosity in the paint industry are cellulose ethers, such as hyroxyethyl cellulose, and these are subject to enzymic hydrolysis by bacterial and fungal cellulases[4]. In addition, recent research has shown that small amounts of cellulases (0.1 ppm) in paints can cause significant (2%) decrease in paint viscosity [3]. Thus, the control of microbial population level and its damaging implication on viscosity and other physico-chemical properties of paints is a significant issue that must be addressed.

Bacillus species have been most frequently isolated from paints [5, 1-2]. For several years, conventional biocides have been successfully used to prevent microbial attack and sustain paint preservation. The application of low-level biocides have proved effective in inactivating most vegetative bacteria, certain fungi and some viruses while high-level biocides are effective in inactivating microbial spores when properly applied with prolonged exposure times [6]. However, the emergence of biocide-resistant microorganisms has necessitated the development of improved, sophisticated and more effective antimicrobial agents for the paint industry. Bacteria have adopted some resistance strategies such as energy-dependent efflux pumps, changes in envelope composition, altered target sites, possible biocide metabolism in the periplasmic space and altered membrane fluidity which all promote effective impermeability to biocides [6].

Therefore, in connection with the phenomenon of biocide resistance development amongst paint microorganisms, the expansion of spectrum of effective bactericidal agents such as application of nanomaterials with antibacterial properties in paints has become a high priority. It is in this context that nanotechnological options are considered. Studies have shown that low molecular weight compounds such as nanoparticles generally inhibit a broad spectrum of bacteria [7]. Nanoparticles have received considerable attraction and attention in the past few decades because of their peculiar physical, chemical and biological properties as well as their high surface to volume ratio which makes them interact effectively with

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bacteria [8, 9 -10]. Consequently, this study sets out to compare the antibacterial activity of conventional biocides used in the paint industry with ZrO_2 nanoparticles.

2. EXPERIMENTAL PROCEDURES

2.1. Isolation of Bacteria and Culture Conditions

Bacteria were isolated from dry paint films and in-can paint samples by spread plate method on sterilized nutrient agar (NA) medium following serial dilution technique. The NA plates were incubated at 37^0C for 24 h for proper colony formation. The predominant bacterial colonies were selected, counted, purified and identified by the analytical profile index (API) and the fatty acid methyl ester (FAME) analysis [11]. The fatty acid composition of the bacterial isolates were then compared to a Sherlock Library of known microorganisms to determine the closest match. Overnight Cultures of the selected test organisms were grown in Mueller Hinton (MH) Broth (Beef; dehydrated infusion 300.0g/L; casein hydrolysate 17.5g/L; starch 1.5g/L, pH 7.3) in 18 x 150 mm glass test tubes shaken at 200 rev min^{-1} at 37^0C for 18-24 h. A cell concentration of 10^8 colony forming units (CFU) ml^{-1} (OD of 0.1, 600nm) was evenly spread on Mueller Hinton (MH) (Beef extract 2.0g/L; Acid hydrolysate of casein 17.5g/L; starch 1.5g/L; Agar 17.0g/L pH 7.3) agar plates with sterile beads. The plates were incubated at 37^0C for 24 h and colony counts were determined. Isolates were stored in 10% (v/v) glycerol at -70^0C and were freshly subcultured before each experiment.

2.2. Biocides

The biocides used were obtained in commercial preparations from the paint industry. They include: Acticide BX-H (In-can biocides) and Acticide EPW-1 (Dry film biocides). They were made up of benzimidazole carbamate/2-n--octyl-4-isothiazolin -3-one (OIT)/Urea derivative), (5-Chloro-2-Methyl-4-isothiazolin-3-one and 2-methyl-4-isothizolin-3-one (CIT/MIT); (Carbendazim, octylisothiazolone and Diuron) and (Chloromethyl and methylisothiazolone) respectively.

2.3. Nanoparticles

Zirconia (ZrO_2) nanoparticles were synthesized at the Materials Science and Engineering Department, Iowa State University. ZrO_2 was synthesized by a non-hydrolytic sol-gel reaction between zirconium (IV) isopropoxide ($Zr[OCH(CH_3)_2]_4.(CH_3)_2CHOH$, Aldrich Chemical Co.99.9%) and zirconium (IV) chloride (Aldrich Chemical Co. 99.9%) with purified trioctylphosphine oxide (TOPO, Aldrich Chemical Co. 90%) at 340^0C for 2 h with vigorous stirring [12].

2.4. *In Vitro* Antibacterial Analysis

The antibacterial susceptibility testing was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [13] using the Kirby-Bauer disc diffusion method [14]. Bactericidal experiments were carried out with *Bacillus* species which were most frequently isolated both in dry paint films and in-can paints. Pure cultures of the test organisms were subcultured in Mueller Hinton (MH) broth (Becton Dickinson) at 37^0C on a rotary shaker at 200 rpm. Approximately 100μL of the overnight culture having 10^8 colony forming units (CFU)/mL of each test organism was inoculated unto the MHA plates with the aid of a micropipette. This was spread uniformly on the plates with sterile glass beads and allowed to stand for 10 minutes for the culture to be better absorbed [9]. Wells of 6mm diameter were made on the MH agar plates by gel puncture. Using a sterile micropipette, 100 μL of each nanoparticle suspension was introduced into each of the wells. Similar experiments were done with biocide impregnated discs [15]. After 24 h incubation at 37^0C, the different levels of zone of inhibition were measured.

3. RESULTS

Figures **1** and **2** show the FAME analysis of *Brevibacillus choshinensis* and *Bacillus sphaericus*. The retention times and elution order of 14 methyl esters from C12:0 to C17:0 and 13 methyl esters (C14:0 to C17:0) for *B*. *sphaericus* and *Brevibacillus choshinensis* respectively are represented as revealed by FAME analysis. The differences in the zone of inhibition produced by ZrO_2 nanoparticles against the test organisms as compared with the biocides are shown in Figure **3**. The highest antimicrobial activity was observed with ZrO_2 nanoparticles with 35mm zone of inhibition against *Brevibacillus choshinensis* and 25mm against *Bacillus sphaericus* (Figure **3A**). However, experiments with biocides showed no inhibition against *Bacillus coagulans* and *Bacillus cereus* (Figure **3B**). Antibacterial activity results revealed that ZrO_2 nanoparticles were very effective against the tested organisms compared to the conventional biocides. The result of this study is indicative of higher level of tolerance and/or resistance to biocide by members of the class *Bacilli*. It is clear

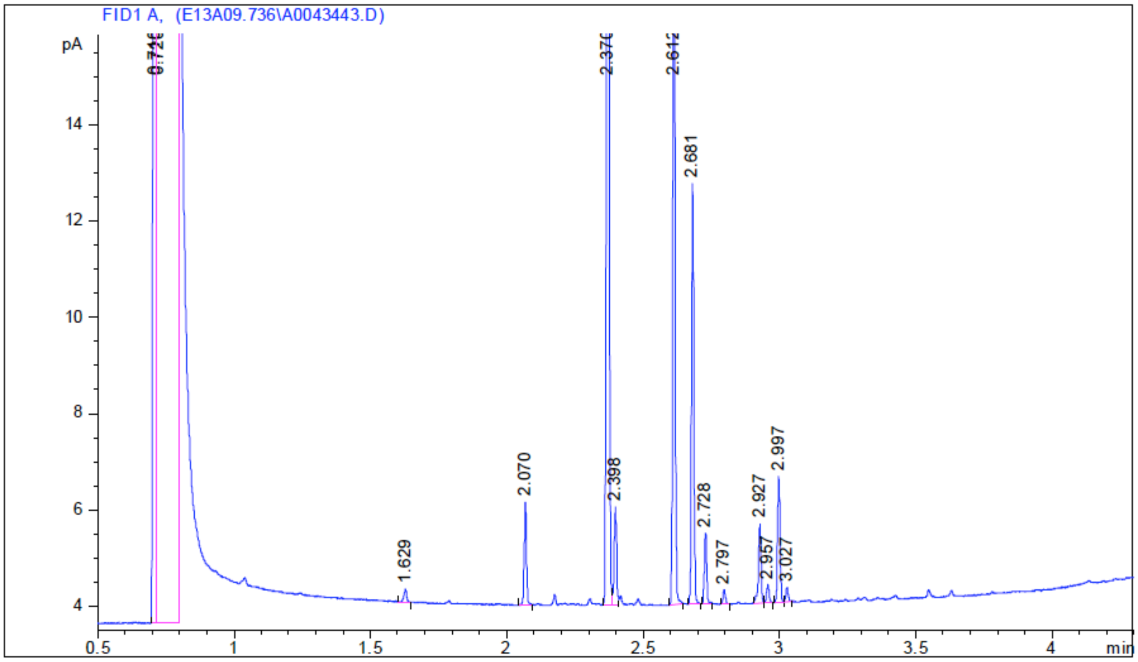


Figure 1: FAME analysis of *Bacillus sphaericus.*

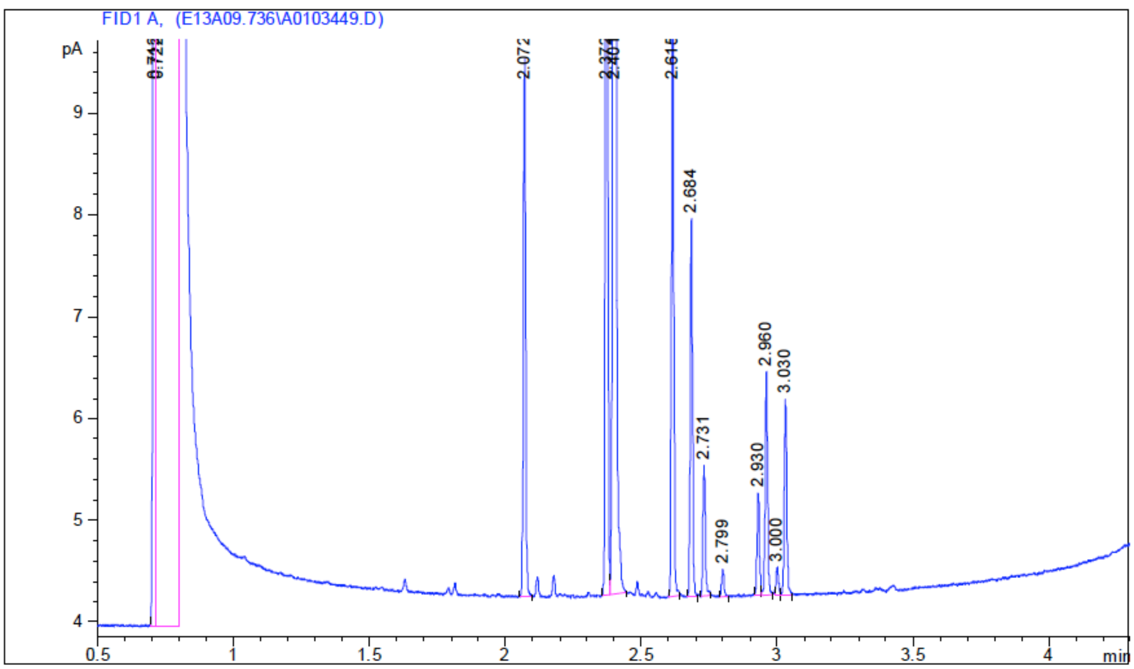


Figure 2: FAME analysis of *Brevibacillus choshinensis.*

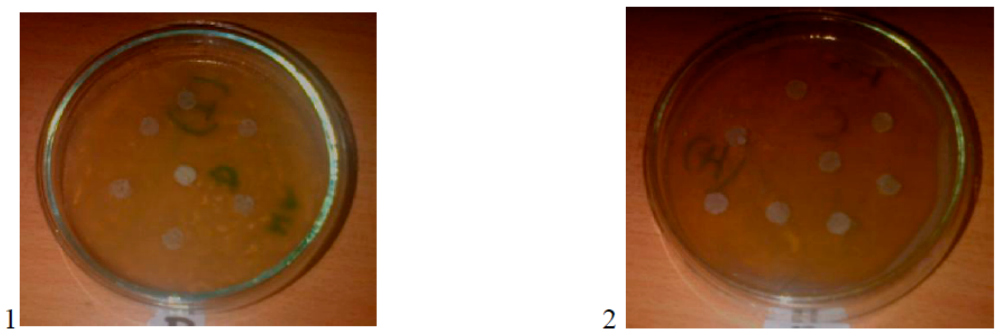
that the nanoparticles had higher antimicrobial activity probably because of their increase in surface to volume ratio due to decrease in their sizes.

4. DISCUSSION

Bacillus species have been reported to be predominantly isolated from paint samples and wall paintings [5, 1-2]. It would not be surprising therefore, if these group of organisms have adopted strategies to enhance impermeability to biocides. The biomarkers which are the predominant fatty acids detected, identified the organisms based on comparison with the profiles of a pre-determined library of known isolates.

Recent research showed that members of the class *Bacilli* such as *B. cereus* and *B. thurigiensis* are able to use volatile organic compounds (VOCs) as a carbon source and degrade the VOCs faster than many other

A: Antibacterial activity of ZrO_2 nanocrystals



B: Antibacterial activity of Biocides

Figure 3: Zone of inhibition produced by different antimicrobial agents. Antibacterial activity of (**A**) ZrO_2 nanocrystals, (**B**) Biocides against bacterial strains (**A1**) *Brevibacillus choshinensis* (**A2**) *Bacillus sphaericus* (**B1**) *Bacillus coagulans*, (B2) *Bacillus cereus.*

organisms [16]. A higher level of resistance of bacteria isolated from spoilt paints to biocide than the isolated fungi was also previously reported [2], emphasising the need for a more potent antimicrobial for paint bacteria. It is however apparent that misuse or excessive use of these biocides could contribute significantly to the evolution and persistence of biocide resistance among *Bacilli* community in paints. Therefore, the need for new, effective antibacterial agents is undisputed. Other nanoparticles such as silver and copper nanoparticles have been severally reported to be highly toxic to *Escherichia coli*, *Staphylococcus aureus* and *B. subtilis* [17, 18, 19-20]. Previous studies have shown the antimicrobial activities of ZrO_2 nanocrystals against *Escherichia coli* (Gram-negative) [21]. The present study however, revealed that ZrO_2 nanocrystals exhibited antimicrobial activity against Gram positive *Bacilli*. This suggests the effectiveness of ZrO_2 nanoparticles in inhibiting a wide range of bacteria. It is obvious that antibacterial activity of the nanomaterials increased with increase in surface to volume ratio due to the decrease in size of the nanoparticles. Furthermore, the interaction between nanoparticles and constituents of the bacterial membrane has been shown to cause structural changes and damage to the membrane, finally leading to cell death [17]. Nanoparticles can be uniformly distributed throughout paint solution, thus providing the paint with a continuous, protective film structure. Previous study reported that if properly applied, nanoparticles can confer corrosion resistance to paints due to the large amount of electrons on their surfaces which can create a sacrificial anode required for corrosion resistance [22]. Although ZrO_2 has been used in making good surface coatings [23] because of its strength, excellent resistance to water, heat and corrosion, it is important to note that ZrO_2 have been implicated in certain biological effects such as bone formation related to implant osseointegration [23]. The incorporation of nanoparticles into coatings have been found to prevent coating damage without resulting in brittleness or reduced chemical resistance and thus, suitable for different types of coatings [22]. Here, we present a comparative study of the bactericidal properties of ZrO_2 nanoparticles on paint *Bacilli.*

5. CONCLUSION

The result of this study suggests that ZrO_2 nanoparticles were highly effective against *Bacillus* species that are commonly encountered in the paint industry and had developed resistance to the conventional biocides. The nanoscale size of the nanoparticles had high impact on their efficacy as they facilitate the contact of a significantly large surface area of the particles with bacteria. This results in effective elimination of the bacteria. The synthesis of these nanoparticles in the formulation of new types of antimicrobials for the paint industry therefore, has profound potentials.

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