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Evaluation of the Role of Bacterial Enzyme, Sulphate, Nitrate and Ultimate Latex Paint Biodegradation

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Abstract: The role of bacterial extracted enzyme, nitrate and sulpahte mobilization in latex paint degradation was investigated. The result revealed that degradation rate i.e. paint utilization was better enhanced by the presence of sulphate (SO_4^{2-}) than nitrate (NO_3^-) though at no significant level ($p>0.01$). However, *Bacillus* species exceptionally mobilized nitrate (19.4mm) than sulphate (18.9mm). The overall mobilization rate for sulphate is represented thus: *Micrococcus* sp (20.2mm) > *Bacillus* sp (18.9mm) > *Pseudomonas* sp (12.6mm) while the reverse occurred for nitrate as; *Bacillus* sp (19.4) > *Pseudomonas* sp (11.1mm) > *Micrococcus* sp (10.6mm). The ultimate degradation of latex paint measured as index of dissolved oxygen (DO) indicated that bacterial contaminants exhibited high rate of paint degradation with significant ($p<0.05$) difference and variation among the bacterial species and in all cases, the level of DO dropped with increase in incubation period. The enzymatic activities on paint breakdown showed a direct correlation ($r=0.01$) on percentage degradation and incubation period. Increase in incubation period corresponded with increase in percentage degradation of paint with no significant difference among the bacterial species. Results obtained from this investigation indicate and confirm that extracellular enzymes, presence of sulphate\nitrate ions and dissolve oxygen in paint as an organic substrate contributed immensely to the bacterial degradation of paint molecule. Therefore, empirical studies aim at controlling these deleterious problems in paint industry is suggested.
Keywords: Mobilization, contaminants, extracellular, proliferation.

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Introduction

Paint is a substance with a complex composition that have a high rate of microbial attack and biodegradability (Ariffin *et al.*, 2006). Its composition enhance the attack by microbial contaminations with concomitant viscosity loss, discolouration, gassings, sedimentation and deordouration (Etim and Antai, 2015).

The bacterial species often implicated in both oil and latex paint degradation are *Pseudomonas*, *Streptomyces*, *Micrococcus*, *Alcalegenes*, *Flavobacterium*, *Norcadia* and *Bacillus.* Their accompanying deleterious effects result to a colossal loss of paint value, cost and aesthetics. A major cause of viscosity loss and other microbial contamination associated

problems of latex paint is because of its organic composition such as thickeners, binders, filters, pigments and the corresponding degradative extracellular enzymes (Badal, 2004).

These extracellular enzymes enable the microorganisms to mobilize large paint molecules found outside the cell (Gibbs *et al.*, 2001). Equally, these enzymes to initiate attack and degradation the microorganisms deploy oxygen-incorporating enzymes that catalyze the insertion of dissolve oxygen (DO) in the paint so that the molecules can subsequently be consumed by cellular metabolism (Nicell, 2001).

As a result of this oxygen very important for the biodegradation of both oil and latex paint since the major pathways for pigment solvent and additives involve molecular dissolved oxygen or oxyginase (Cooney, 1990). However, theoretical calculations indicated that 3 to 4 parts of dissolve oxygen are required to completely oxidized one part of hydrocarbon into carbon dioxide and water (Lee and Levy, 1989).

The presence of sulphate and nitrate cations in paint promote their uptake and mobilization by bacterial contaminants. These ions in paint act as electrons acceptors in the degradation of paint as organic substrate for energy and carbon (Atlas and Bartha, 1997). Therefore, sulphate-reducing bacteria (SRB) use sulphate as terminal electron acceptor during anaerobic respiration through the principal mechanisms such as cathodic deplorization and hydrogen sulphide production (Ibiene *et al.*, 2006).

Therefore, to reduce sulphate into sulphide, the bacteria facilitate a chemical reaction that adds electrons taken from hydrogen or other donors such as iron in the environment to the sulphate (Chamritski *et al.*, 2004).

The study attempts to evaluate the role of bacterial extracellular enzymes, sulphate and nitrate uptake and mobilization and the rate of ultimate paint degradation using dissolved oxygen (DO) as indices for paint microbial degradation.

Materials and methods

Sulphate (SO_4^{2-}) and nitrate (NO_3^-) mobilization

The agar pit method described by Cheesbrough (2000) was adopted. An overnight (18hrs) broth cultures (approximately 0.5 McFarland Standard, 1.5 ×10 cfu mL^{-1}) of *Pseudomonas* sp, *Micrococcus* sp and *Bacillus* sp were formulated and aseptically dispense into the well. After 48hrs of incubation at room temperature (28± 2^oC).

The diameter of each zone around the well was measured with a caliper rule for accuracy as the degree of sulphate (SO_4^{2-}) and nitrate (NO_3^-) mobilization in milliliters by the bacterial species.

Paint degradation by bacterial extracellular enzyme

The submerged fermentation enzyme production by microorganisms in a liquid medium as described by Ugochukwu *et al.*, (2008), Agrahara and Wadhwa (2010 and Praveen *et al* (2012) was used to produced the extra and intracellular enzymes. The paint degradation assay followed the method described by Odukuma and Dickson (2002). The percentage analysis of degradation was done every 5days (incubation period) during which period a flask was removed and the rate of degradation determined with a spectrophotometer (721G Visible Spectrophotometer, Searchtech Instrument) and calculated as described by Etim *et al.*, (2007).

Determination of ultimate (dissolve oxygen level (DO) degradation of latex paint

In this procedure, 20mL of filter sterilized latex paint was dispensed into twenty 50mL capacity Erlenmeyer flasks. A set of 5 flasks representing each test bacterium were inoculated with respective bacterial (*Pseudomonas, Micrococcus* and *Bacillus*) species.

The last set of 5 flasks were left uninoculated to serve as controls. The sets of inoculated and uninoculated flasks were incubated at room temperature (28^{o}C) for 25 days incubation period. After every 5days, the content of a set of representative flasks including the control were assayed for dissolved oxygen (DO) using a calibrated Dissolved Oxygen meter as index of ultimate biodegradation of latex paint.

Statistical analysis

The data collected were subjected to mean calculation, percentage determination and correlation analysis of variance (ANOVA) using Statistical Analysis System Generalized Linear Model (SASGLM, SAS version 8.02 (SAS<2000). Results are discussed based on the various statistical conclusions and recommendations put forward accordingly.

Results

The result as presented in table I indicates that sulphate and nitrate mobilization by the bacterial species was significant ($p<0.01$) and varied among the species. However, sulphate was mobilized by the organism than nitrate. The trend of sulphate mobilization rate follows; *Micrococcus sp* > *bacillus sp* > *Pseudomonas sp.* while the reverse occurred with nitrate; *Bacillus sp* > *Pseudomonas sp* > *Micrococcus sp* statistically, there is no significant difference ($P<0.01$) between the rates of sulphate and nitrate mobilization among bacterial species.

The result presented in figure1 showed the ability of the test organisms to elaborate the necessary enzymes for degradation of paint. The result for each bacterium indicates a significantly ($P>0.5$) high level of paint degradation with increase in incubation period. Notably on the 25 day period, *Pseudomonas sp* caused about 92% degradation, followed by B*acillus* sp with 90% and *Micrococcus* sp with 83%.

Figure II represent the level of dissolve oxygen (DO) as index of ultimate biodegradation of latex paint in humid temperature. The results indicate that the bacteria species exerted significant ($P>0.5$) high degree of paint degradation.

However, at the 25 day period of incubation, the DO for *Bacillus* sp = 3.6 suggesting that *Bacillus* sp has higher degradability potential than *Pseudomonas* sp = 3.8 and *Micrococcus* sp = 4.1. The figure also demonstrated a high level ($r=0.01$) of correlation between the DO, incubation period and percentage rate of paint degradation.

Table 1. Sulphate (SO_4^{2-}) and Nitrate (NO_3^-) mobilization by bacterial isolates

Isolates	Agar absorption technique	
	Sulphate (SO_4^{2-})	Nitrate (NO_3^-)
	Mean zone of clearance (mm)	
Pseudomonas sp	12.60	11.10
Micrococcus sp	20.20	10.60
Bacillus sp	18.90	19.40
Values are mean of 3 readings		

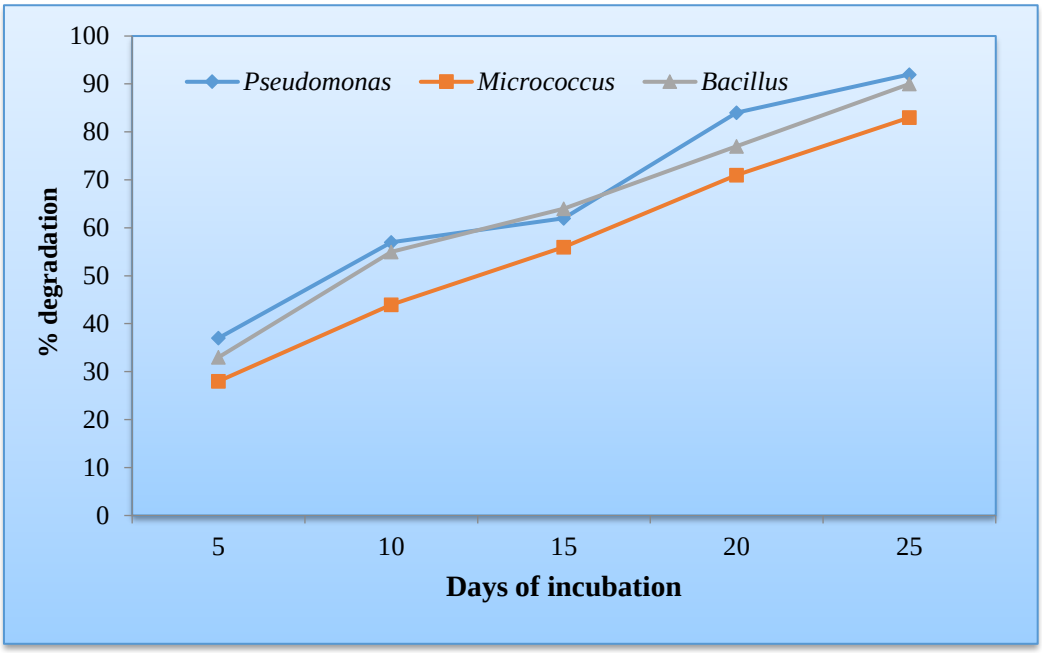

Figure 1. Enzymes production and degradation of latex paint by bacterial isolates

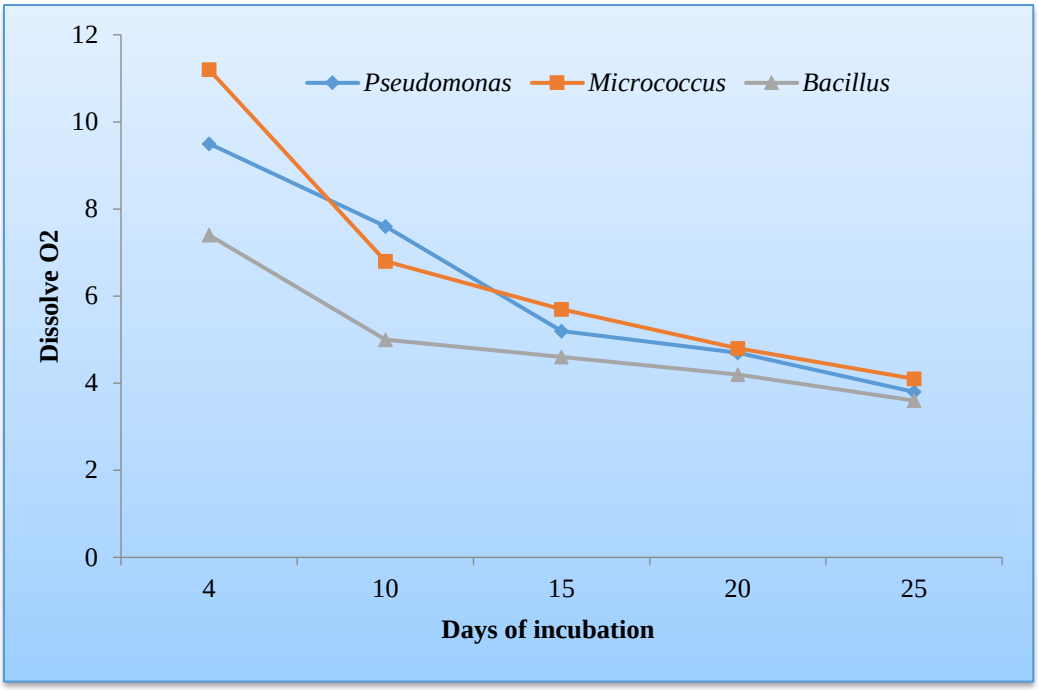

Figure 2. Ultimate latex paint degradation rate by the bacterial isolates

Discussion

The presence of detectable cations and anions as reported by Akanigbo and Jidere (2002) is indication that paint may serve as efficient source of carbon, nitrogen, phosphates, sulphates and nitrates for microbial activities and metabolism.

Therefore, the significant rate ($P>0.10$) of sulphate (SO_4^{2-}) and nitrate (NO_3^-) noted in this study makes paint as a substrate acidic in a highly humid and moisture laden condition. The effect of these ions as micro and macro nutrients for bacterial growth and proliferation has been previously demonstrated by Bock *et al.*, (1988).

The author stated that *Pseudomonas* sp and *Bacillus* implicated in this study has been considered a sulphur-cycling and nitrifying bacteria hence their ability to mobilized sulphate and nitrate as shown in this study. Also the level of sulphate and nitrate uptake by *Pseudomonas, Micrococcus and Bacillus* spp is in concert with the report of Nicell (2001) and Naranjo *et al.,* (2007). The authors reported that at a certain range of pH brought about by the presence of sulpahte ions supported microbial proliferation in a carbon base medium, excessive biomass production and increase rate of enzyme catalyzed reaction.

The results obtained showed that the cells; *Pseudomonas, Micrococcus and Bacillus* spp produced the necessary enzymes for the breakdown of the paint. Kang *et al.,* (2004) and Josephine *et al.,* (2012) had reported that the breakdown of latex paint is a function of the following extracellular enzymes; cellulose, protease, hemicellulase, liquase, chitinase and glucase. These authors further confirmed that enzymes produced as those produced by the test bacterial cells; *Pseudomonas, Micrococcus and Bacillus* spp are in direct correspondence to the material composition of the paint as a substrate.

In other words, Praveen *et al.,* (2012) and Charitha and Kumar (2012) agreed that the breakdown of paint by *Pseudomonas and Bacillus* spp have been considered to be an enzymatic reaction. The results obtain in this study using extracellular enzymes agrees with the above reports. The selected test bacteria *Pseudomonas, Micrococcus and Bacillus* spp are bacteria already identified as bacteria with high capacity to produce large quantity of different kinds of enzymes even in a commercial scale (Ariffin *et al.,* 2006). Secondly, the rates of catalyzed reactions by the enzymes are high but differ among the organisms. The study has shown that the enzymatic degradation rate varied among the three test bacteria. From the results, *Pseudomonas* species degraded paint more than *Bacillus* while *Micrococcus* exhibited the least. Badal (2004) in his investigations on the role of enzymes in the remediation of polyphenol, nitrite, PAHs and heavy metals in soil demonstrated that enzymes extracted from *Pseudomonas* species exhibited higher oxidative degradation potential on the pollutants than *Bacillus* species.

Biodegradation is predominantly an oxidation process. The evaluation of dissolve oxygen (DO) as an index of paint ultimate biodegradation has been established. The result obtained indicated that the level of dissolved oxygen declined in correspondence with increase in incubation period (days). This affirmed the report put forward by Linder (2005) that the oxidative reaction caused by microbial activities in paint could result to paint discoloration, viscosity loss, gassing, pH changes, frothing and deordouration. Similarly to this, the autotrophic and nitrifying bacteria including those implicated in this study oxidized ammonia to nitrate hence the biological induced corrosion of painted structures (Bock *et al*.,1988). Equally, Nweke and Okpokwasili (2003) had suggested that aerobic biodegradation of hydrocarbon and paint leads to the production of organic acids with its attendant toxic effects on the activities of the bacteria.

Conclusion

The study results attest to the facts that mobilized sulphate and nitrate ions acted as sources of electron acceptors for the bacterial degradation of paint. Similarly, the bacterial produced enzyme initiated the attack on the paint. Therefore, the bacterial enzyme catalyzed the insertion of dissolved oxygen into the paint so that the paint molecules were consumed by cellular metabolism as index of ultimate paint degradation. The dissolved oxygen (DO) is important for paint degradation because the major pathways for pigment solvents and additives involved molecular oxygen or oxynase among other extracellular enzymes.

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