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Paint Viscosity Evaluation and Preservation against Bacterial Species Contamination Using Extracts of *Bryophyllum Pinnatum*

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Abstract:

Viscosity and viscosity control of microbial contaminated latex paint was evaluated using the ball and cylinder method, MBC and MIC. The contaminated bacterial genera isolated were that of *Pseudomonas*, *Bacillus* and *Micrococcus*. The viscosity instability and loss caused by the organisms was obvious and significant ($p < 0.01$) in the order; *Pseudomonas* > *Micrococcus* > *Bacillus* spp. Their susceptibility to the phytoextract of *B. pinnatum* as a means of preservation was high, positively correlated ($r = 0.05$) and significant ($p < 0.01$). Also, the phytoactive activity significantly ($p < 0.01$) reduced viscosity loss as compared with the control. The MIC and MBC levels ($250\text{mg}/100\text{mL} < 500\text{mg}/100\text{mL}$) and ($500\text{mg}/100\text{mL} > 250\text{mg}/100\text{mL}$) demonstrated an excellent preservative properties of *B. pinnatum* against activities of *Pseudomonas* sp, *Micrococcus* sp and *Bacillus* sp. The result obtained indicated that latex paint subjected to bacterial viscosity loss can be controlled with phytoactive agents that could significantly bring about paint preservation. Therefore, empirical formulation of plants extract with high antibacterial potency can be supplemented in paint manufacturing process to control at first hand bacterial paint viscosity loss that often resulted to paint quality and financial loss.

Keywords: Viscosity loss, phytoextract, paint molecule, degradation, preservation

1. Introduction

Paint is a mastic composition of pigments, filters, thickeners, binders, oil and solvents. Each of these components contain a wide range of organic and inorganic constituents that provide different ecological niches exploited by a large variety of microbial species. These molecules as a substrate support the growth of microorganisms such as bacteria, fungi and algae under favourable conditions e.g. humidity, temperature, light and to some extent pH (Etim and Antia, 2014). The microbial attack on paint often results in colossal loss in value, aesthetics and financial loss.

Microbial degradation problems associated with paint include viscosity loss, malodouration, discoloration, gassing, frothing, sedimentation and pH change. Viscosity loss occurs as a result of pH changes and the presence of cellulose as the source of carbon-energy required for the growth of bacteria. Bacterial utilization of the cellulosic ether is considered the primary cause of the decrease in paint viscosity. Most microbial cellulases as adapting enzymes occur as a typical extracellular enzyme (Banik and Prakash, 2004).

These extracellular enzymes enable the organisms to metabolize large paint molecules found outside the cell. Therefore, cellulosic bacteria including *Pseudomonas* species produced cellulase either in a solution or on painted film surfaces (Naranjoret al., 2007).

Bacterial growth on paint and painted surfaces is invisible to the eye compared to that of algae and fungi. Bacteria are often the first to colonize paint and painted surfaces. The bacteria species frequently isolated from paint and painted objects are *Pseudomonas*, *Arthrobacter*, *Streptomyces*, *Sarcina*, *Bacillus*, *Micrococcus*, *Norcadia*, *Flavobacterium* and *Alcaligenes* spp (Atenburger et al., 1996; Etim and Antai, 2014). Therefore, bacterial colonization of paint film destroys the decorative properties, durability potential of paint film, can paint and irreversible loss of viscosity. *Bryophyllum pinnatum* is a succulent perennial shrub implicated in ethnomedicinal practices (Ofokansiet al., 2005; Etim et al., 2016). The plant is confirmed in various studies to contain a high level of polyphenolic compounds with antimicrobial potency. *B. pinnatum*'s antimicrobial potency favours the treatment of bacterial infections caused by *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella aeruginosa* and *Klebsiella pneumonia* (Okwu and Josiah, 2006). The study therefore attempts to evaluate viscosity loss control and paint preservation against bacterial species contamination using phytoactive properties of *Bryophyllum pinnatum* extract.

2. Materials and Methods

2.1. Paint Effluent

Paint effluent used for the isolation of the test cells (*Pseudomonas* sp, *Bacillus* sp, *Micrococcus* sp) species was collected from a paint industry located at Calabar Free Export Processing Zone (EPZ). The sample was collected into a 2.0L capacity sterile plastic bottle in an ice packed cooler (4°C) and transported to the laboratory for microbial analysis within 2 hours.

2.2. Paint Sample

Four (4.0L) liters sealed container of latex (Everbright) paint was bought from the paint store located within the Calabar municipality. The container was thoroughly checked for possible leakage and damage that may lead to the contamination of the paint sample.

2.3. Phytochemical Agent

Fresh leaves of *Bryophyllum pinnatum* was obtained from a botanical garden, in Calabar, Nigeria. The leave sample was washed, pulverized and dried in an oven at 45°C to dehydrate the sample for excess water for 2 weeks (Shahidi, 2004; Etim et al., 2016). The dried material was milled into fine powder and preserved in an airtight plastic bottle and stored at room temperature (28 ± 2°C).

2.4. Isolation and Identification of Bacterial Contaminants

One (1.0mL) milliliter of paint industrial effluent was cultured on nutrient agar for 18 hours. The colonies developed were discretely picked and purified by subculturing on nutrient agar. Subsequently, the method described by Bharathi and Vasuduvan (2001) was adopted to conduct microscopic, macroscopic, biochemical reactions and sugar fermentation on the 18 hours old isolates as a means of identification.

2.5. Determination of Viscosity Loss in Paint Contaminated by Bacterial Species

The viscosity loss of paint by *Pseudomonas*, *Bacillus*, *Micrococcus* spp contamination was determined using the ball and cylinder method described by Okeke et al. (2006). In this method, a tall glass graduated cylinder and a metal ball of a known weight (density) and diameter was used. Ten 250mL capacity Erlenmeyer flasks were filled with 200mL of filter-sterilized latex paint. A set of 5 flasks were inoculated with 5.0mL McFarland standard (approximately 1.5 to 2.0×10^8 cfu mL⁻¹) of an overnight (18 hours) broth culture of *Pseudomonas*, *Bacillus*, *Micrococcus* spp. Another set of 5 flasks containing paint were uninoculated to serve as controls. The contaminated and control flasks were incubated on a shaker at room temperature (28 ± 2°C) for 25 days. Every 5 days, duplicate flasks of contaminated and control samples were removed and the extent of viscosity loss was determined in triplicate using the ball and cylinder method.

2.6. Control of Viscosity Loss in Paint Using Phytoactive Agents of *B. Pinnatum* against the Activities of *Pseudomonas*, *Micrococcus*, *Bacillus* Spp

The ball and cylinder method described above was used. Ten 250mL capacity Erlenmeyer flasks were filled with 200mL of filter-sterilized latex paint. A set of 5 flasks were inoculated with 5.0mL of an overnight (18 hours) broth cultures of *Pseudomonas* spp, *Micrococcus* sp, *Bacillus* sp and supplemented with 500mg of *B. pinnatum* aqueous extract. Another set of 5 flasks without cells and extract treatments were prepared and set aside as control. Then the inoculated and extract treated flasks were incubated at room temperature (28 ± 2°C) on a shaker for 25 days. Every 5 days, each representative flasks and control were removed and the extent of viscosity was determined.

2.7. Antibacterial Potential of *B. Pinnatum* on the Isolates

The aqueous extracts of *B. pinnatum* were prepared using soxhlet extraction to obtain the bioactive compound as described by Brooks and Etim (2004) and Achi (2006). The dehydrated extracts were resuspended in dimethylsulfoxide (DMSO) to prepare various concentrations of aqueous extract which was subsequently used in the susceptibility assay (Achi, 2006). An 18-hour broth culture of each organism was diluted with sterile physiological saline (0.85% w/v sodium chloride) and the cell concentration compared to 0.5 McFarland standard (approximately 1.5 to 2.0×10^8 cfu mL⁻¹). Then 3.0mL portion of the test cell was placed onto the surface of a pre-dried Mueller Hinton (Biomark Laboratory, India) agar plates and spread around evenly with a sterile bent glass rod. Then cut sterilized 6mm Whatman No. 1 filter disks were used to absorb approximately 1.5mg/100mL of the suspended (DMSO) extract and was placed on top of the agar surface (Aida et al., 2001).

The plates were allowed to stand for 15 minutes at room temperature (28 ± 2°C) for the extracts to diffuse across the surface of the agar before incubation at 35°C for 18 hours. The diameter of the zone of inhibition was measured with a meter rule in triplicate (Choet al., 1995).

2.8. Determination of the Preservative Property (MIC, MBC) Of *B. Pinnatum* phyto bioactive Agents against Bacterial Species Contaminated Paint

The preservative potential of *B. pinnatum* extract on paint contaminated by microbial species was determined by estimating the total viable count (TVC) of paint contaminant as described by Achi, (2006). Fifteen 250mL capacity flasks were suspended with 98.0mL of distilled water and autoclave at 121°C and 15psi. On cooling, 5.0mL of pressurized filter

sterilized latex paint, 0.2ml of *Pseudomonas* cells (approximately 1.5 to 2.0×10^8 cfu/mL) and 250mg/100mL and 500mg/100mL of aqueous extracts of *B. pinnatum* were added separately to 2 subsets of 5 flasks respectively. Then the third subsets of 5 flasks without the extract were set aside as controls. Each of the flasks were arranged and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 25 days on a shaker, rotating at 110rpm.

At interval of 5 days, 10.0mL of representative sample from each flask was aseptically drawn and was used to determine the total viable count (TVC) in ten-fold serial count as an index of paint preservation against the bacterial contaminants.

2.9. 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.

3. Result

The rate of viscosity loss created by *Pseudomonas*, *Micrococcus*, and *Bacillus* spp as contaminants is presented in Figure 1. *Bacillus* sp exerted the greatest loss then followed by *Micrococcus* and *Pseudomonas*. The extracts produced strong growth inhibitory effect on *Pseudomonas* sp than *Micrococcus* and *Bacillus* spp as presented in Figure 2. The rate of viscosity loss by each contaminant and the inhibitory effect of the plant extract were significant ($p > 0.10$) with increase in incubation period. As a result, the paint became liquidified and less viscous. Secondly, the time the ball travel through the contaminated paint column varied tremendously and positive in ($r = 0.10$) relation to incubation period.

The antibacterial and preservative efficacy of phytoextracts of *B. pinnatum* against the activity of paint degraders is presented on Figures 3 and 4. Results obtained indicated that the extract concentration as MIC (250mg/100mL and 500mg/100mL) exhibited different degrees of toxic effect on the paint degraders. The 500mg/100mL concentration exhibited twice the toxic effect on the cells than 250mg/100mL. *Pseudomonas* sp (16.6-23.8cm) appeared to be the most sensitive with significant ($p > 0.01$) inhibitory or preservative effect at 500mg/100mL. For example, on day 25, the mean effect of *B. pinnatum* for the isolates were *Pseudomonas*=23.8 > *Bacillus*=14.6 > *Micrococcus*=14.3. with significant ($p > 0.01$) shift in cell count. In each case, the cell count (MBC) significantly ($p > 0.01$) dropped with increase in incubation period with reference to the control. The control of the test cells induced viscosity loss in latex paint by *B. pinnatum* phytoactive aqueous extract is presented in Figure 5.

4. Discussion

Paints composed of inorganic and organic compounds are eminent substrates for the growth and multiplication of microorganisms. Sliveet al., (1999) reported that microbial flora, transient or permanent colonizing paint and painted structures depends among other factors on the chemical nature of the substrate and biochemical reactions catalyzed by the microbial species e.g. *Pseudomonas*, *Micrococcus* and *Bacillus*. This therefore accounts for the presence of *Pseudomonas*, *Micrococcus* and *Bacillus* spp obtained in the study.

Bryophyllum pinnatum is reported to possess a variety of secondary bioactive phytochemicals such as bryophyllin, potassium malates, ascorbic, malic, citric acids, phenolic compounds, riboflavin, thiamin and niacin (Ofokansiet al., 2004). The presence of phenolic compounds and flavonoids indicates that the *B. pinnatum* is antimicrobial and antioxidant. Recently many authors have established that these phenolic properties of *B. pinnatum* were responsible for the effective treatment of *Pseudomonas aeruginosa* and other Gram-positive organisms' infection (Okwu, 2001, 2003, 2004). The result obtained correlate with these reports. *B. pinnatum* in this study demonstrated high degree of antimicrobial potency on these test organisms. The degree of antibacterial potency of *B. pinnatum* inhibits significant growth of the bacterial species. Consequently, reduction in growth rate significantly correlates ($r = 0.05$) with the decrease in paint viscosity loss. This suggests that the activities of the organisms against the paint thickener (the organic material responsible for paint body stability) was relatively inhibited and controlled by the phenolic compounds in *B. pinnatum*. The preservative properties of *B. pinnatum* on paint against bacterial contamination are considered a function of extract concentration (MIC and MBC), type of extract and incubation duration. The higher extract concentration (500mg/100mL) is considered to contain higher amount of the phenolic compounds. The higher extract concentration is considered to be responsible for the high toxic level resulting in double cell count reduction per incubation time.

5. Conclusion

Latex paint by its composition is rich in various forms of carbon that encourage bacterial growth and energy. The presence of *Pseudomonas*, *Bacillus*, and *Micrococcus* species as contaminants resulted in paint viscosity loss. However, the use of the phytoactive agent from *Bryophyllum pinnatum* extract effectively reduced the rate of this loss. Therefore, empirical formulation of the extract could be applied in paint preservation measures.

Figure 1: Determination of Viscosity Loss of Latex Paint by Bacterial Isolates

Figure 2: MIC (250mg) *B. Pinnatum* Extract on Bacterial Isolates: Preservative Effect of *Bryophyllum Pinnatum* Extract against *Pseudomonas* Cultures on Latex Paint

Figure 3: MIC (500mg) *B. Pinnatum* Extract on Bacterial Isolates

Figure 4: Preservative Effect of 500mg (MIC) of *B.Pinnatum* Extract on Bacterial Induced Viscosity Loss on Paint

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