Microorganisms Survive In Paints

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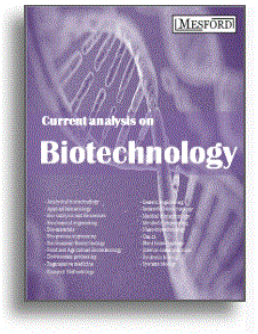

Abstract:

Paints are great carriers of microbes because several factors act on it. Bacterial and fungal growth in painted surfaces and unused paints were studied. Bacteria isolated from these paints were Bacillus sp., Pseudomonas sp., Micrococcus sp. and Serratia sp. while fungi isolated were Aspergillus niger, Aspergillus fumigatus, Rhodotorula sp and Aspergillus flavus. Emulsion paints had total viable counts ranging from 0 to 5.4 x 105, its optical density ranged from 0 to 4.4 and reduction in pH was from 8.5 to 4.8. While for the gloss paints, total viable counts were from 0 to 6.2 x 105, its optical density was from 0.46 to 5.6 and its pH reduced from 9.1 to 5.6. Statistical analysis indicated that there was a significant difference ($p>0.05$) in the growth of microorganisms between painted surfaces and unused paints and between emulsion and gloss paints. Combined effects of microorganisms (bacteria and fungi) had a greater effect on paints than either of them reaching individually. Microorganisms utilized painted surfaces more than unused paints. Temperature is one of the environmental factors that promote paint degradation. The use of biocides and reduction of contamination by microorganisms during the production of paints should be compulsory to ensure that paints are safe from microbial attacks. This research compares microbial growth between gloss and emulsion paints.

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Paints, unused, used, painted surfaces, bacteria, fungi.

1. INTRODUCTION

A synthetic substance which gives texture to infrastructure, furniture, and utensil of everyday life is called paint [1]. Paints are correctly distributed mixtures that have a thin liquid to a semi-solid paste viscosity and have a pigment such as oil or water which serves as the vehicle. Paint can be applied as a thin coat to various surfaces such as wood, metal, or stone with a brush, roller or spray gun. It is basically made of pigments, binder, solvents, and certain additives. Paints can be in either emulsion or oil based formulations [2] i.e., Paints basically protect surfaces from corrosion, oxidation, environmental weathering or other types of deterioration and they also provide decorative finish [3,4]. Paints are made up of vehicle, pigment, additive and solvent [3]. These act as a carbon source for many species of microorganisms. The contamination of paints by microorganisms can come from sources such as raw materials, manufacturing plant process units and packaging materials [5]. Melzer stated that "Paint has become the most essential item in modern times, whether it is meant for residential purposes or industrial applications" [6]. Therefore, it is important that paint-life; as a dry film, has to be as long as possible. Bacterial, fungal and algal attacks are of concern to the paint industry. Therefore to control these microorganisms, makers of coatings add microbiocides or paint preservatives such as bactericides, fungicides and algaecides. A good biocide provides an in-can protection and a consolidated layer on applied surfaces [7]. Microorganisms damage the layers painted surface by causing discoloration, by increase of porosity of the layer, decrease in physical resistance and also by allowing moisture to easily penetrate through the surface [8]. The stages at which contamination occurs in paints are the manufacturing stage and in storage as a product [9]. Microorganisms and its activities can degrade water based house paints and this can reduce the shelf-life of the paints [10].Thus, quality of paints gets negatively affected.

Nutritionally, non-exacting fungi and bacteria attack water-borne coating paints because of the use of recycled water which can be a source of contamination. Paint is a liquor blend

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and is the main source of volatile organic compounds (VOCs). Hence, it is very harmful to the environment and human beings. The deterioration of painted surfaces causes it's components to be mineralized, resulting in surface corrosion. This surface corrosion results in the release of harmful degradation products in environment causing a negative effect. Surface corrosion of paints also lead to economic loss [11]. A volatile organic compound (VOCs) which is an organic chemical is used in paints as solvents [12] and they may cause short and long-term environmental effects [13] and may also lead to respiratory, allergic, or immunogenic defects in humans [14]. Microorganisms use the constituents of paints as a source of food and energy. The spoilage of these paints lead to unpleasant looks on the buildings.The objectives of this study were to compare the microbial counts between emulsion and gloss paints and also between painted surfaces and unused paints, to identify the class of paint that harbors more microorganisms between samples and to also monitor the optical density, pH, and temperature for a period of time so as to make a conclusion on the spoilage potential of microorganisms.

2. MATERIALS AND METHOD

Unused emulsion and gloss paints were bought from paint shops at Timber market, Umuahia, Abia State, Nigeria while used emulsion and gloss paint scrapings, were obtained from surfaces that were painted 6 month ago. Three to four drops of the unused paints were added to 9ml tryptone broth, this was incubated for 48hours after which serial dilution was conducted. One gram of the painted surface sample was also soaked into 9ml of tryptone broth. This was done to promote the growth of microorganisms. From this test tube (stock solution), a serial dilution was carried out.

Spread plating of the 0.1ml aliquot was allowed into Petri dishes containing nutrient agar and Sabouraud Dextrose agar for the growth of bacteria and fungi respectively. Colony counts were done using a colony counter. The microorganisms grown on these Petri dishes were isolated as pure cultures and stored in the appropriate slants. Proper identification of microorganisms was carried out from this using biochemical tests.

2.1. Growth of Isolates at Various Temperatures

Isolates were examined for their ability to grow at different temperatures. These isolates were inoculated singly into tubes containing nutrient broth. The tubes were incubated at 30oC, 45oC, 55oC, 60oC and 70oC.

2.2. Screen test for the Utilization of the Paints by the Bacterial and Fungal Isolates

Paint utilization was checked for using each bacterial or fungal isolates and also using the modified mineral salt medium of Mills et al. [15] containing paint samples as the sole carbon and energy source. Modified mineral salt medium contained: NaCl: 10.0 g, MgSO4.7H2O: 0.42 g, KCl: 0.29 g, KH2PO4: 0.83 g, NaHPO4: 1.25 g, NaNO3: 0.42 g, distilled water: 1L, pH 7.2. The mineral salt medium was put into 9.9 ml test tubes. This was shared into halves. To half of these tubes, 0.1ml each of painted surfaces was added and to the other half, 0.1ml each of unused paints was added. The tubes were sterilized by autoclaving at 121°C for 15 minutes and allowed to cool. After cooling, each set of tubes were inoculated with the corresponding isolates. The tubes were incubated at room temperature for 18 days and turbidity was checked after the incubation period.

2.3. Growth Monitoring of the Microbial Isolates in Paint Samples

The number of positive tubes from the screen test was used to determine the number of mineral salt medium Erlenmeyer flasks. 1ml of used paint was added to the 250ml flask and the mixture was autoclaved at 121°C for 15 minutes and then allowed to cool. The isolates with the highest turbidity from the screen tests were used as pure cultures to inoculate the different flasks. The control flask remained uninoculated. The procedure was some for unused paint samples. The flasks were incubated at room temperature on a rotary shaker at 140 rpm/min for 18 days. The optical density (OD) was at 560 nm using a spectrophotometer, total viable counts and pH of the culture in each flask using a benchtop pH meter was monitored at intervals at day 0, day 6, day 12 and day 18. The graphs are shown as Figs. (**1-8**).

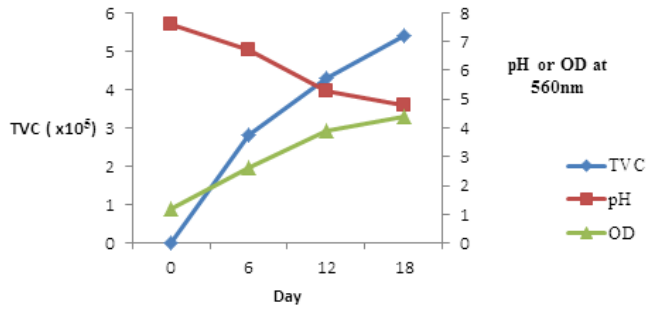

Fig. (1). A graph of TVC, pH and OD against Day of mixed bacterial and fungal colonies from saclux emulsion painted surfaces.

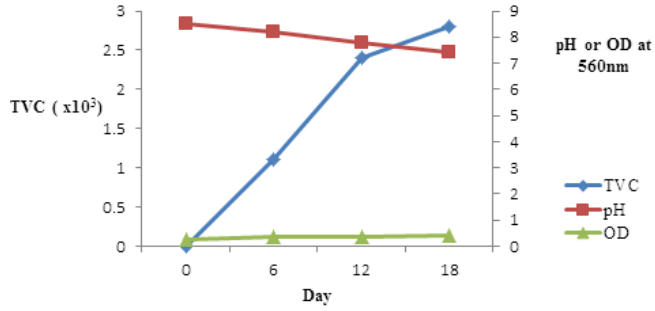

Fig. (2). A graph of TVC, pH and OD against Day of mixed bacterial and fungal colonies from unused saclux emulsion paint.

3. RESULTS

Total viable counts for unused paints ranged from 1.1 x 103 to 3.6 x 103 cfu ml-1 while total viable counts from painted surfaces ranged from 2.8 x 105 to 6.4 x 105 cfu ml-1. Bacillus sp., Pseudomonas sp., Micrococcus sp. and Serratia sp. were the bacteria isolated from the paint samples and the fungi were also isolated. These fungi were A. niger, A. flavus, Rhodotorula sp., and A. fumigatus. The result after screen test for the utilization of painted surfaces and unused paints as sole source

Table I. Result after a Screen Test

Microbial Isolates		Painted Surface with S.E	Unused B.E	Painted surface with B.O	Unused S.O
Bacterial isolates	*Bacillus* sp.	+++	+	+++	+
	Pseudomonas sp.	+++	+	++	-
	Micrococcus sp.	++	+	-	
	Serratia sp.	++	-	+	+
Fungal isolates	*Aspergillus fumigatus*	++	-	+	-
	Aspergillus flavus	++	-	-	+
	Rhodotorula sp.	+	-	-	-

Key: +++ for high growth, ++ for moderate growth, + for little growth, - for no growth. S.E for Saclux emulsion, B.O for B-Lux Oil, B.E for B-Lux Emulsion, S.O for Saclux Oil.

of carbon and energy by bacterial and fungal isolates is presented in Table **I**.

Serratia sp., Aspergillus fumigatus, Aspergillus flavus, Rhodotorula sp. did not show any turbidity in the screen test culture for unused paints. Microorganisms that did not show any turbidity were streaked out on the appropriate agar plates to check for viability. The growth that occurred on all plates, streaked showed that the organisms were still alive but were not able to use the paint as the only carbon source for their growth.

At 30oC and 45oC, all microorganisms that were isolated, grew. The growth began to decrease at 550C. This is represented in Table II. Organisms which did not grow at 60oC to 70oC are microorganisms that cannot survive harsh environmental conditions (temperatures). Some organisms were alive but did not grow. These microorganisms grow properly when the temperature becomes favorable.

As optical density and total viable count increased, pH decreased and this research also showed that used paints had higher counts than unused paints and gloss paints also had better growth than emulsion paints. This also revealed the rate at which these microorganisms used these paints as the source of carbon and energy (Figs. **1** to **8**).

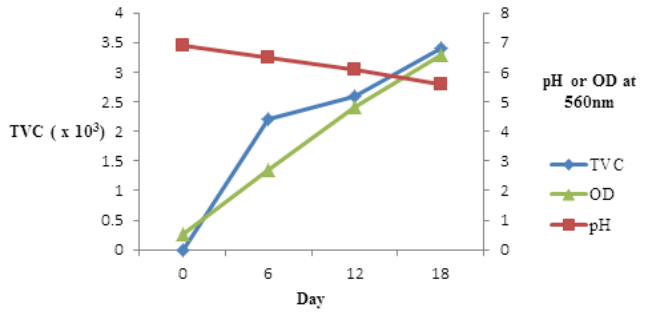

Fig. (3). A graph of TVC, pH and OD against Day of mixed fungal colonies from painted surfaces with B-lux emulsion paint.

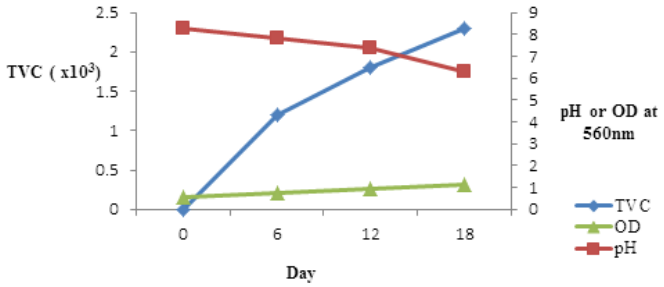

Fig. (4). A graph of TVC, pH and OD against Day of mixed fungal colonies of unused B-lux emulsion paint.

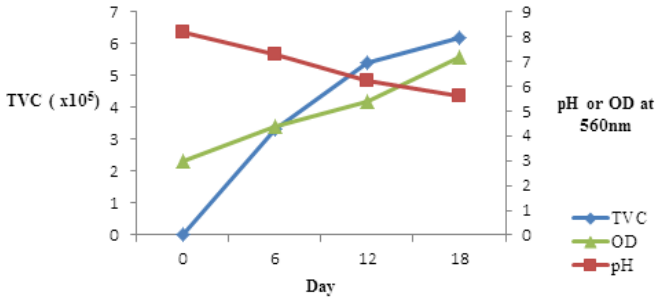

Fig. (5). A graph of TVC, pH and OD against Day of mixed bacterial and fungal colonies from painted surfaces with saclux gloss paint.

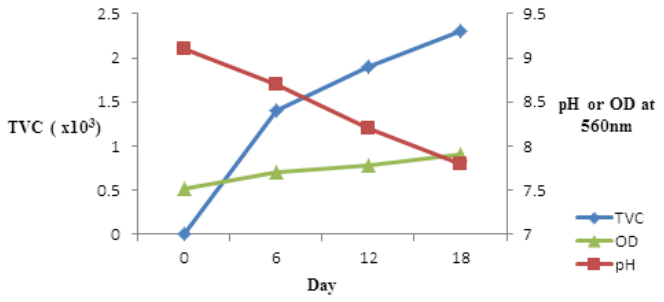

Fig. (6). A graph of TVC, pH and OD against Day of mixed bacterial and fungal colonies of unused saclux gloss paint.

Table II: Microbial growth at different temperatures at 48 hours interval.

Isolates	30°C	45°C	55°C	60°C	70°C
Bacillus sp.	+	+	+	+/-	+/-
Pseudomonas sp.	+	+	+/-	-	-
Micrococcus sp.	+	+	+/-	-	-
Serratia sp.	+	+	+/-	-	-
Aspergillus niger	+	+	+/-	-	-
Aspergillus fumigatus	+	+	+	+/-	+/-
Aspergillus flavus	+	+	+	+/-	-
Rhodotorula sp.	+	+	+/-	-	-

Key: + for growth, - for no growth, +/- for viable but no growth
No growth was noted at 80oC and 90oC for all isolates

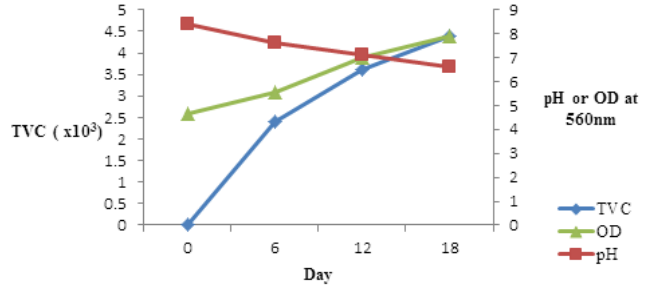

Fig. (7). A graph of TVC, pH and OD against Day of mixed fungal colonies from B-lux gloss painted surfaces.

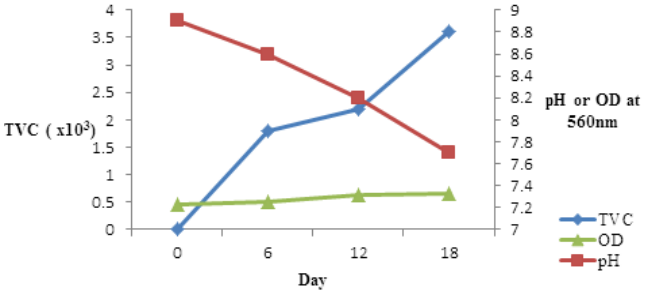

Fig. (8). A graph of TVC, pH and OD against Day of mixed fungal colonies of unused B-lux gloss paint.

4. DISCUSSION

Some microorganisms recorded in the paint samples could be due to the packaging of paints or during usage on surfaces. Obidi et al. [16] recorded that bacterial contaminants isolated in the paint-products included Bacillus brevis, B. polymyxa, B. laterosporus, Lactobacillus gasseri, L. brevis, Esherichia coli, and Proteus mirabilis and the fungal contaminants detected in the paints were mainly Aspergillus niger, A. flavus, and Penicillium citrinum. Ma et al. [17] in their research from ancient cave wall paintings of the Mogao Grottoes exhibits signs of biodeterioration isolated the bacterial groups such as Firmicutes, Proteobacteria, Actinobacteria, Acidobacteria, Cyanobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, and Chloroflexi were found and the fungal groups which were also isolated were Euascomycetes, Dothideomycetes, Eurotiomycetes, Sordariomycetes, Saccharomycetes, Plectomycetes, Pezizomycetes, Zygomycota, and Basidiomycota. Oyeleke et al. [18] in their work titled. "Isolation and characterization of microorganisms" associated with paints deterioration in storage identified microbes associated with the deterioration of paints which were bacteria such as Bacillus cereus, Bacillus subtilis, and Staphylococcus aureus and the fungi isolated were Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium notatum, Trichophyton megninii, Trichophyton rubrum, Pullularia species, Mucor species, Candida stalletoides, and Alternaria species.

Ogbulie and Obiajuru [19] identified Pseudomonas, Bacillus, Micrococcus, Staphylococcus, Enterobacter, and Streptomyces as bacterial genera and they also isolated fungal genera such as Rhizopus, Penicillium, Cladosporium, Aspergillus, Alternaria, Fusarium and Curvularia and they recorded the occurrence of microbial isolates in deteriorated painted surfaces as Pseudomonas (90:100%), Bacillus (80:100%), Rhizopus (60:100%) and Aspergillus (50:90%), Penicillium, Staphylococcus, Enterobacter, Aspergillus niger, Cladosporium, Alternaria, Streptococcus, Fusarium, Curvularia, and Micrococcus had between 20 and 70 % in the biodeteriorated samples. Oyeleke et al. [18] in their work had mean bacterial counts ranging from 3x105 to 3x106 while fungal counts were from 1.5x 103 to 4.0x 105. Shinkafi and Haruna [20] recorded mean bacterial counts between1.05x 103 to 9.4x 104.

Table **II** shows the test growth for isolated microorganisms at different temperatures. It was observed that at 30°C and 45°C, all isolates grew, at 55°C, 37.5% of the microorganisms grew, at 60°C, 62.5% did not grow, at 70°C, 62.5% did not grow and at 80°C and 90°C, 100% of the microorganisms did not grow

at all . At 55°C - 70°C, some microorganisms were viable but did not grow. Bacillus species which are spore formers can resist adverse temperature conditions by going into spore stage [21].

The screen test and monitoring test revealed that the microorganisms used for screening grew after the initial attack by primary microorganisms which utilized the breakdown of products of the paint after the previous attack had occurred (Figures 1 and 2). bacillus sp. and pseudomonas sp. showed the highest turbidity in the tubes, Micrococcus sp. and Serratia sp. were next (Table 1). This suggests that one of the causes of initial bacterial degradation of the paint may be by these strains [21].

Bacterial and fungal growth is usually seen in paints and coatings especially in the liquid state because it allows mainly fungi, algae, and cyanobacteria to attack after paints and coatings are used [22]. On a surface which appears clean, bacteria can be found in sufficient numbers and that lead to negative results. One of such example is the negative effect of the production of inorganic acids by concrete and metal corrosion[23, 24, 25] and by any other microbial metabolic activities which include the blistering of paint. Chemolithotrophic and oligotrophic bacteria can lead to a surface to be colonized by other microorganisms [25]. A fungus causes discoloration of building materials which can be seen with the natural eyes because they are highly colored.

5. CONCLUSION

Gloss paints are better habitats for microorganisms than emulsion paints. It is advisable that gloss painted surfaces should not be used for a longer period of time before re-painting. It is easier to observe the deterioration of emulsion paints and such a change of paint is necessary. Together, bacteria and fungi have greater negative effect than each of them acting alone. It is also advisable to purchase paints containing biocides. This will help to reduce microbial growth on painted surfaces to the lowest minimum.

CONFLICT OF INTEREST

There is no conflict of interest.

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