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Bacterial degradation of paint

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Abstract
Paint is mainly used for two purposes like decoration and protection but it is the main source of volatile organic compounds which may cause short or long term impacts on environment, which also leads to health problems such as nerve and kidney damage. Ozone is formed when nitrogen oxides, carbon monoxide, and VOCs react in atmosphere in the presence of sunlight these chemicals cause respiratory problems and carcinogenic. Bacterial organisms such as Pseudomonas spp, Bacillus spp and Staphylococcus spp were isolated from the paint flakes. Shake flask experiment was carried out to assess the paint degradation ability of these microbes. The experiment was carried out for 14 days under 37°C with continuous shaking to provide good aeration to for the degradation and growth of microorganism. Characterization of paint sample before and after treatment were studied using FT IR and SEM analysis. FTIR analysis reflected a shift in the functional groups, due to degradation activity of microbes. SEM analysis clearly showed the adherence and bacterial growth on paint flakes. The treated samples evidently showed distorted / ruptured surface when compared to the untreated samples which had intact structures of the paint compounds.

Keywords: paint, bacterial organisms, shake flask experiment, FTIR and SEM analysis

1. Introduction
Paint is one of the oldest synthetic substances known to mankind. During pre-historic time's clay and chalk were mixed with animal fat and used to depict hunts on the cave wall. Paint has become the most essential item in the modern times. Decoration and protection are the two main purposes of all paints. Paint is used to protect the surface to which they are applied from corrosion, oxidation, and decoration. It is the main source of volatile organic compounds which may cause short and long term environmental impacts. Paint also contains high level of mercury and which may lead to health risk when ingested (Mendell, 2007) [4]. Ingestion may lead to serious health problems such as nerve and kidney damage, some paints also containing Antifouling compounds like Tributylin [TBT] which is highly toxic to marine life. (Omae I, 2003), (Talbert & Rodger, 2007) [2].

1.1 Components of paint
Paint is essentially a mixture of binder, pigments, solvents and additives. The binder is to hold the pigment particles together and provide adhesion to paint surface. Water based paints use acrylic emulsion as their binder (Bently, J, 1997) [9]. The pigments gives paint a colour, opacity and occasionally prevent corrosion. Also helps to provide colour, hiding and control gloss. The solvents make the paint spreadable to acts as a carrier for the pigments and resin. The solvent may be organic or water. The additives helps to enhance certain properties such as brushing, mould resistance, resistance, drying, and sag resistance. (Spurgeon, 2006) [3].

1.2 Biodegradation of paint
Paints deterioration on surface or in the environment causes its components to be mineralized. This corrosion on the surface is not only an economic loss, but also results in release of harmful products into the environment causing an alarming situation. Various microbial species are reported for paint degradation. Major group of microbes involved in paint degradation are bacteria and fungi. Example: *Pseudomonas sp., Aerobacter sp., Enterobacter sp., Proteus sp., Micrococcus sp., and* Streptomyces sp., etc., in Gram negative and *Clostridium* and *Lactobacillus sp.,* etc., in Gram positive bacteria. (Altenburger *et al.,* 1996) [5].

1.3 Enzymatic biodegradation
Due to lack of water solubility and large size of polymer molecules the microorganisms are unable to transport the polymeric materials directly into the cells, where most biochemical processes takes place. So they are forced to produce certain enzymes which could penetrate the polymer and degrade them into smaller transportable molecules. Enzymes play an important role in metabolism and detoxification. The enzymes may be extracellular or intracellular and their mode of action will be different. Enzymatic degradation occurs by catalytic process. The enzyme can be denatured quickly by changes in temperature or solvent or pH. Some enzymes require coenzymes. Coenzymes act as a donor or acceptor for a specific group. Hydroxylases and oxygenases are the main enzymes used. (Cifferi, O. 1999) [7].

2. Materials and methods
2.1 Collection of paint flakes
Different kinds of paint flakes were collected. The samples were collected, using a cutting tool. The paint flakes were removed from the substrate. Sample spillage was avoided and collected in separate containers**.** The paint flakes containers

were sealed. Similar paints were also commercially purchased for comparison study. The sealed individual containers were labelled neatly and transferred to the laboratory for further analysis.
C of microbial isolates:

2.2 Isolation of bacteria from paint flakes
Mineral Salt medium was used to isolate the bacteria from paint samples MSM Composition:

- Di potassium hydrogen phosphate - 1.0g
- Potassium di hydrogen phosphate - 0.2g
- Sodium chloride - 1.0g
- Boric acid - 0.005g
- Calcium chloride - 0.002g
- Ammonium sulphate - 1.0g
- Magnesium sulphate - 0.5g
- Copper sulphate - 0.001g
- Distilled water - 100 ml

MSM media was prepared by mixing the above composition in 100ml of distilled water, where paint acted as the sole source of carbon. Media was sterilized for 20minutes at 121°C at 15lbs. The flask was allowed to cool to reach the room temperature. The pH is checked and adjusted to 7.2 using 1N NaOH or 1N HCl. 1 gm of the paint flakes were added into the medium aseptically and incubated at 37°C for 24hours.

2.3 Enrichment of isolated of bacteria
Nutrient agar was prepared. 2.8g of nutrient agar and 1.5g of Agar agar was added to 100ml of distilled water. Sterilized at 121°C for 20minutes at 15lbs and plated in pre-sterilized petriplates and left for solidification. After the media solidified 0.1ml incubated MSM was transferred to plates and spread plate technique was carried out. The inoculated plates were incubated at 37°C for 24 hours.

2.4 Characterization of the isolated bacteria
The identification of bacteria was based on morphological characteristics and biochemical tests carried out on the isolates. Morphological characteristics observed for each bacteria colony after 24 h of growth included colony appearance; shape, elevation, edge, optical characteristics, consistency, colony surface and pigmentation. Biochemical characterizations were done according to the method of Fawole and Oso 2004 [12].

2.5 Shake flask experiment with Bacterial inoculum
The components of MSM were weighed and added to the conical flask. Different types of paint flakes were added aseptically. Later paint emulsion was added aseptically to flasks containing different organisms. Isolated and characterized bacterial organisms were also inoculated in the flasks. The flasks were incubated at the shaker for 14 days at 37°C. The optical density was noted at 600nm and analysed for the degradation ability of the isolated bacteria. The results were noted and checked for the degradation ability of the isolated bacteria.

2.6 FTIR (Fourier transform infrared spectrometer)
The chemical changes in the paint flakes due to biodegradation caused by the bacterial species was studied using Fourier Transform Infrared Spectroscopy (FTIR). The paint flake sample before and after degradation were studied after 14 days of incubation, the Shift in the functional groups before and after degradation and the structure of the paint were noted. The pieces of paint flakes were fixed to the FTIR sample plate. Spectra were taken at 400 to 4000 wave-numbers cm^{-1}.

2.7 Scanning electron microscope (SEM)
It is used to analyse the surface morphology. The samples were poured into eppendorffs. Centrifuged at 1000 rpm for 5 to 10 minutes. The pellet was collected, dried and washed with phosphate buffer solution. Later few drops of gluteraldehyde was added, left for overnight and rinsed with water, poured onto the surface of aluminium foil placed on petri plate and kept it in hot air oven for few minutes until dried. The sample was then given for analysis for SEM analysis to study the sample's surface topography, composition, and other properties such as electrical conductivity.

3. Results and Discussion
3.1 Isolation and Characterization of Bacteria from paint samples
Visual observation of bacterial treated paint samples compared with control indicates that paint color was faded and surface became ruptured and bacterial growth was also observed. Bacterial strains were screened out for the biodegradation of the paint on the basis of theits adherence on the MSM medium with paint as the only carbon source. Plate assay results showed the bacterial growth of *Pseudomonas sp.*, *Staphylococus sp. and Lactobacillus sp.*confirmed by morphological and biochemical studies.(Fig 2), (Table 1).
Optical density with bacterial cultures showed an increase in bacterial growth from 0 to 8th day. However, on 10thday, an inconsistency in growth was observed and decreased in growth rate. Maximum growth was detected on 6th and 8th day. Microorganism under stress condition utilizes synthetic organic matter as a energy source.

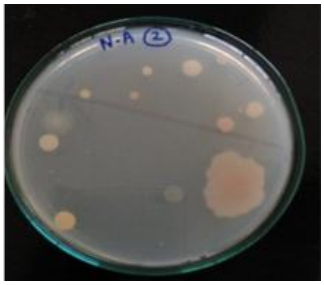
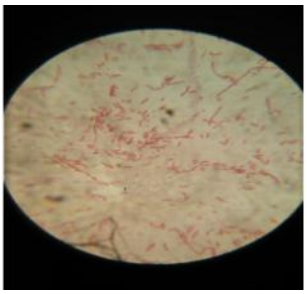


Fig 1: Nutrient agar plat showing the growth of colonies



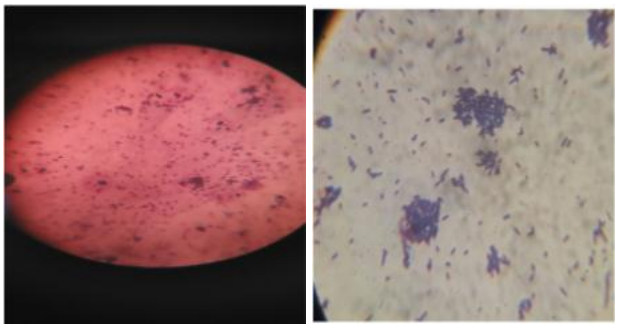


Fig 2: Microscopic view of organisms from different colonies obtained in Nutrient agar plate

Table 1: Morphology and biochemical characterization of the bacterial isolates

Biochemical Characters	Bacteria 1	Bacteria 2	Bacteria 3
Gram's staining	+	-	+
Shape	Cocci	Rod	Rod
Indole	-	-	-
MR	+	-	-
VP	+	-	+
Citrate	-	+	+
TSI	A/A	K/K	A/A
Gelatinase	+	+	+
Catalase	+	+	+
Oxidase		+	+/- (variable)
Urease	-	-	-
Probable Identity	*Staphylococcus* sp.	*Pseudomonas* sp.	*Bacillus* sp

Key: (+) = Positive, (-) = Negative, sp.= Species, A= Acid, AG=Acid-Gas, K- Alkaline

3.2 Shake flask experiment

Shake flask experiments were performed to degrade the paint sample. Flask A was used as control contained MSM medium and 1ml paint emulsion, Flask B - MSM with *Staphylococcus sp* and 1 ml paint emulsion, In flask C - MSM with *Pseudomonas Sp* and 1ml paint emulsion was added. In flask D- MSM with *Bacillus sp* with 1ml paint emulsion was added, The flasks were kept in shaker at 35°C for 14 days and the OD was taken at 600nm to access the degradating ability of the organisms isolated. (Table 2). Microorganisms under stress condition utilizes synthetic organic matter as a energy source for their growth. All the three organisms exhibited almost the same degradation ability in emulsion paint.

Table 2: Shake flask experiment optical density

Flasks	Optical Density at 600nm
Flask A	Control
Flask B	0.31
Flask C	0.36
Flask D	0.34

3.3 Fourier Transform Infrared Spectroscopy of the samples

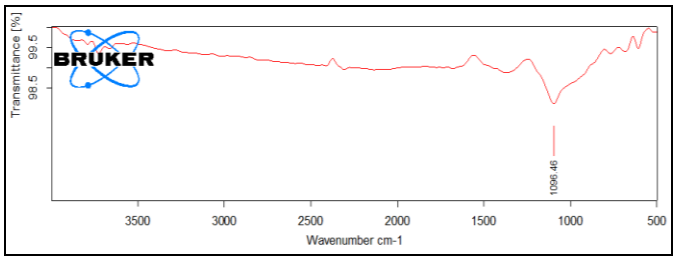


Fig 3: FTIR analysis of untreated paint sample in Flask A (Control).

Peak	Chemical bond
1096.41	Alkoxy C-O group

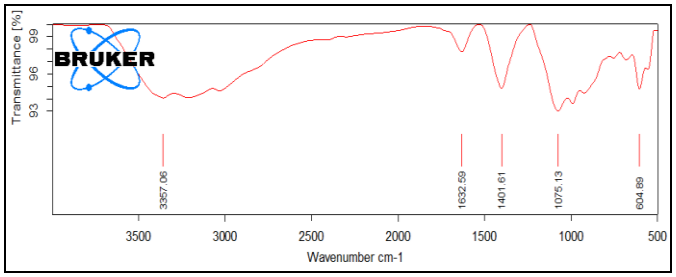


Fig 4: FTIR of *Staphylococcus* sp treated paint sample in Flask B

Peak	Chemical bond
3357.06	Alcohol O-H
1632.59	C-X double bonds
1401.61	C-F Fluro compound
1075.13	C-O
604.89	C-I halo compounds

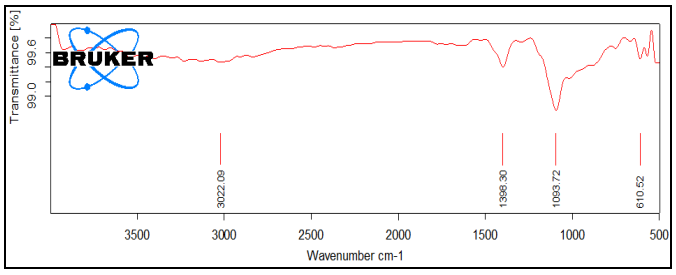


Fig 5: FTIR of *Pseudomonas* sp treated paint sample in Flask C.

Peak	Chemical bond
3022.09	C-H
1398.30	O-H bending
1093.72	C-O

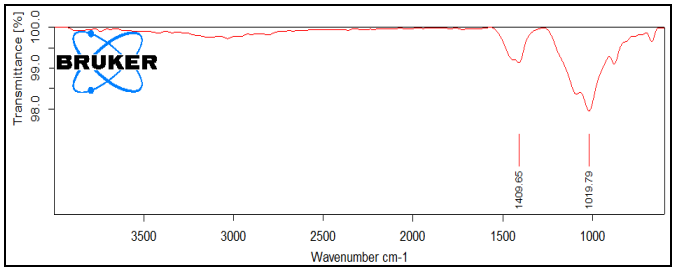


Fig 6: FTIR of *Bacillus* sp treated paint sample in Flask D.

Peak	Chemical group
1409.65	CH_2 Bending
1019.79	Si-OR silicon groups

Biodegradation of paint samples in the flasks using different organisms showed the shift in the prominent peak where, its exhibited more than one peak in all the samples with different bonds such as C-H, O-H, C-I separated halo compounds etc., indicating the degradation of the paint sample. The untreated sample in Flask A (Fig 3) showed a single intact peak, indicating Poly(ethyl acrylate) an important constituent of paint which has a characteristic single Alkoxy C-O group was reflected in the FT IR results of untreated paint sample as single peak at 1096.41. (Saxon.R., 1964)., whereas after subjecting to degradation by different microorganisms the breakdown of chemical groups due to microbial degradation activity led to the increase in number of peaks in each sample clearly indicating that the organisms has utilized the synthetic organic matter as energy source.(Fig 4,5,6). (Cappitelli et al., 2005) [8].

3.4 Scanning Electron Microscope

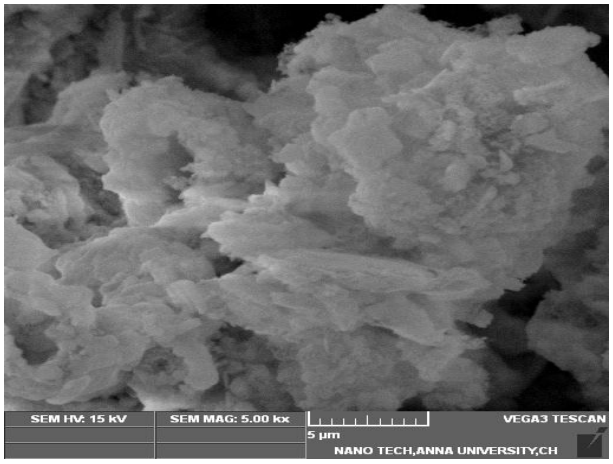


Fig 7: SEM of untreated paint sample in Flask A (Control)

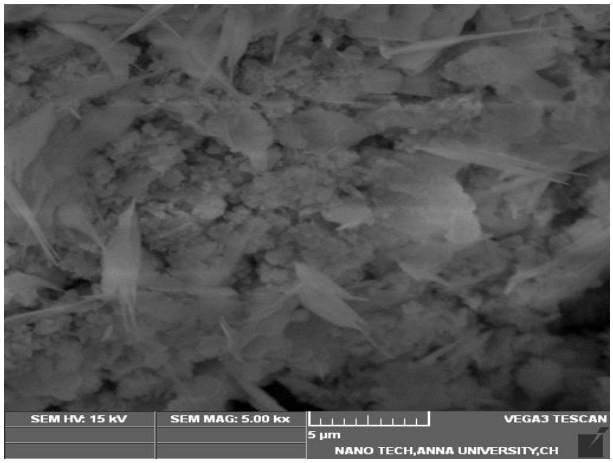


Fig 8: SEM of *Staphylococcus s*p treated paint sample in Flask B

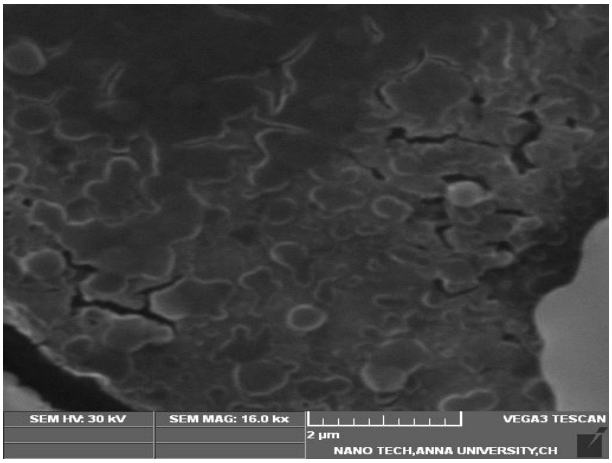


Fig 9: SEM of *Pseudomonas s*p treated paint sample in Flask C

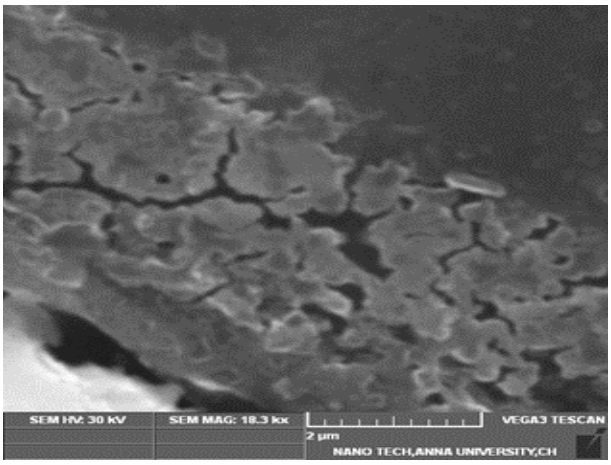


Fig 10: SEM of *Bacillus s*p treated paint sample in Flask D

SEM photographs showed that surface roughness increased in treated samples due to degradation as compared to control(Fig 7,8,9,& 10). Even some holes were observed in biodegraded samples of paint after treatment (English, S.E, 2003) [11]. Surface roughness was less in 14 days treated samples, but it would have been more prominent if the samples were treated for a longer period. A similar pattern of biodeterioration of paint observed earlier (Aecio *et al.,* 2011) [1].

4. Conclusion

The present study concludes that microorganisms such as bacteria, not only cause discoloration of paint surfaces but also they can directly cause degradation of the materials through their metabolic activities. The comprehensive distribution of the bacterial isolates also vary with the type of paint used. The microbes isolated in the study, has used the paint as sole source of carbon for its growth and metabolic activity and contributed towards the degradation of the paint materials. The presence of acrylic compound in the paint samples inhibits growth of many organisms which made the isolation process tedious. Reports on fungal isolation of microbes from paint are quite common than bacteria. Hence

the present study was a unique attempt to explore it. The presence of various polymer compounds used in paint manufacturing makes it resistant to degradation and continue to be a potential hazard to the environment. Use of nonabrasive and environmentally safe methods, to reduce the impact of microbial activities can further reduce the damage as well as help in bioremediation of paint contaminated water, soil and environments to clean up.

5. Acknowledgement
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6. References

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