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STUDY ON ACRYLIC PAINT DEGRADING ACTIVITY AND ITS EFFECT IN ENVIRONMENT

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ABSTARCT:

Microorganisms can degrade a wide range of substrates and can use it as one of the nutrient sources. Acrylic paints could be one of the examples. Due it its similarity in chemical structure with the wall paints, it can be used to paint walls. Wall paints are a source of Organic compounds including Volatile organic compounds (VOCs), resins, etc. Thus, in this study, it can be expected that the isolation of degrading acrylic could have potential to degrade wall paints. Four degrading microflora was isolated from acrylic paints and studied along-side the standard culture known to degrade paint namely, *Bacillus* and *Aspergillus* spp. and its consortium. The endospores and spores of the *Bacillus* spp and fungus respectively are ubiquitous in environment. Thus, degradation can be expected in unmaintained or closed environment. The degradation is assessed by plate assay and broth assay using Mineral medium with paint flakes as the only Carbon source. Presence of growth is an indication of the potential of the microflora in degrading the wall paint. The availability of carbon source increases the biomass, which is expected to keep on increasing following the incubation. The degradation can also be attributed to the production of acids, which results in lowering of the pH. When degraded, leads to changes in the chemical structure, these changes can be detected used Fourier Transform Infrared Spectroscopy (FTIR). Also, calcium carbonate is the base upon which the paints are painted. Thus, calcite dissolution can be expected by the followed by the degradation of paint. These, all together can lead to wall degradation and ultimately weaken them.

Keywords: Acrylic paint, Paint degraders, VOCs, *Bacillus*, *Aspergillus*, FTIR.

INTRODUCTION:

Paint is a synthetic substance commonly used to provide texture to infrastructure, furniture and utensil of everyday life. It is used to protect all sorts of buildings and structures from the effects of water and sun. Wooden buildings such as houses are usually painted because a coat of paint prevents water seeping into the wood and making it rot. The paint also helps to prevent the wood from drying out in the hot sun. It is also coated on to the metallic surfaces to prevent its corrosion. Primarily, it is composed of pigments (prime pigments to impart colour and opacity), extender (larger pigment particles added to improve adhesion, strengthen the film and save binder), binder (a polymer, often referred to as resin, forming a matrix to hold the pigment in place, solvents (sometimes called a thinner- either an organic solvent or water is used to reduce the viscosity of the paint for better application, water-borne paints are replacing some paints that use volatile organic compounds such as the hydrocarbons which are harmful to the atmosphere) and certain

additives (used to modify the properties of the liquid paint or dry film). Paints are found either in the form of Emulsion or Oil based formulations. Organic chemicals such as volatile organic compounds (VOCs) including benzene, toluene and xylene (BTX), and even more complicated compounds, i.e., chlorinated benzenes and toluene are used in paints as solvents. The solvents used may cause short and long-term environmental impacts, and may lead to respiratory, allergic, or immunogenic defects in humans (Ishfaq et al., 2015, Lan et al., 2020). Frequently, paints also contain a high level of mercury or lead and their ingestion may lead to serious health problems such as nerve and kidney damage. In addition, other metals such as chromium and cadmium are also reported to provide many health risks. Further, some paints also contain antifouling compounds like Tributyline (TBT) which has proven to be highly toxic to marine life (Ishfaq et al., 2015).

Thus, if this protective layer is wears off, it might expose the inner surface to the environment and probably lead to its deterioration by physical and chemical means such as water, oxygen, atmospheric gases, might be acids and alkalis, temperature, even microorganisms. This would lead to weakening of the infrastructure and loss of property.

As paint is a complex compound made from pigments, extender, binder, solvents and other additives. It is an excellent source of inorganic and organic matter. These available nutrients would facilitate the growth of various microorganisms. However, the paints are also supplemented with various chemical preservatives which would take part in inhibiting the microorganisms. But, some microorganisms have various strategies to overcome the barrier of these preservatives and utilizing the components as a source of energy. However, not all paint can be degraded even being these microorganisms ubiquitous in nature because of the environmental conditions being unfavourable. Once favourable would support the growth of these decaying microflora.

The use of the components of the paint would could alter the structural integrity of the paint as an whole and making the protective coating weak. Also, atmosphere has a complex microbial population which could work in synergism and damage could be in collective form.

Thus, isolating this degrading microflora would help to formulate newer preservation techniques to protect its integrity. Also, the leaching of paint in environment is recalcitrant in nature and is harmful to various biota including animals, marine life and humans. These microorganisms can help in removal of paints from environment.

LITERATURE SURVEY:

Paint is a synthetic substance commonly used to provide texture to infrastructure, furniture and utensil of everyday life. Primarily, it is composed of pigments, extender, binder, solvents and certain additives. Heterotrophic microorganisms such as bacteria and mould grow on the surface of paintings that contain a wide range of organic and inorganic constituents and provide different ecological niches that are exploited by a large variety of microbial species (Pepe et al., 2010). The activity of fungal and bacterial species is supported by many factors such as relative ambient humidity, temperature fluctuations, light, the nature of nutrients on the material, its moisture content, physical properties of the surface of the object, moisture adsorption-emission mechanisms in the support, pH, dust, oxygen and carbon dioxide concentration in the atmosphere, and the presence of microclimates that may induce condensation.

Thus, it is important to characterize the microorganisms involved in biodeterioration processes to understand their effects on cultural assets and to define an efficient strategy for protecting artworks, monuments, and buildings from microbiological recolonization. The microbial communities dwelling easel painting were analysed. Cultivable bacteria and fungi colonizing the painting were isolated and identified and they belonged to the *Staphylococcus* and *Bacillus* genera. Furthermore, culture-dependent techniques and

SEM/EDS analyses revealed the presence of filamentous fungi of the genera *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* (Caselli et al., 2018).

Investigating the microbial community (bacteria and fungi) colonising an oil painting on canvas, which showed visible signs of biodeterioration caused by the bacterial strains belonged to species of the phylum *Firmicutes*, as *Bacillus* sp. (Caselli et al., 2018) and *Paenisporosarcina* sp., whereas the majority of the non-cultivable members of the bacterial community were shown to be related to species of the phylum *Proteobacteria*, as *Stenotrophomonas* sp. Fungal strains belonged to different genera of the order Eurotiales, as *Penicillium* and *Eurotium*, and the non-cultivable belonged to species of the order Pleosporales and Saccharomycetales. The cultivable microorganisms, which exhibited enzymatic activities related to the deterioration namely *Arthrobacter* sp. as the representative bacterium and *Penicillium* sp. as the representative fungus (López-Miras et al., 2013).

Evidence of hyphal penetration and disruption of paint was observed and it was found that the changes in the surface roughness increased over the microbial exposure. SEM analysis showed that interaction between the hyphae of *A. pullans* and the paint are such that the hyphae may initiate the breakdown of the paint flakes (English et al., 2003).

Biogenic secretion or release of inorganic and organic acids by a great number of microorganisms is considered as the probable cause of biocorrosion of monumental stone surfaces. The destruction processes induced by the released inorganic and organic acids are respectively known as acidolysis and complexolysis. The process of acidolysis is associated with the chemolithotrophic bacteria like nitric and sulfuric acid producing bacteria. Apart from this, the release of carbon dioxide produced during cellular respiration by lichens and mosses is also a potent corrosive agent. The formation of organic acids like oxalic acids, citric acids etc by certain chemoorganotrophs and lichens have strong corrosive property (Dakal and Singh 2012). The production of organic or inorganic acids and production of pigments may lead to discoloration of wall paints.

Discolouration of wall paints was sometimes associated with detachment of the paint layer and/or to the development of efflorescence or a patina. Heterotrophic filamentous microbes like fungi, belonging to the genera *Aspergillus* and *Penicillium* and actinomycetes, belonging to the genera *Streptomyces*, which contribute to mechanical destruction of wall paintings due to mycelia production. The hyphae penetrate the painted layer, degrading some of its components (especially glues and binders), which results in a reduction in its cohesion, thus giving rise to exfoliations, cracking and loss of the paint (Pepe et al., 2010).

Microorganisms induce carbonate precipitation through different metabolic pathways, such as photosynthesis, ureolysis, ammonification, denitrification, sulfate reduction, anaerobic sulfide oxidation, and methane oxidation, either increasing pH or dissolved inorganic carbon (Zhu and Dittrich 2016).

Paint is used as a sole source of Carbon this would bring variations in its structure. To analyse the degraded layer FTIR is used to check the functional groups which is actually consumed. Paint film biodegradation was confirmed by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) studies. The loss in intensity of the bands at a wavelength of 1115.7 cm^{-1} and 1065.67 cm^{-1} for ester linkages indicated degradation of the paints through the breaking of the ester group. A loss in intensity of bands at a wavelength of 3286.87 cm^{-1} (corresponding alcoholic peak) due to breakage of alcoholic linkages. Scanning electron micrographs clearly showed the adherence and fungal growth on paint flakes and the distorted / ruptured surface was also observed in three months treated paint samples (Ishfaq et al., 2015).

The FTIR-spectra of mock paintings revealed that neither *Arthrobacter* sp. nor *Penicillium* sp. alone, were able to induce chemical changes on the various materials used to prepare mock paintings, but only when inoculated together, could a synergistic effect on the FTIR-spectra be observed, in the form of a variation in band position on the spectrum (López-Miras et al., 2013).

Spray paint exhaust gas contains recalcitrant volatile organic compounds (VOCs), such as benzene, toluene and xylene (BTX). Three strains for BTX degradation were isolated and identified as *Pseudomonas putida*, *Bacillus cereus* and *Bacillus subtilis* by using 16S rRNA sequencing technology. A consortium of relatively suitable ratio of *P. putida*, *B. cereus* and *B. subtilis* was obtained. An efficiency of over 90% was achieved in the biofilter with VOC concentration of 1000 mg/m^3 through inoculation with the microbial community after only 10 days of operation. Analysis of intermediate products by gas chromatography–mass spectrometry indicated that BTX was degraded into short-chain aldehydes or acids via ring opening reactions (Lan et al., 2020).

MATERIALS AND METHODS:

A. SAMPLE COLLECTION

The sample was taken from the acrylic paint which showed the signs of deterioration such as visible fungal growth or the separation of the paint components. Make a saline suspension of the sample for further enrichment.

B. ENRICHMENT OF THE SAMPLE

The saline suspension (1 ml) of the paint sample was enriched in Trypticase Soy broth for bacteria and Sabouraud Dextrose broth for fungi and incubated at 28°C for 2 weeks for the enhanced growth of the degrading microflora.

C. ISOLATION OF THE PAINT DEGRADERS

Enriched sample was isolated directly onto agar plates containing Sabouraud Dextrose Agar (SDA) and Trypticase Soy Agar (TSA) and were incubated at 28°C, over a total period of two weeks. During this period, colonies exhibiting different morphology and appearance were transferred to new culture plates of TSA medium for bacteria and SDA for fungi to obtain pure strains. All purified strains were stored in 0°C for conservation.

D. PREPARATION OF STANDARD CULTURE AND ITS CONSORTIUM

To compare the results obtained from the isolates, *Aspergillus* and *Bacillus* species known to degrade paint were taken as the Standard culture. The pure cultures were freshly cultured for on Sabouraud Dextrose Agar (SDA) and Nutrient Agar (NA). For Preparation of the consortium both the cultures were taken in a ratio of 1:1.

E. FUNGAL BIOMASS QUANTIFICATION EXPERIMENT

It represents the ability of selected microorganism to grow and utilize component of the paint blend as sole carbon source. The quantification was done for the screening of isolates from degradation experiments by examining the growth of microorganisms on the paint flakes. The isolates and standard organisms were cultured in 50 ml flask containing mineral salt medium with paint sample as a sole carbon source. Along with the test, the medium without the inoculum was deemed as control. Fungal biomass was quantified on a weekly basis till Day 21 in shaking incubator at 155 rpm at 30°C. The whole contents of flask containing the fungal culture were filtered through pre weight Whatman filter paper no.1. Biomass on filter paper was dried in oven at 50°C to constant weight. The filter paper was re-weighed overnight to get the dry biomass.

F. OPTICAL DENSITY OF BACTERIA

Shake flask experiment with bacterial culture (1ml) in 50 ml mineral medium with paint flasks was carried out. The optical density of the bacterial inoculum at wavelength 600 nm of colorimeter was recorded weekly till Day 21 to check bacterial growth.

G. SHAKE FLASK EXPERIMENT

Shake flask experiment with 50 ml mineral salt medium, 1 ml inoculum and paint flakes were carried in a shaker at 37°C. Control was run as well. Samples of paint flakes were collected after intervals of 10, 20 days. Flakes surface was analysed by Fourier transform infrared spectroscopy (FTIR).

H. ANALYSIS OF DEGRADATIVE SUBSTANCES

i.Carbonate Dissolution Test

The potential ability of the tested microorganisms to solubilize calcite was screened using $CaCO_3$ glucose agar plates of the following composition, per litre of deionized water: calcium carbonate, 5 g; glucose, 10 g; agar, 15 g; and deionized water, 1000 ml. The prepared medium was sterilized at 121°C for 15 min, after which pH was adjusted to 8.0 with 10 N HCl and the mixture was cooled to 45°C with gentle stirring to resuspend $CaCO_3$. After pouring into Petri plates, the plates were then kept in a cool place. Petri plates with agarized $CaCO_3$ glucose agar were inoculated with 10 µl of microbial suspensions and incubated for 21 days at 25 ± 2°C. Positive strains displayed a clear zone around the colony, thus confirming calcite dissolution.

ii. Acid And Alkali Production

To determine the capacity of microorganisms to affect the pH value of the substrate on which they grow, isolates were cultivated in a liquid minimal medium of the following composition, per litre of deionized water: sodium nitrate, 3 g; dipotassium phosphate, 1 g; magnesium sulphate heptahydrate, 0.5 g; potassium chloride, 0.5 g; iron (II) sulphate heptahydrate, 0.01 g; glucose, 10 g; and deionized water, 1000 ml. Titration flasks with 100 ml of medium, sterilized at 114°C for 25 minutes and having its pH readjusted to 7.0 with 10 N HCl, were inoculated with 10 µl of microbial suspensions and incubated for three days on a platform shaker under conditions of room temperature (22 ± 2°C) and rotation of 300 rpm. After the incubation period, cultures were filtered through Whatman No. 4 filter paper and a pH meter was used to measure pH values of the filtrates, i.e., the culture medium.

iii. Pigments Production

The potential ability of the tested isolates to produce and secrete organic pigments under nutrient-limited conditions (ideally present on the surface of clean, properly maintained mural paintings), and consequently to induce alterations in the original colouration of the painted layer, was assayed by cultivation on Czapek-Dox minimal medium of the following composition, per litre of deionized water: sodium nitrate, 2 g; dipotassium phosphate, 1 g; magnesium sulphate heptahydrate, 0.5 g; potassium chloride, 0.5 g; iron (II) sulphate heptahydrate, 0.01 g; glucose, 10 g; agar, 20 g; and deionized water, 1000 ml. The medium was sterilized for 25 minutes at 114°C, after which its pH was adjusted to 5.5 with 10N HCl. Petri plates inoculated with 10 µl of microbial suspensions, were incubated for 21 days at 25 ± 2°C. Secretion of fungal pigments was confirmed by changes in colour of the transparent medium. The colour of the produced pigment was determined by comparing it with the ISCC-NBS colour palette.

RESULTS:

A. ENRICHMENT AND ISOLATION OF THE PAINT DEGRADERS

The degraded paint sample was enriched in Tryptic Soy broth for bacteria and Sabouraud Dextrose broth for fungi for 2 weeks 28°C. Following the enrichment, the enriched samples were isolated on the Tryptic Soy broth for bacteria and Sabouraud Dextrose broth for fungi for 3-4 days. A total of 4 isolates were selected in which 2 were bacteria and the other 2 were fungi. The isolates were purified on the slants and stored at 0℃ for further analysis.

B. FUNGAL BIOMASS QUANTIFICATION EXPERIMENT

The fungal isolates along with the standard culture of *Aspergillus*, was grown in minimal mineral media which contained paint flakes as the only carbon source and biomass was quantified weekly till day 21. Increase in biomass is an indication of the paint being used as a source of carbon. For both the fungal isolates and Aspergillus, the biomass increased with increase in period of incubation. However, Isolate 1 has the highest dry weight of biomass and the Isolate 4 accounts the least.

Sr. No.	Test Organism	Dry weight of the biomass (gm)		
		Day 7	Day 14	Day 21
1	Isolate 1	0.2	0.5	0.6
2	Isolate 4	0.01	0.1	0.15
3	*Aspergillus*	0.1	0.35	0.5

Table 1: Fungal Biomass quantification.

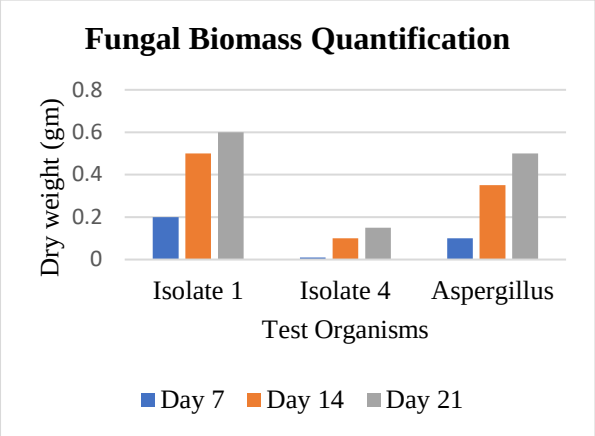
Fig 2: Enrichment of the degraded paint sample in Tryptic Soy broth (left) and in Sabouraud Dextrose broth (right), Day 0.

C. OPTICAL DENSITY OF BACTERIA

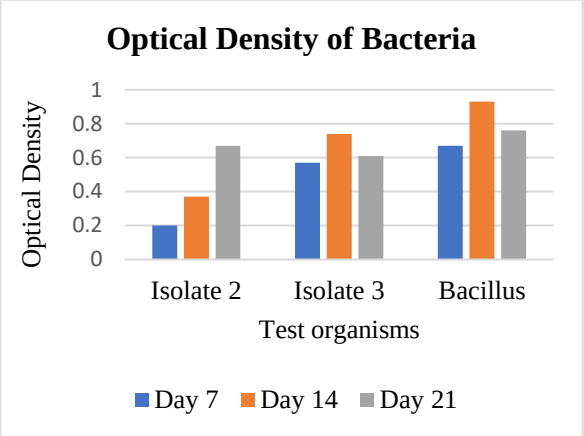
The optical density of the bacterial inoculum at wavelength 600 nm of colorimeter was recorded to check bacterial growth. Increase in optical density an indication of the paint being used as a source of carbon.

Sr. No.	Test Organism	Dry weight of the biomass (gm)		
		Day 7	Day 14	Day 21
1	Isolate 2	0.2	0.37	0.67
2	Isolate 3	0.57	0.74	0.61
3	*Bacillus*	0.67	0.93	0.76

Table 2: Optical Density of Bacteria.



Graph 1: Fungal Biomass Quantification.



Graph 2: Optical Density of Bacteria.

D. SHAKE FLASK EXPERIMENT

Shake flask experiment with 50 ml mineral salt medium, 1 ml inoculum and paint flakes were carried in a shaker at 37°C. Control was run as well. Microbial growth was observed in the flask which varied from organism to organism and also with the increase in day of incubation. The turbidity is an indication of use of the paint sample as a source of the carbon.

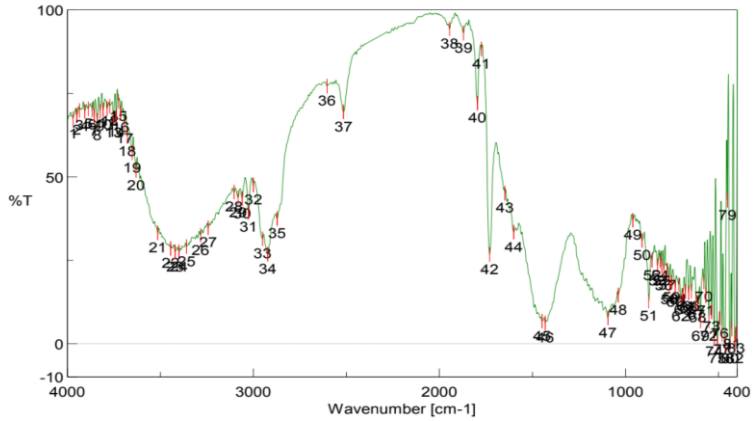
Fig 1: Shake Flasks at Day-10.

Fig 2: Shake Flasks at Day-20.

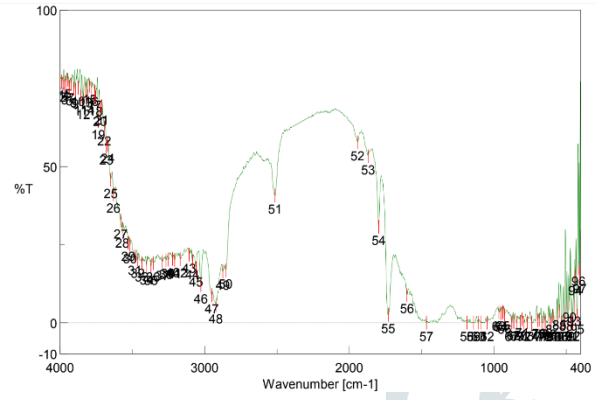
Samples of paint flakes were collected after intervals of 10, 20 days. The flaks were softened when compared to the flasks in the control, which could be an indication of degradation. Flakes surface was analysed by Fourier transform infrared spectroscopy (FTIR).

E. FTIR ANALYSIS

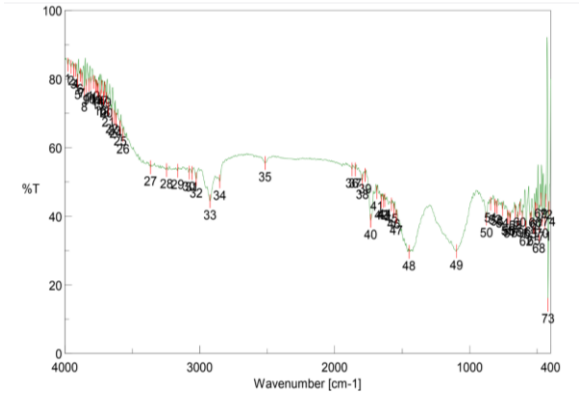
Graph 3 is the FTIR spectrum of the control sample peaked at various wavelengths suggesting presence of various functional group present. The various functional group includes alcohols, phenols, primary and secondary amines, amides, alkanes, aldehydes, carboxylic acids, esters, etc. When undergoes degradation, these functional groups are catalysed to extract energy by the degrading microflora. The degraded paint samples can be compared for the loss of the peak at that particular wavelength as an indication of loss of the functional group.



Graph 3: FTIR Spectrum of the control paint sample.

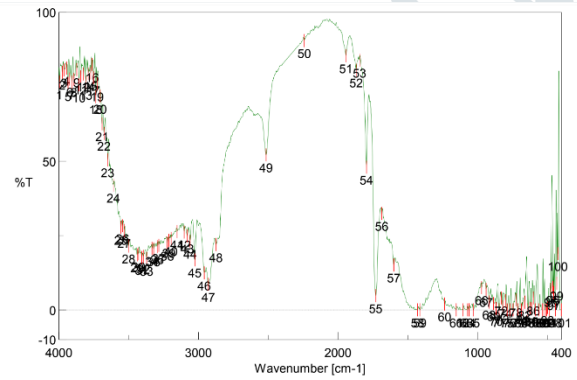


Graph 4: FTIR Spectrum of the Day 10 paint sample degraded by Isolate 1.

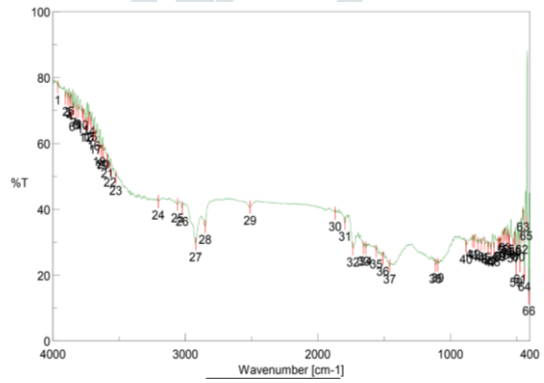


Graph 5: FTIR Spectrum of the Day 20 paint sample degraded by Isolate 1.

On comparing the day 10 (Graph 4) spectra with the control, not much difference is observed. However, on day 20 (Graph 5) the loss of the peaks at 3445cm^{-1}, 2999cm^{-1}, 2604 cm^{-1}, 1038 cm^{-1} position from the degraded sample by Isolate 1 indicates the loss of primary amine, alkane, aldehyde, carboxylic acid/ alcohol/ ester/ ether respectively, lost when compared to the spectra of control sample.

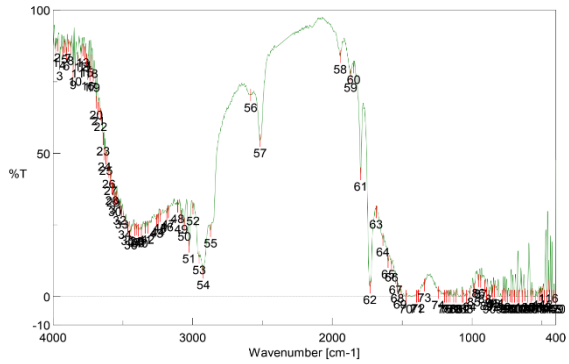


Graph 6: FTIR Spectrum of the Day 10 paint sample degraded by Isolate 2.

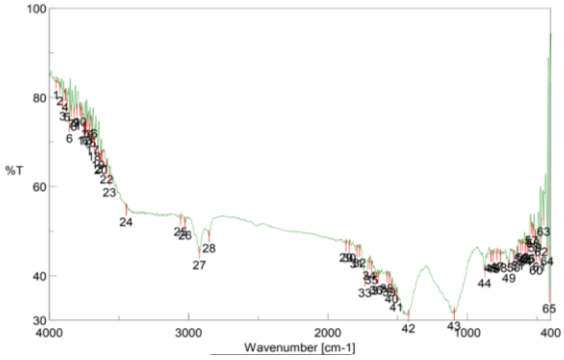


Graph 7: FTIR Spectrum of the Day 20 paint sample degraded by Isolate 2.

Again, not much difference was seen in the spectra of the day 10 (Graph 6) and the control sample. The loss of peak 3445 cm^{-1}, 2950 cm^{-1}, 1038 cm^{-1}, 911 cm^{-1}, 857 cm^{-1} at day 20 (Graph 7), indicating the degradation of functional group primary amines, alkanes, alcohols, carboxylic acid, aromatic compounds respectively.

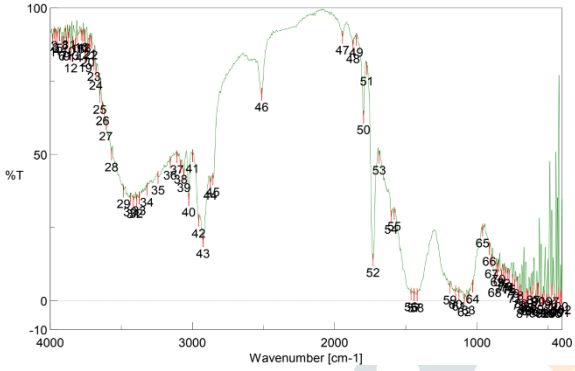


Graph 8: FTIR Spectrum of the Day 10 paint sample degraded by Isolate 3.

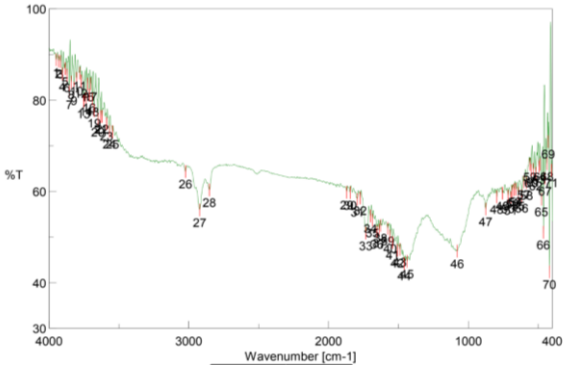


Graph 9: FTIR Spectrum of the Day 20 paint sample degraded by Isolate 3.

Not much of difference in the loss of peak is observed on comparing the day 10 (Graph 8) degraded sample by Isolate 3 with control sample. On day 20 (Graph 9), the loss of the peak at wavelength 2999 cm^{-1}, 1038 cm^{-1}, 911cm^{-1}, 775 cm^{-1} was observed indicating the degradation of the functional group alkanes, aliphatic amines, carboxylic acids, alkyl halides, respectively.



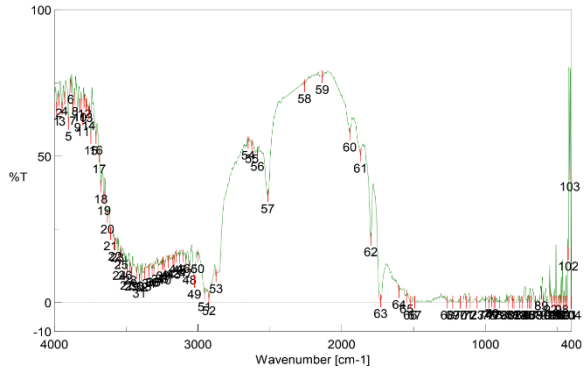
Graph 10: FTIR Spectrum of the Day 10 paint sample degraded by Isolate 4.



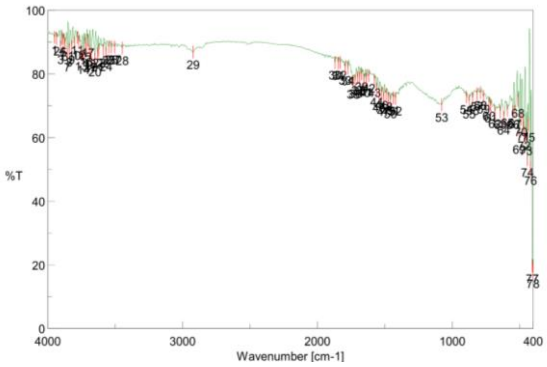
Graph 11: FTIR Spectrum of the Day 20 paint sample degraded by Isolate 4.

On day 20 (Graph 11), a lot many peaks belonging to the functional group-alcohol was lost. Along will the loss of the peaking on the wavelength 1038 cm^{-1} belonging to the alcohol, carboxylic acids, esters and ether; alkyl halides in the paint were also degraded by Isolate 4 as the loss of the peak at 857 cm^{-1} was observed.

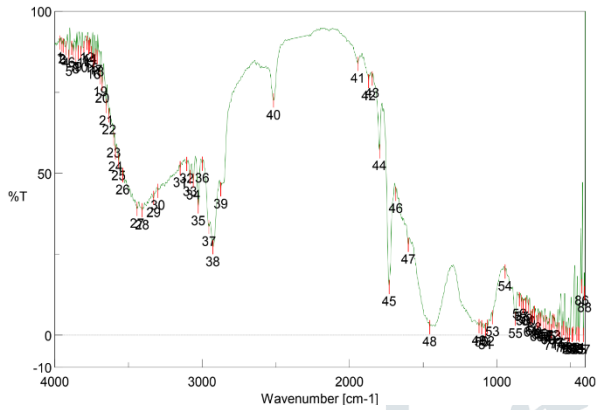
At day 20 (Graph 13), the peak lost at wavelength 2999 cm^{-1}, 2872 cm^{-1}, 2514 cm^{-1}, 1094 cm^{-1}, 578 cm^{-1} indicating loss of alkanes, aldehydes, carboxylic acid, aliphatic amine, alkyl halides probably due to degradation by the *Aspergillus*.



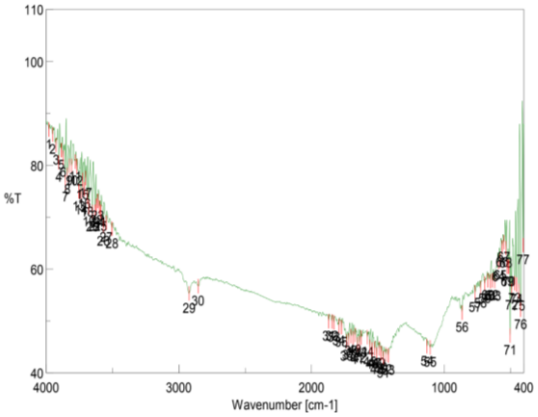
Graph 12: FTIR Spectrum of the Day 10 paint sample degraded by *Aspergillus*.



Graph 13: FTIR Spectrum of the Day 20 paint sample degraded by *Aspergillus*.

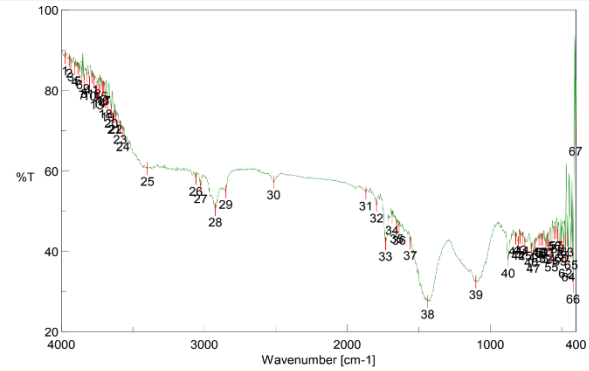


Graph 14: FTIR Spectrum of the Day 10 paint sample degraded by *Bacillus*.

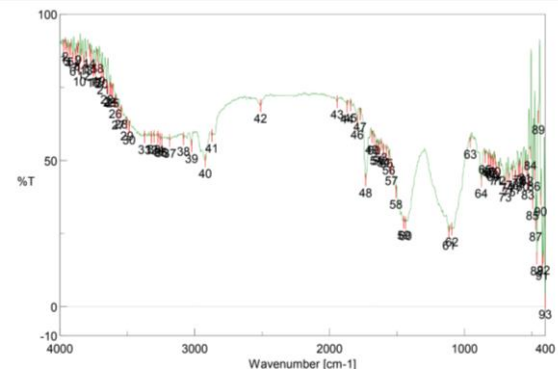


Graph 15: FTIR Spectrum of the Day 20 paint sample degraded by *Bacillus*.

Loss of the peak at 3999 cm^{-1} on day 10 (Graph 14) due to utilization of primary amines by *Bacillus* was observed. Whereas, on day 20 (Graph 15) a lot many peaks lost was observed- 3445 cm^{-1}, 3284 cm^{-1}, 3242 cm^{-1}, 2999 cm^{-1}, suggesting the degradation of alcohols/ phenols, primary amines, alkynes, alkenes and alkanes by *Bacillus*.



Graph 16: FTIR Spectrum of the Day 10 paint sample degraded by consortium of Aspergillus and *Bacillus*.



Graph 17: FTIR Spectrum of the Day 20 paint sample degraded by consortium of *Aspergillus* and *Bacillus*.

In contrast to other organisms, the consortium on day 10 (Graph 16) degraded comparatively more carbon compounds which resulted in the peak loss at 3445 $cm^{-1,}$ 2499 cm^{-1}, 1094 cm^{-1}, 960 cm^{-1} which represents alcohols/ phenols, carboxylic acid, aliphatic amines and alkenes. On day 20 (Graph 17) as well the degradation of the similar compounds was observed.

F. ANALYSIS OF DEGRADATIVE SUBSTANCES

i. Carbonate Dissolution Test

Fig 3: Clear zones produced by the consortium of *Aspergillus* and *Bacillus* in Carbonate Dissolution test.

The potential ability of the tested microorganisms to solubilize calcite was screened using $CaCO_3$ glucose agar plates. Positive strains should display a clear zone around the colony, to confirming calcite dissolution. However, the isolates from the paint did not show clear zones nor did the Standard culture of *Aspergillus* and *Bacillus*. However, clear zones could be observed in case of the consortium.

i. Pigments Production

The potential ability of the tested isolates to produce and secrete organic pigments under nutrient-limited conditions, and consequently to induce alterations in the original colouration of the painted layer, was assayed by cultivation on Czapek-Dox minimal medium. However, no colouration was observed because of no production of pigment in any of the isolates nor it the Standard culture and their consortium.

iii. Acid And Alkali Production

Fig 4: Acid-alkali production test.

To determine the capacity of microorganisms to affect the pH value of the substrate on which they grow, isolates were cultivated in a liquid minimal medium with pH readjusted to 7.0, were inoculated with 10 µl of microbial suspensions and incubated for three days on a platform shaker under conditions of room temperature (22 ± 2°C) and rotation of 300 rpm. After the incubation period, cultures were filtered through Whatman No. 4 filter paper and a pH was measured of the culture medium. In all the cases the pH drop was observed in acidic range. However, the drop of pH was not much.

Sr. No.	Organisms	Day 0	Day 7
1	Isolate no 1	7	5.0
2	Isolate no 2	7	5.7
3	Isolate no 3	7	6.0
4	Isolate no 4	7	5.0
5	*Aspergillus*	7	4.8
6	*Bacillus*	7	5.0
7	Consortium	7	5.0

Table 2: pH value on the Day-0 and Day-7.

DISSCUSION

Paint is a complex compound thus, to acquire nutrients as energy source from it requires specialised catabolic pathway. The degrading microflora is rather in a nutrient stress condition. To overcome this stress the degraded paint samples must be enriched. The nutritive media such as Tryptic Soy broth for bacteria and Sabouraud Dextrose broth for fungus are used as enrichment media. After enrichment, the enriched samples were isolated.

Dry cell biomass experiment was carried out for paint degradation with three fungal strains and cell dry mass was calculated after 7, 14, 21 days. The data obtained depicted that the fungal was increased between 7-21 days. Where isolate no 1 accounted the maximum increase in the biomass of 0.6 for 21st day, which was followed by *Aspergillus* and finally the isolate no 4 which accounted the least. This increase in dry cell mass of fungal strains could be due to the fact that the fungal strains used the paint as the sole carbon source in the minimal salt media and with the passage of time they adjusted themselves on this carbon source resulting in significant increase in growth.

Optical density bacterial cultures showed that *Bacillus* growth increased from 7th to 14th day with a maximum optical density of 0.93 and decreased on the day 21. Similar trend was observed the optical densities obtained by isolate no 3 with the maximum at 0.74 on the 14th day and decreased the for the 21st day. However, isolate no 2 rather increased with the time with maximum optical density of 0.67 for the 21st day. The slow growth rate can result in slow biodegradation.

The paint flakes in the shake flask experiment shows softening of the paints compared to the flakes in the control. This could be the sign of degradation. FTIR analysis of the treated flakes with the microorganisms showed changes in the test sample spectra as compared to the control spectrum. When comparing the individually degraded samples with the degraded sample in consortium, right from day 10 the changes can be observed indicating the synergistic degradation by *Aspergillus* and *Bacillus*. Various functional groups were degraded including alkanes, alkenes, alkynes, amines, alcohols, phenols, ethers, esters, carboxylic acid, alkyl halides, etc. The degradation due the action of various enzymes. Rhodococci are frequently isolated from petroleum hydrocarbon-contaminated environments where they often play an important role in the degradation of aliphatic hydrocarbons, such as alkanes. However, the presence of the alkane 1-monooxygenase (alkB) gene, which is a key catabolic gene of alkane degradation. Thus, the organisms in our studies degrading the alkanes may possess alkane monooxygenases. It was found that TIBETAN4 showed enzymatic activity of phenol hydroxylase and catechol 1,2-dioxygenase after induction by phenol, which means there could be a degradation pathway of phenol through the ortho-pathway (Wu et al., 2018). Thus, utilization of phenols could be attributed due to presence these enzymes. While, alcohol dehydrogenase (ADH) is important for the degradation of alcohols to their aldehydes or ketones and Aldehyde dehydrogenase converts aldehydes to ketones. Dehalogenases are important for degradation of halides, Aldehyde dehydrogenase for the conversion of (Bhandari et al., 2021). Ethers are degraded due to action of dealkylase activity (Kim et al., 2004).

When paint is degraded leads to compromise in the structural integrity. To further add on to the damage, degraders may also possess the ability to degrade the limestone present beneath the paint layers. Limestone dissolution induced by microbial metabolites is a well-known and thoroughly studied natural phenomenon in the terrestrial environment. Solubilization of limestone and consequent deposition of secondary carbonates induced by various microorganisms is the primary cause of structural alterations of carbonate substrata (Albertano and Urzì 1999). In two very similar investigations, (Pangallo et al., 2012) cultivated fungi isolated from stone monuments, indoor artwork, wall paintings and ambient air on $CaCO_3$ glucose agar and showed that many species of the genera *Aspergillus* and *Penicillium* dissolve calcite. The results obtained from this study reveals that none were capable to dissolve the calcite. However, when the

consortium of *Aspergillus* and *Bacillus* dissolved the calcite to a much larger extend. This suggests the synergistic role of both the organisms. This is also likely due to the fact that the primary way carbonate dissolution occurs is through synthesis and secretion of various organic acids, although other mechanisms have also been proposed, for example enzymatic dissolution, ligand activity, and oxidation-reduction of redox-sensitive elements (only when CO_2 is used as a carbon source for autotrophy, as $CaCO_3$ is not a redox sensitive compound). It follows that in cultures of other positive isolates, $CaCO_3$ dissolution either occurred as a result of acid production induced by the presence of Ca ions, or by one of the other mentioned mechanisms. In most cases, to produce acids, an abundant carbon source, i.e., sugars, is needed, since intensive growth results in production of more organic acids than is necessary for normal metabolism, the excess being excreted into the substrate. Cultured in Czapek-Dox minimal broth, an essentially oligotrophic medium, only a very small number of isolates, one of *Aspergillus* caused considerable acidification of the broth medium, possibly owing to acid production. *Aspergillus* being one of the strongest producers, primarily of oxalic, gluconic and citric acids. However, the acid production in our experiment did in fact lower pH of the broth medium by values on the order, of one pH unit. Such modest changes in pH, it cannot be definitely concluded that acid synthesis is responsible for carbonate dissolution.

Microorganisms are known producers of a large variety of organic pigments, each with its own structure, composition, and colour, which are regulated by a number of things such as the available carbon and nitrogen source, metals in the substrate, light (UV), and other environmental factors that limit growth. Many isolates have been shown to secrete variously coloured pigments, as indicated by changes in colour of the employed transparent medium, something which has been previously confirmed several times under nutrient-limited conditions with many *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, and *Penicillium* species isolated from wall paintings, documents, books, paintings, and photographs. Furthermore, it was recently shown that same fungal species, albeit isolated from a variety of substrata, can produce pigments of different colour or lack pigment production at all, which would account for the inconsistencies in colour production documented here and in previously published research. An additional complication is the fact that produced pigments can be secreted into the substrate. In our results, no pigment production was observed. The ability of microorganisms to produce organic acids and pigments is crucial in discolouration of the paints (Rojas et al., 2012).

CONCLUSION

The microorganisms degrading the acrylic paints were isolated. The isolates were compared the known paint degrading species of *Aspergillus* and *Bacillus*. As these microorganisms can degrade acrylic paint and them being ubiquitous in nature is a risk for degradation of wall paints. These isolates were potent enough for the degradation of the paint and produced similar results to the known paint degraders *Aspergillus* and *Bacillus*. The FTIR analysis revealed the utilization of various functional groups viz. alkanes, alkenes, alkynes, carboxylic acids, esters, ethers, amines, aldehydes and halides as a source of energy. The degradation is attributed to production various degradative enzymes which leads to degradation of paint. The rate of degradation increases with the time and also the synergistic effects of the consortium. The degraded paint samples were softer than the paint samples of control. Thus, these are potent enough for degradation and weakening of the walls.

To add on to degradation, consortium of *Aspergillus* and *Bacillus* were capable enough to dissolve carbonate beneath the walls making it further weaken. However, the isolate organisms did not dissolve carbonate, but the consortium did. No pigments were produced by either and the acid production was not enough to produce discolouration of paint. As these have potential in degrading paint *in vitro*, these can be used to degrade the paint which is released into the environment through various means and which are recalcitrant in nature. Thus, approaches are to be taken to prevent this degradation and to maintain the integrity of the wall paints.

REFERENCES

[1] **Albertano, P., & Urzì, C.** (1999). Structural Interactions among Epilithic Cyanobacteria and Heterotrophic Microorganisms in Roman Hypogea. *Microbial ecology*, *38*(3), 244–252. https://doi.org/10.1007/s002489900174

[2] **Alford, K. L., & Kumar, N.** (2021). Pulmonary Health Effects of Indoor Volatile Organic Compounds-A Meta-Analysis. *International journal of environmental research and public health*, *18*(4), 1578. https://doi.org/10.3390/ijerph18041578

[3] **Alvarez J. M., Seijas C. J., Bianchi G. L.** (2020). Elimination of volatile organic compounds in paint drying by absorption reaction in water combined with the ozone oxidation technique. *Environmental Advances*, *2*, 100017. https://doi.org/10.1016/j.envadv.2020.100017

[4] **Bhandari, S., Poudel, D. K., Marahatha, R., Dawadi, S., Karan Khadayat, K., Phuyal, S., Shrestha, S., Gaire, S., Basnet, K., Khadka, U., Parajuli, N.** (2021). Microbial Enzymes Used in Bioremediation, *Journal of Chemistry*, 8849512, 17 pages https://doi.org/10.1155/2021/8849512

[5] **Babitha, S., Soccol, C. R., & Pandey, A.** (2007). Effect of stress on growth, pigment production and morphology of Monascus sp. in solid cultures. *Journal of basic microbiology*, *47*(2), 118–126. https://doi.org/10.1002/jobm.200610261

[6] **Bollmann, U. E., Minelgaite, G., Schlüsener, M., Ternes, T. A., Vollertsen, J., & Bester, K.** (2017). Photodegradation of octylisothiazolinone and semi-field emissions from facade coatings. *Scientific reports*, *7*, 41501. https://doi.org/10.1038/srep41501

[7] **Cappitelli, F., Vicini, S., Piaggio, P., Abbruscato, P., Princi, E., et al.** (2005) Investigation of fungal deterioration of synthetic paint binders using vibrational spectroscopic techniques. *Macromol Biosci* 5: 49–57. https://doi.org/10.1002/mabi.200400134

[8] **Caselli, E., Pancaldi, S., Baldisserotto, C., Petrucci, F., Impallaria, A., Volpe, L., D'Accolti, M., Soffritti, I., Coccagna, M., Sassu, G., Bevilacqua, F., Volta, A., Bisi, M., Lanzoni, L., & Mazzacane, S.** (2018). Characterization of biodegradation in a 17th century easel painting and potential for a biological approach. *PloS one*, *13*(12), e0207630. https://doi.org/10.1371/journal.pone.0207630

[9] **Ciferri, O.** (1999). Microbial degradation of paintings. *Applied and environmental microbiology*, *65*(3), 879–885. https://doi.org/10.1128/AEM.65.3.879-885.1999

[10] **Dakal, T.C., Cameotra, S.S. Microbially induced deterioration of architectural heritages: routes and mechanisms involved** (2012). *Environ Sci Eur*, 24, 36 https://doi.org/10.1186/2190-4715-24-36

[11] **Garg KL, Kamal KJ, Mishra AK**. Role of fungi in the deterioration of wall paintings. *Sci Total Environ*. 1995; 167: 255–271. doi:10.1016/0048-9697(95)04587

[12] **González-Cabrera, M., Domínguez-Vidal, A., & Ayora-Cañada, M. J.** (2021). Monitoring UV-accelerated alteration processes of paintings by means of hyperspectral micro-FTIR imaging and chemometrics. *Spectrochimica acta. Part A, Molecular and biomolecular spectroscopy*, *253*, 119568. https://doi.org/10.1016/j.saa.2021.119568

[13] **Goodier, M. C., Siegel, P. D., Zang, L. Y., & Warshaw, E. M.** (2018). Isothiazolinone in Residential Interior Wall Paint: A High-Performance Liquid Chromatographic-Mass Spectrometry Analysis. *Dermatitis: contact, atopic, occupational, drug*, *29*(6), 332–338. https://doi.org/10.1097/DER.0000000000000410

[14] **Horemans, B., Smolders, E., & Springael, D.** (2013). Carbon source utilization profiles suggest additional metabolic interactions in a synergistic linuron-degrading bacterial consortium. *FEMS microbiology ecology*, *84*(1), 24–34. https://doi.org/10.1111/1574-6941.12033

[15] **Ishfaq, S., Ali, N., Tauseef, I., Khattak, M. N. K., Shinwari, Z. K., & Ali, M. I.** (2015). Analysis of paint degradation by fungal and bacterial species. *Pakistan Journal of Botany*, *47*(2), 753-760

[16] **Kim, Y. H., & Engesser, K. H.** (2004). Degradation of alkyl ethers, aralkyl ethers, and dibenzyl ether by Rhodococcus sp. strain DEE5151, isolated from diethyl ether-containing enrichment cultures. *Applied and environmental microbiology*, *70*(7), 4398–4401. https://doi.org/10.1128/AEM.70.7.4398-4401.2004

[17] **Kennes, C., & Veiga, M. C.** (2004). Fungal biocatalysts in the biofiltration of VOC-polluted air. *Journal of biotechnology*, *113*(1-3), 305–319. https://doi.org/10.1016/j.jbiotec.2004.04.037

[18] **Lan, H., Qi, S., Yang, D., Zhang, H., Liu, J., & Sun, Y.** (2020). Combination of highly efficient microflora to degrade paint spray exhaust gas. *Scientific reports*, *10*(1), 6027. https://doi.org/10.1038/s41598-020-62972-2

[19] **Ławniczak, Ł., Woźniak-Karczewska, M., Loibner, A. P., Heipieper, H. J., & Chrzanowski, Ł.** (2020). Microbial Degradation of Hydrocarbons-Basic Principles for Bioremediation: A Review. *Molecules (Basel, Switzerland)*, *25*(4), 856. https://doi.org/10.3390/molecules25040856

[20] **López-Miras, M., Martín-Sánchez, I., Yebra-Rodríguez, A., Romero-Noguera, J., Bolívar-Galiano, F., Ettenauer, J., Sterflinger, K., & Piñar, G.** (2013). Contribution of the microbial communities detected on an oil painting on canvas to its biodeterioration. *PloS one*, *8*(11), e80198. https://doi.org/10.1371/journal.pone.0080198

[21] **Pandey, Padmini & Kiran, UV.** (2020). Degradation of Paints and its Microbial Effect on Health and Environment. Journal of Critical Reviews. 7. 4879-4884. https://www.researchgate.net/publication/344321477_DEGRADATION_OF_PAINTS_AND_ITS_MICROBIAL_EFFECT_ON_HEALTH_AND_ENVIRONMENT

[22] **Pandey, Padmini & Kiran, UV.** (2020). Solvent Based Paint and its Impact on Environment and Human Beings, Environment and Society. https://www.researchgate.net/publication/344677732_SOLVENT_BASED_PAINT_AND_ITS_IMPACT_ON_ENVIRONMENT_AND_HUMAN_BEINGS_ENVIRONMENT_AND_SOCIETY

[23] **Pangallo, D., Chovanová, K., Simonovicová, A., & Ferianc, P.** (2009). Investigation of microbial community isolated from indoor artworks and air environment: identification, biodegradative abilities, and DNA typing. *Canadian journal of microbiology*, *55*(3), 277–287. https://doi.org/10.1139/w08-136

[24] **Pangallo, D., Kraková, L., Chovanová, K., Simonovičová, A., De Leo, F., & Urzì, C.** (2012). Analysis and comparison of the microflora isolated from fresco surface and from surrounding air environment through molecular and biodegradative assays. *World journal of microbiology & biotechnology*, *28*(5), 2015–2027. https://doi.org/10.1007/s11274-012-1004-7

[25] **Pepe, O., Sannino, L., Palomba, S., Anastasio, M., Blaiotta, G., Villani, F., & Moschetti, G.** (2010). Heterotrophic microorganisms in deteriorated medieval wall paintings in southern Italian churches. *Microbiological research*, *165*(1), 21–32. https://doi.org/10.1016/j.micres.2008.03.005

[26] Petersen, K., Yun, Y., & Krumbein, W. E. (1995). On the occurrence of alkalitolerant and alkaliphilic microorganisms on wall paintings and their interaction in restoration/consolidation. In *Biodeterioration of cultural property 3: proceedings of the 3rd international conference on biodeterioration of cultural property, July 4-7, 1995, Bangkok, Thailand* (pp. 371-386)

[27] **Rojas, T. I., Aira, M. J., Batista, A., Cruz, I. L., & González, S.** (2012). Fungal biodeterioration in historic buildings of Havana (Cuba). *Grana*, *51*(1), 44-51 https://doi.org/10.1080/00173134.2011.643920

[28] **Singh, N., & Turner, A.** (2009). Leaching of copper and zinc from spent antifouling paint particles. *Environmental pollution (Barking, Essex: 1987)*, *157*(2), 371–376. https://doi.org/10.1016/j.envpol.2008.10.003

[29] **Soroldoni, S., Vieira da Silva, S., Castro, Í. B., de Martinez Gaspar Martins, C., & Leães Pinho, G. L.** (2020). Antifouling paint particles cause toxicity to benthic organisms: Effects on two species with different feeding modes. *Chemosphere*, *238*, 124610. https://doi.org/10.1016/j.chemosphere.2019.124610

[30] **Taft WSJ, Mayer JW.** The structure and analysis of paintings In:Taft WSJ, Mayer JW, editors. *The science of paintings*. New York: Springer-Verlag New York; 2000. p. 1–11.

[31] **Táncsics, A., Benedek, T., Szoboszlay, S., Veres, P. G., Farkas, M., Máthé, I., Márialigeti, K., Kukolya, J., Lányi, S., & Kriszt, B.** (2015). The detection and phylogenetic analysis of the alkane 1-monooxygenase gene of members of the genus Rhodococcus. *Systematic and applied microbiology*, *38*(1), 1–7. https://doi.org/10.1016/j.syapm.2014.10.010

[32] **Unković, N., Dimkić, I., Stupar, M., Stanković, S., Vukojević, J., & Ljaljević Grbić, M.** (2018). Biodegradative potential of fungal isolates from sacral ambient: In vitro study as risk assessment implication for the conservation of wall paintings. *PloS one*, *13*(1), e0190922. https://doi.org/10.1371/journal.pone.0190922

[33] **Unković, N., Erić, S., Šarić, K., Stupar, M., Savković, Ž., Stanković, S., Stanojević, O., Dimkić, I., Vukojević, J., & Ljaljević Grbić, M.** (2017). Biogenesis of secondary mycogenic minerals related to wall paintings deterioration process. *Micron (Oxford, England: 1993)*, *100*, 1–9. https://doi.org/10.1016/j.micron.2017.04.004

[34] **Wiesinger, R., Pagnin, L., Anghelone, M., Moretto, L. M., Orsega, E. F., & Schreiner, M.** (2018). Pigment and Binder Concentrations in Modern Paint Samples Determined by IR and Raman Spectroscopy. *Angewandte Chemie (International ed. in English)*, *57*(25), 7401–7407. https://doi.org/10.1002/anie.201713413

[35] **Wu, L., Ali, D. C., Liu, P., Peng, C., Zhai, J., Wang, Y., & Ye, B.** (2018). Degradation of phenol via ortho-pathway by Kocuria sp. strain TIBETAN4 isolated from the soils around Qinghai Lake in China. *PloS one*, *13*(6), e0199572. https://doi.org/10.1371/journal.pone.0199572

[36] **Yoshikawa, M., Zhang, M., & Toyota, K.** (2017). Biodegradation of Volatile Organic Compounds and Their Effects on Biodegradability under Co-Existing Conditions. *Microbes and environments*, *32*(3), 188–200. https://doi.org/10.1264/jsme2.ME16188

[37] **Ytreberg, E., Lagerström, M., Nöu, S., & Wiklund, A. E.** (2021). Environmental risk assessment of using antifouling paints on pleasure crafts in European Union waters. *Journal of environmental management*, *281*, 111846. https://doi.org/10.1016/j.jenvman.2020.111846

[38] **Zarnt, G., Schräder, T., & Andreesen, J. R.** (1997). Degradation of tetrahydrofurfuryl alcohol by Ralstonia eutropha is initiated by an inducible pyrroloquinoline quinone-dependent alcohol dehydrogenase. *Applied and environmental microbiology*, *63*(12), 4891–4898. https://doi.org/10.1128/aem.63.12.4891-4898.1997

[39] **Zhu, T., & Dittrich, M.** (2016). Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review. *Frontiers in bioengineering and biotechnology*, *4*, 4. https://doi.org/10.3389/fbioe.2016.00004