Pak. J. Bot., 47(2): 753-760, 2015.

ANALYSIS OF PAINT DEGRADATION BY FUNGAL AND BACTERIAL SPECIES

SHUMAILA ISHFAQ[1], NAEEM ALI[1], ISFAHAN TAUSEEF[2], MUHAMMAD NASIR KHAN KHATTAK[3], ZABTA KHAN SHINWARI[4,5], MUHAMMAD ISHTIAQ ALI[1*]

[1]Microbiology Research Laboratory, Department of Microbiology, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[2]Department of Microbiology, Hazara University, Mansehra, Pakistan
[3]Department of Zoology, Hazara University, Mansehra, Pakistan
[4]Department of Biotechnology, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[5]Pakistan Academy of Sciences, Islamabad-Pakistan
**Corresponding author e-mail: ishi_ali@hotmail.com Phone: +925190643196; Fax: +925190643156*

Abstract

Paint is a liquor blend, used as a decorative or protective coating. Paints are the main source of volatile organic compounds (VOCs), very harmful for the environment and human beings. In the present study, fungal and bacterial growth on paint flakes sandwiched between the mineral salt medium agar layers were subjected to various analysis. Dry cell mass quantification was carried out by shake flask experiment with fungal inoculum. The maximum growth of 0.7g observed on 28th day. Further evidence of 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. The current research study represents the significant trends of paint biodegradation by isolated microorganism.

Key words: Biodegradation, Dry cell mass, Alcoholic linkage, Fungal adherence.

Introduction

Paint is a synthetic substance commonly used to provide texture to infrastructure, furniture and utensil of everyday life (Bently & Turner, 1997). Primarily, it is composed of pigments, binder, solvents and certain additives. Paints are found either in the form of Emulsion or Oil based formulations (Talbert & Rodger, 2007). Organic chemicals such as volatile organic compounds (VOCs) are used in paints as solvents (Goldstein & Galbally, 2007). These solvents may cause short and long-term environmental impacts (Spurgeon, 2006), and may lead to respiratory, allergic, or immunogenic defects in humans (Mendell, 2007). 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 (Omae, 2002).

Paint's deterioration on surfaces 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 the release of harmful degradation products into the environment causing an alarming situation. Although there are certain chemical approaches available for removal of degraded products, these methods have some disadvantages. Microorganisms are renowned for their potential to degrade synthetic compounds, various microbial species reported for paint degradation (Vanderberg-Twary *et al*., 1997; Cifferi, 1999; Ahmad *et al.*, 2011).

Major groups of microbes, involved in paints degradation, are bacteria and fungi. Various fungi, e.g., species of *Penicillium*, *Aspergillus*, *Cladosporium*, *Chaetomium*, and *Alternaria* are reported to play a vital role in such degradative processes (Altenburger *et al.*, 1996; Cifferri, 1999; Ravikumar *et al*., 2012). However, few bacterial species belonging to genera of *Pseudomonas*, *Arthrobacter*, and *Streptomyces* have also been reported (Altenburger *et al.*, 1996). The present study was carried out in order to evaluate the biodegradation of paint when it is released as waste in the environment. This involved isolation and screening of different bacterial and fungal strains to testify their biodegradation abilities of paint.

Materials and Methods

Screening of microbial isolates by agar plate assay: Mineral salt medium containing the paint as the only carbon source used to monitor the growth of microbial isolates. Paint flakes (Berger paint) were kept between two layers of mineral salt agar in petri plates. The mineral salt medium (MSM) (K_2HPO_4: 1.0g, KH_2PO_4:0.2g, NaCl: 1.0g, Boric acid: 0.005g, $CaCl_2$. $2H_2O$: 0.002g, $(NH_4)_2SO_4$:1.0g, $MgSO_4$. $7H_2O$:0.5g, $CuSO_4$:0.001g,) Agar 2%was used for determining the deteriogenic properties of the isolates. Fungal strains were maintained on slant of Sabouraud's agar. Media was sterilized by autoclaving at 121ºC and 15 Ibs pressure for 20 minutes. The pH of the media was adjusted prior to sterilization with 0.1 M sodium hydroxide or hydrochloric acid.

The plates were inoculated with 1ml of fungal spore suspension and kept in incubator at 35°C for 7 days in the incubator for further analysis.

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 fungal isolates were cultured in 50 ml flask containing mineral salt medium with paint sample as a sole carbon source. Fungal biomass was quantified on a weekly basis in shaking incubator at 155 rpm at 30ºC. The whole contents of flask containing the fungal culture were filtered through pre weight Whitman filter paper # 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.

Shake flask experiment with fungal inoculum: Shake flask experiment with 100 ml mineral salt medium, 1 ml fungal spore suspension and oil paint flakes was carried in a shaker at 35°C. Samples of paint flakes were collected after intervals of 7, 14, 21, 28 and 35 days. Flakes surface was analyzed by Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM).

Shake flask experiment with bacterial inoculum: Shake flask experiment with bacterial inoculum (1 ml) with 1ml paint emulsion was carried out in100 ml mineral salt medium, at 37°C for 14 days. The optical density of bacterial inoculum at 600nm wavelength of spectrophotometer was recorded to check the bacterial growth.

Analysis by fourier transform infra red (FTIR) spectroscopy: Analysis of chemical changes in the paint flakes biodegradation during shake flask experiments was performed. Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the changes in the functional groups and the structure of the paint. The pieces of paint flakes were fixed to the FTIR sample plate. Spectra were taken at 400 to 4000 wave-numbers cm^{-1}.

Analysis by scanning electron microscopy (SEM): The surface morphology of the paint flakes was analyzed through scanning electron microscopy after shake flask experiment. Samples were coated for 30 seconds in sputter coater SP1-Module TM. Then the images were withdrawn by using JEOL JSM-5910 scanning electron microscope. The images of control and test samples were observed and recorded.

Results and Discussion

Visual observation results: Visual observation of fungal treated paint samples compared with control indicates that paint color was faded and surface became ruptured and fungal growth was also observed (Fig. 1). The

Fungal strains were screened out for the biodegradation of the paint on the basis of the fungal adherence on the MSM medium with paint as the only carbon source. Plate assay results showed the fungal growth of *Aspergillus, Phanerochaete chrysosporium and Rhizopus* on the MSM agar plate containing paint as shown in Fig. 2. Fungal hyphae were observed on the plates (Arquiaga *et al.,* 1995; Obidi *et al.,* 2005). The fungal species *Aspergillus niger*, *Cladosporium sp.* growth is reported common on painted wall (Adeleye & adeleye, 2000; Gaylarde & Gaylarde, 2005; Shirakawa *et al.,* 2010;).

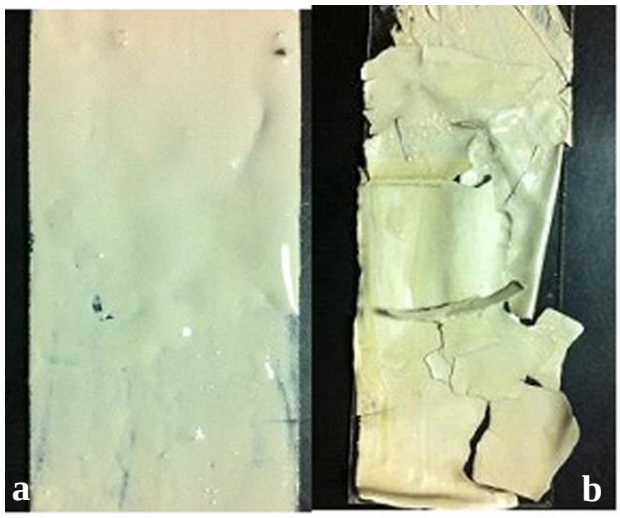

Fig. 1. Visual observation of paint samples (a) Control (b) Fungal treated.

Dry cell mass experiment was carried out for paint degradation with three fungal strains and dry cell mass was calculated after 7, 14, 21, 28 and 35 days. The data obtained depicted that fungal dry cell mass was increased from 0.1 to 0.7g between 7 to 28 days and then decreased (Fig. 3). This increase in dry cell mass of fungal strains could be due to the fact that fungal strains used the paint as the sole carbon source in minimal salt media and with passage of time, they adjusted themselves on this carbon source resulting in a significant increase in growth (Kim, 2003; Aina *et al*., 201; Ravikumar and Karigar, 2012).

Optical density with bacterial cultures showed an increase in *Bacillus* sp. growth from 0 to 8^{th} day (Fig. 4). However, on 10^{th}day, an inconsistency in growth was observed when it decreased to an ~ 25% lower level. The growth level resumed and increased again after 10^{th} day of analysis. Maximum growth of *Bacillus* culture in emulsion paint was detected on 6th and 8th day. Microorganism under stress condition utilizes synthetic organic matter as a energy source (Prescott *et al.,* 2002;Gaylarde and Gaylarde, 2005).

An increase in growth of *Pseudomonas* sp., was observed up to 4^{th} day (Fig. 4). However, initially a decrease in growth was observed for *Bacillus* sp., up to 6^{th} day (Fig. 4) followed by an increase till 10^{th} day (Fig. 4). The slow growth rate was observed with *Bacillus subtilus* leading to a slow biodegradation rate.

FTIR analysis of the treated paint films with three fungal strains showed few changes in sample spectra, as compared to control spectrum (Fig. 5, Fig. 6a, b & c). In control, the peak at wavelength 3286.87 cm-1 (alcoholic) decreased in treated samples of paint after 7, 14 and 21 days (Fig. 6 and Fig. 7). An increase in the peak length at wavelength 2923.8cm-1 (CH_3) was observed in treated samples of 14 and 21 days, but there is no significant change in 7 days treated sample. The peak at wavelength 1722.92cm-1 (aldehydes) conjugation with ketonic group, increased in treated samples of 7, 14 and 21 days as compared to control. FTIR analysis can be used for the characterization of biodeterioration of paint (Cappitelli *et al.,* 2005).

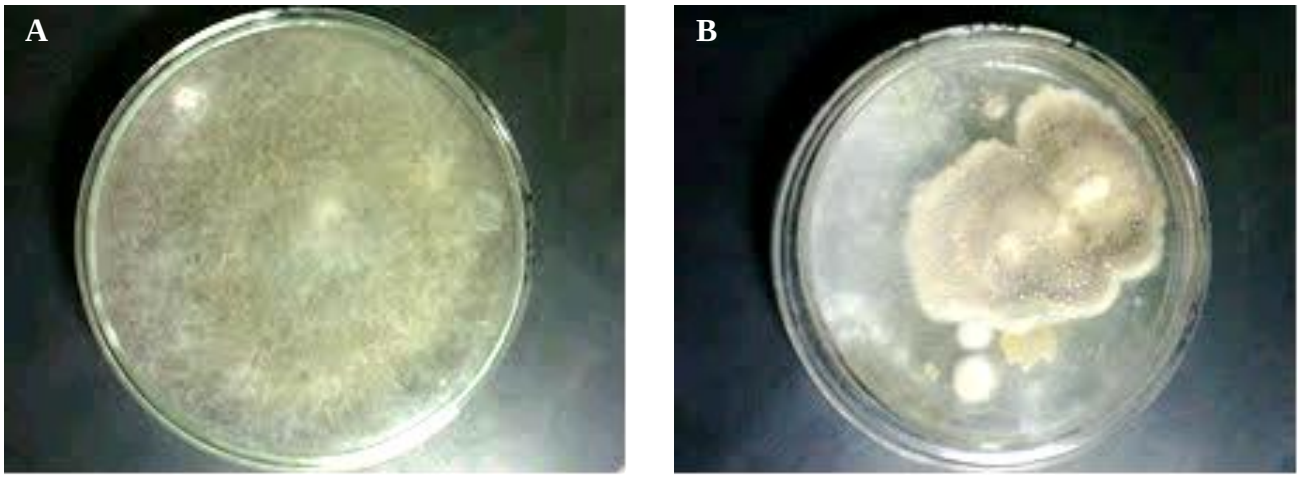

Fig. 2. Fungal adherence and growth on agar plates (A) *Aspergillus niger*, (B) *Phanerochaete chrysosporium*.

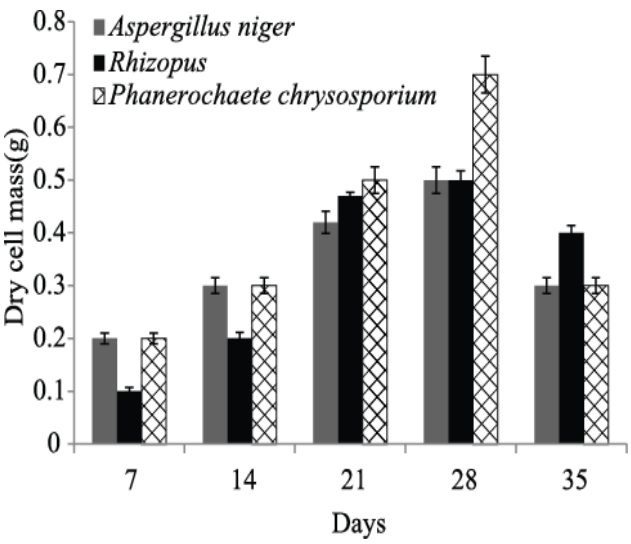

Fig. 3. Dry cell mass of emulsion sample with *Aspergillus niger*, *Rhizopus*, *Phanerochaete chrysosporium*.

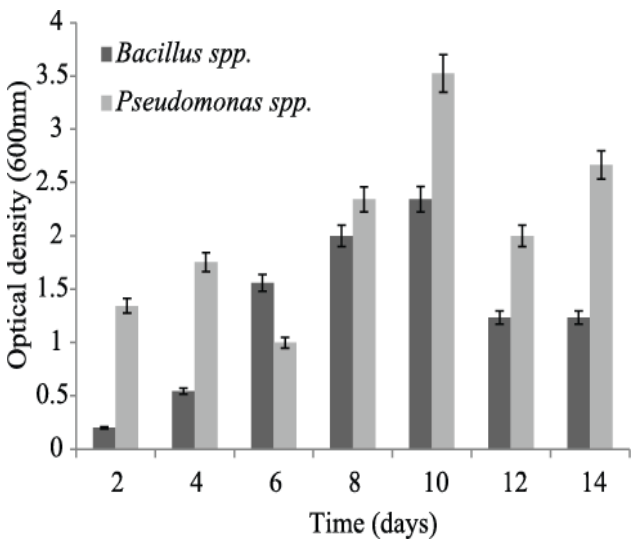

Fig. 4. Growth changes of *Bacillus* sp. and *Pseudomonas* sp. in paint emulsion.

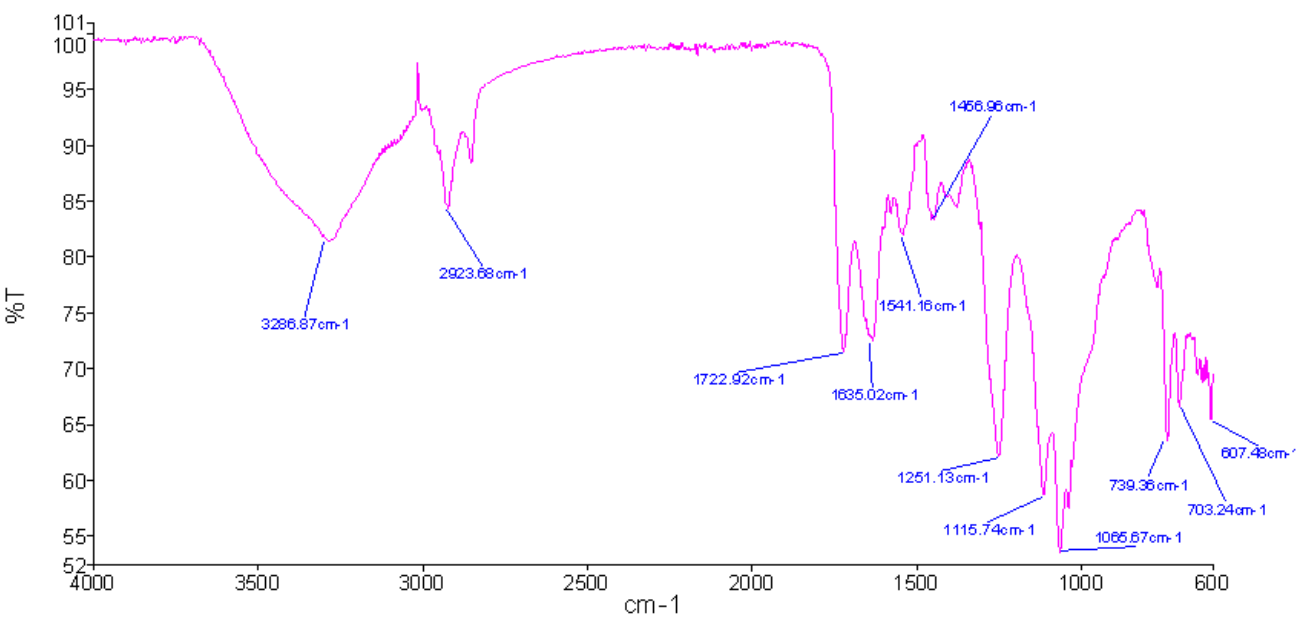

Fig. 5. FTIR spectra of paint sample as a control.

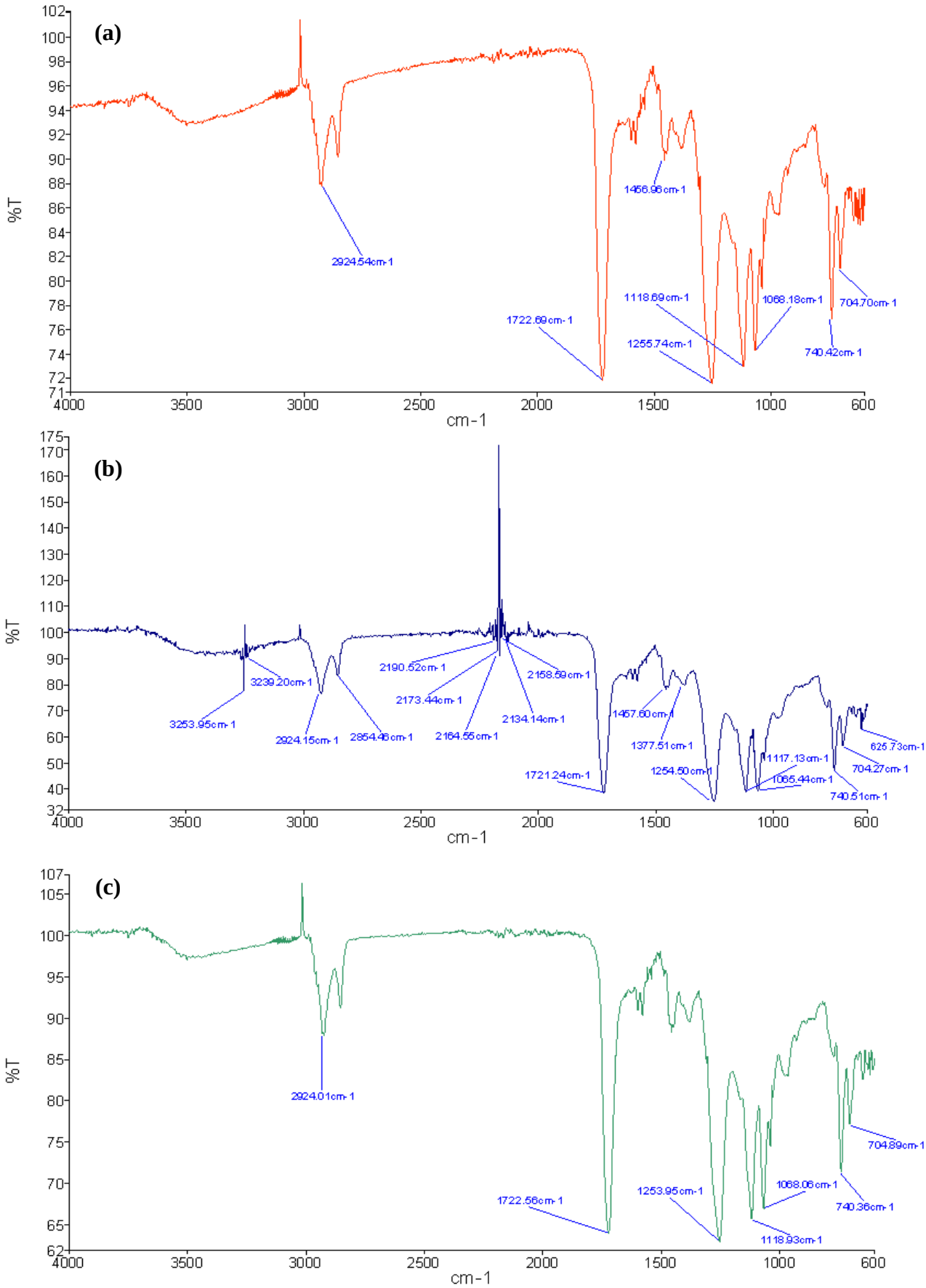

Fig. 6. FTIR spectra of 14 days treated paint sample of (a) *Rhizopus* sp. (b) *Phanerochaete chrysosporium* (c) *Aspergillus niger*.

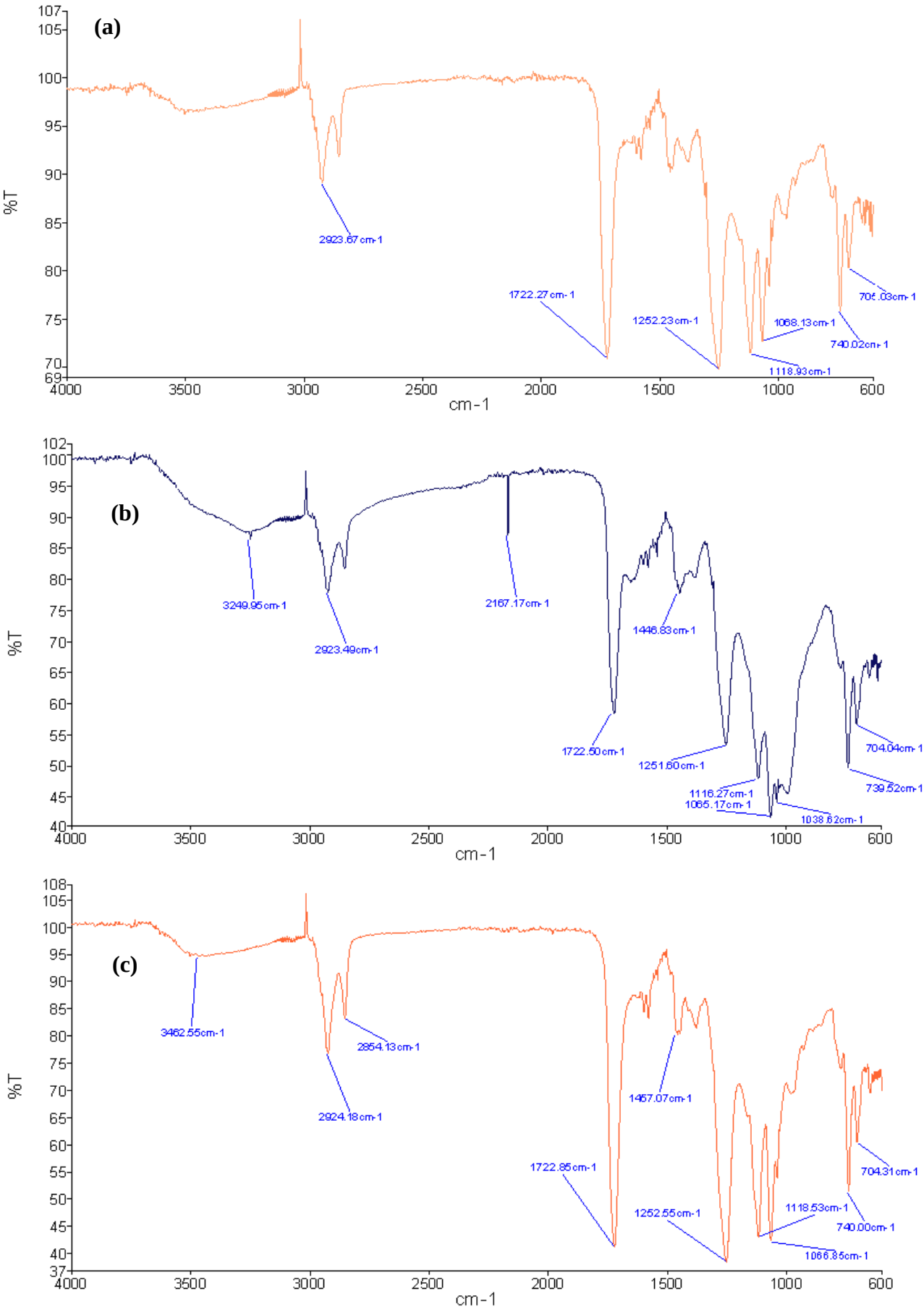

Fig. 7. FTIR spectra of 21 days treated paint sample of (a) *Rhizopus* sp. (b) *Phanerochaete chrysosporium* (c) *Aspergillus niger*.

The peak at wavelength 1635 cm-1 indicates the primary amine completely disappeared in treated samples. Carboxylic acid peak at wavelength 1251 cm-1 is increased in 7, 14 and 21 days samples as compared to control. The decrease in alcoholic peak was not much greater in 7 days treated paint samples but it was more prominent decrease in 14 and 21 days treated paint samples. The peaks wavelength 115.7 cm-1 and 1065.67 cm-1 indicates the ester stretching and these peaks were decreased in 21days treated paint samples. The peak at wavelength 1541.16 cm-1 indicates alkane group was present in control paint sample, but was completely disappeared in treated paint samples. The Fourier transform infrared spectroscopic (FTIR) analysis of the fungal treated paint films showed few changes in sample spectra as compared to control spectrum in our studies. Our results were consistent with the previous studies suggesting that Phthalic acid is converted to protocatechuic acid, which degrades to ß-carboxy-cis, cis-muconate via the ortho-cleavage pathway and is immediately transformed into γ-carboxy muconolactone leading to the TCA cycle. The gradual diminution in intensity of ester bands after attack of microorganism in our studies could be due to change in polymeric structure by rupturing the ester linkages. Similar results were also observed in paint films biodegradation by Dutta (Dutta *et al.*, 2005).

Samples of paint flakes were collected after intervals of 21 days and 3 months and biodegradation was analyzed by Scanning electron microscopy. The analysis indicates the surface changes in paint treated samples of 14 days and three months compared with the control (Figs. 8, 9, 10 & 11). The SEM photographs showed that surface roughness increased in treated samples due to degradation as compared to control. Even some holes were observed in biodegraded samples of paint after three months. A similar pattern of biodeterioration of paint observed earlier (Aecio *et al.*, 2011).

The rough and wrinkled texture, increased in treated samples as compared to control. Surface roughness was less in 14 days treated samples, but it was more prominent in three months treated samples of paint. Evidence of hyphal penetration and disruption of the paint was observed and it was found that changes in the surface roughness increased over the duration of the microbial exposure. SEM analysis has shown that interactions between the fungal hyphal of *A. pullans* and the paint flakes are such that the hyphae may initiate the breakdown of the paint film (English *et al.*, 2003).

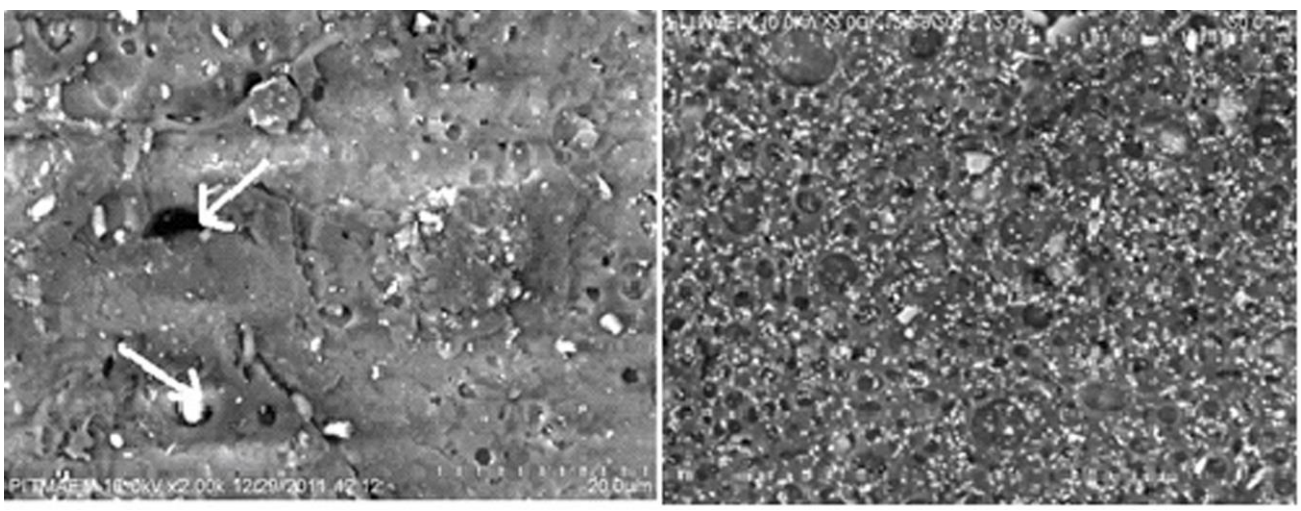

Fig. 8. SEM photograph of paint sample with *Aspergillus niger* arrows indicating the holes (21, 90 days).

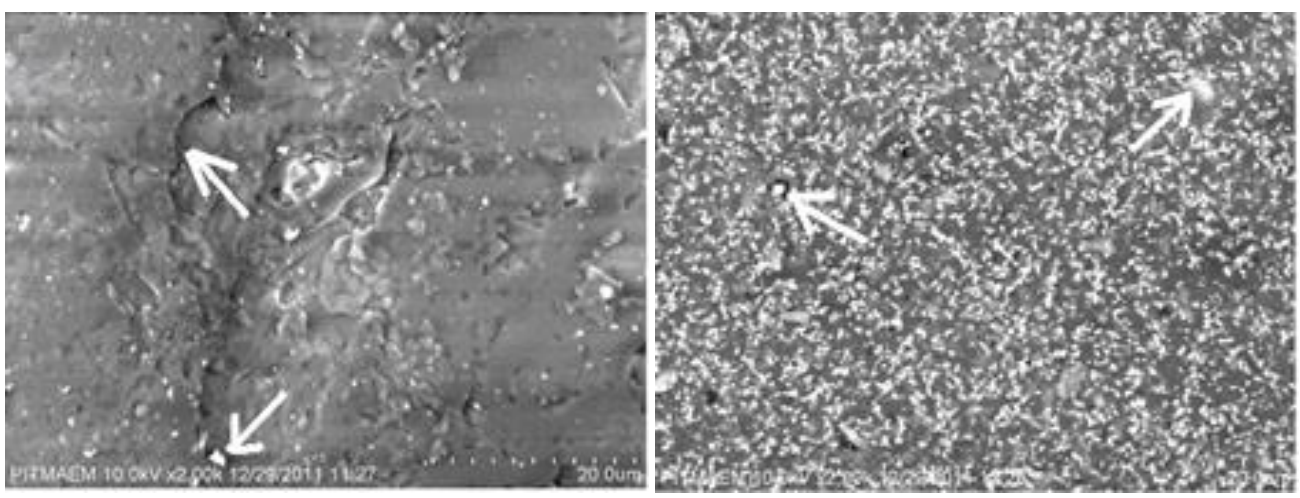

Fig. 9. SEM photograph of paint sample with *Rhizopus* sp. Arrows indicating the cracks (21, 90 days).

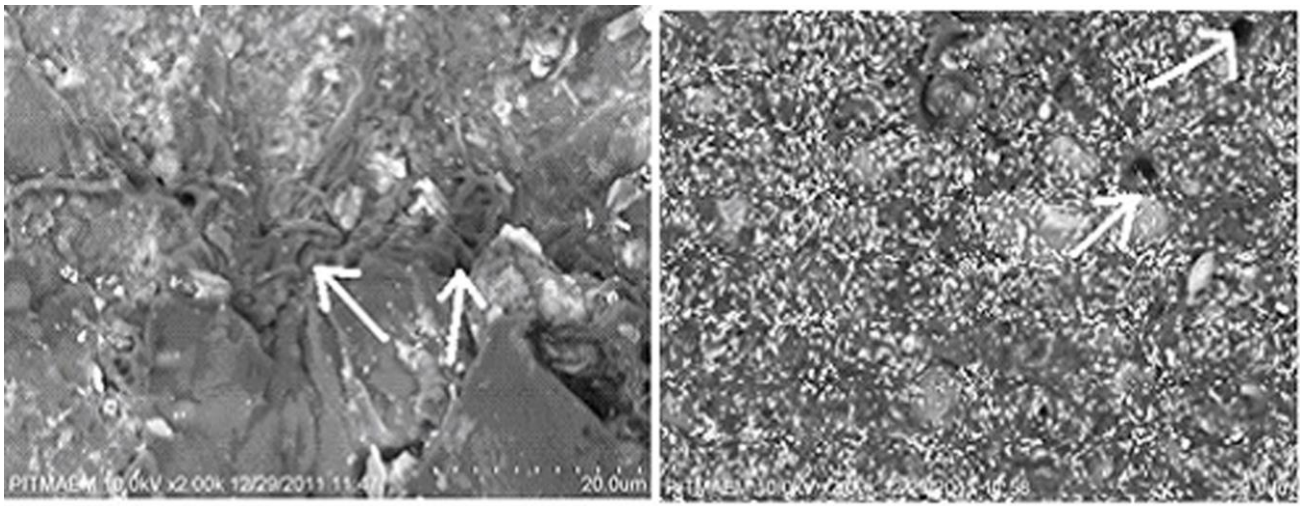

Fig. 10. SEM photograph of paint sample with *Phanerochaete chrysosporium* arrows indicating the holes and pits (21, 90 days).

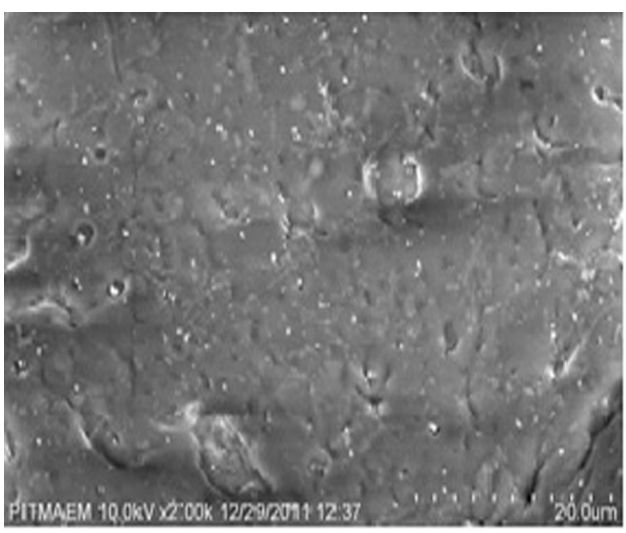

Fig. 11. SEM photograph of control.

In conclusion selected fungal and bacterial strains have shown the ability to adhere and degrade the paint flakes under stress conditions. Fungal strains showed more promising results for paint degradation in term of surface changes and surface and chemical structure change when evaluated by SEM and FTIR respectively.

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(Received for publication 15 October 2013)