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THE EFFECTS OF TEMPERATURE AND pH ON BACTERIAL DEGRADATION OF LATEX PAINT IN HUMID ENVIRONMENT

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ABSTRACT

The goal of this study was to integrate the activities of paint deterioration of microbial communities (*microcosms*) on the basis of environmental factors. The effect of temperature and pH on bacterial degradation of latex paint under humid condition by bacterial isolates was studied. Results obtained revealed that paint industrial waste (PIW) and paint film scrap (PFS) contained approximately 28.2% to 37.3% of dry organic content (DOC), pH 6.6 to 8.3, optical density (OD) 2.5 to 3.9 and undetermined amount of Ca^{2+}, Na^{+}, K^{+}, SO_4^{2-} and NO_3^{-}. Thirteen (13) isolates were obtained from PIW and fifty two (52) from PFS. The main heterotrophic count ranged from 8.7 to 9.4 × 10^8 $cfuml^{-1}$ for PIW and 3.4 to 6.8 × 10^6 $cfuml^{-1}$ for PFS. The bacterial genera and their percentage occurance for PIW and PFS were: *Pseudomonas* (40:32%), *Bacillus* (26:44%), *Norcadia* (9:6%), *Streptomyces* (0:6%), *Alcaligenes* (11:3%), *Micrococcus* (14:7%) and *Flavobacterium* (0:27%) respectively. The organisms exhibited various degree of paint degradation under different temperature and pH points. *Pseudomonas* demonstrated the highest rate of degradation at pH 7.4 and the lowest at pH 4.2. *Bacillus* had its highest rate at pH 6.3 and lowest at pH 3.5 while that of *Micrococcus* occurred at pH 4.2 and 8.1 respectively. Equally, *Pseudomonas* and *Bacillus* had double peaks of degradation at 28°c and 40°c while that of *Micrococcus* occurred only at 40°c. However, 28°c and 40°c are considered optimal and maximal temperature for biodegradation of paint. Also, the effect of pH and temperature was independent and insignificance at $P < 0.05$. The study therefore, indicated that paint and painted surfaces (objects) can be preserved from bacterial contamination, deterioration and degradation by controlling the storing pH and temperature.

KEYWORDS: Paint, temperature, pH, biodegradation, significance.

INTRODUCTION

Paint is described as a liquefiable mastic material capable of being applied in a thin layer over a surface. On application, the thin layer is converted into an opaque solid film (Kappock,1977; Linder,2005). Paint is made of two distinct phases; the liquid phase which is mainly oil and a powdered solid phase which gives colour and body to the mixture. Paint therefore is used either for protection to prevent environmental weathering or for decorative and aesthetic finish on woods, metal and sculpture (Ogbulie and Obiajuru,2004).

Paint is composed of organic and inorganic pigments, solvents, binders and thickeners (Realimi *et al* 1988; Gettens *et al,* 1990, Strezelezyk, 1981, Montegut *et al;* 1991 and Guglieminette *et al;* 1994). These composit parts of paint and painted film act as substrate with a wide range of organic and inorganic constituents. The constituent provide different ecological niches that are exploited by a large variety of microbial species as nutrient.Given the wide range of these substrates, many microorganisms may grow provided that favourable environmental conditions such as; temperature, oxygen concentration, humidity, pH changes and light are met (Lazar and Dimitru, 1973. Krumbein and Large 1978, Realimi, 1988).

Temperature and pH exert tremendous effect on paint stability and preservation. The four major effects of pH on paint and painted surfaces includes viscosity loss, pigment dispersion stability, hydrolytic stability of resins and surface wetting that often result to various level of corrosion (Realimi *et al.,*1988). Degradation of paint occurs at a wide range of temperature ranging from as low as below 0°C to as high as 70°C (Atlas,1981). Microbial growth rate on paint is a function of temperature (Gibb *et al.,*2001) and rate of degradation decreases with decreasing temperature. Higher temperature increases the rate of paint metabolism to a maximum typically in the range of 30°C to 40°C. At this temperature, enzymatic activities is reduced and membrane toxicity by metabolites is increased (Leah and Colwell,1990). Equally, higher fractions of paint become less volatile, thereby leaving the paint constituents that are toxic to microbes in the medium for a long time with resultant depression of microbial activity. Paint therefore becomes more viscous at low temperature, hence, less spreading occurs and less surface area is available for colonization by microbes (Etim *et al.,*2007).

The three main classes of fouling microorganisms that colonize paint are fungi, algae and bacterial. The presence and survival of these organisms

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result to a number of degradation problems such as; viscosity loss, malodouration, discolourization, gassing, sedimentation, pH change and loss of adhesion (O'neill, 1986, Agravel *et al;* 1988, Nugari *et al;* 1996 and Gferri, 1999).

The fouling problems impose significant and tremendous economic loss to paint manufacturers and consumers in the humid southern region of Nigeria and other part of the world. This study therefore was designed to investigate the role of bacterial and environmental elements in paint degradetion in the tropical humid environment.

MATERIALS AND METHOD

Sample collection:
Paint film scrapings of deteriorated painted surfaces aged 2 – 5 years were collected from twenty-five (25) buildings located within 3 distinct catchment areas. The areas were; Calabar water front, interland area approximately 12 km from the water front and the interior approximately 30km from Calabar metropolis. The film scraps were collected with a sterile scapel blade and plastic bags as described by Okpokwasili and Ituen(1996) and Ogbulie and Obiajuru(2004). The samples collected were transported aseptically to the laboratory for bacteriological investigations.

Determination of some physicochemical properties of paint film scrap.

The physicochemicals analysed were; pH, optical density(OD), dissolved organic contents(DOC). Others include the pH of the homogenized PFS and PIW in deionized sterile water were determined following the protocols outlined by Eckerts and Sims(1995) with an electronic pH meter (Sankin PHB-3 model,Japan) the cations: Na^+, K^+ and anions: SO^-_4, NO^-_3 and Cl^-.

The film pH in deionized water was measured with an electronic pH meter (Sankin PHB-3 model, Japan).
The turbidity was measured as optical density(OD) at 450nm with spectrophotometer (model spectronic 20 Genesys, spectronic Inst. Inc. Rochesli, NY). The dissolve organic content was determined as described by Tack *et al*;(1996). The cations and anions were determined as described by APHA(1998).

Bacterial characterization and identification.

The heterotrophic bacterial load was determined by the pour plate method on tryptone soy agar (TSA) (Atlas and Bartha 1982, Ogbulie *et al*;1998 and Etim *et al*;2007). The isolates obtained were purified by repeated subculturing and characterized based on their cultural, morphological and biochemical reactions described by Buchanan and Gibbon(1974), MacFaddin(1980) and Cowan(1985).

Effect of pH on bacterial degradation of latex paint:

One(1.0ml) millilitre of an overnight (18hours) pure culture of *Pseudomonas, Bacillus* and *Micrococcus* were inoculated into fifty(50ml) milliliters of mineral salt medium (Titan BIOTECH: K_2HPO_4 1.8g, KH_2PO_4 1.2g, NaCl = 0.1g, NH_4Cl = 4.0g, $MgSO_4.7H_2O$ = 0.2g, $FeSO_4.7H_2O$ = 0.01g in 1 liter of water) and adjusted to different pH points of 3.5, 4.2, 6.3, 7.4 and 8.1 respectively by addition of varying proportions of K_2HPO_4 and KH_2PO_4 as described by Cruickshank *et al*;(1975), Antai(1990) and Etim *et al*;(2007).

Each sample was inoculated in triplicate and incubated at room temperature (30 ± 1^oC) for 14 days (Antai,1990) on the bench. The setup received daily shaking agitation to enhance aeration. The extend of bacterial degradation at each pH point was gravimetrically determined and total viable count (TVC) (Etim *et al*;2007).

Effect of temperature on bacterial degradation of latex paint:

The mineral salt medium (MSM) as previously prepared was dispensed in 50ml amount into thirty nine sterile 100ml capacity flasks. Filter sterile latex paint was added in 2.0ml amount to each flask. Overnight broth culture of *Pseudomonas, Bacillus* and *Micrococcus* spices was added in 1.0ml amount to a set of 12 flasks respectively. Three flasks were left unincubated to serve as controls.

The thirty nine (39) flasks were divided into four(4) groups: group one was incubated at 20^oc, group two at 28^oc, the third group at 37^oc and the fourth group was incubated at 40^oc. Choice of temperature on paints reflects the temperature usually observed in Nigeria during rainy and dry seasons. Incubation was for 14 days with a daily shaking agitation. The extent of bacterial degradation was gravimetrically determined.

RESULTS

A total of sixty-three (63) heterotrophic bacterial isolates were obtained as presented in Table 2. Paint industrial waste from two locations labeled A and B had thirteen isolates while paint scrap from twenty-five buildings had fifty-two isolates. The average heterotrophic count ranged between 3.4×10^6 to 9.4×10^8 $cfuml^{-1}$ of paint waste. The heterotrophic isolates were seven and of the genara *Pseudomonas, Bacillus, Micrococcus, Alcaligenes, Norcadia, Streptomyces* and *Flavobacterium.* The percentage abundance of the isolates is represented in Table 3.
The physiocochemical index of the paint scraping are represented in Table 4. The average range of the dry organic content (DOC) of the paint industrial waste and paint scraping were PIW = 28.2 to 34.8% and PS = 31.6 to 37.3. This indicated that paint scraping had more organic content than paint effluent. The pH remains slightly the same in both cases at pH 6.8 to 8.3 respectively.

The effects of pH and temperature on degradation of the paint by bacterial isolates (*Pseudomonas* and *Bacillus*) are represented in Tables 4 and 5 and Figures I and 2 respectively. The pH and temperature range had different degree of effect on each of the organisms. At pH 3.5 and 8.1, *Micrococcus* had the highest cell count and degradation than *Pseudomonas* and *Bacillus* and was significant at $p > 0.05$. But at pH 4.2 and 28^oC, *Pseudomonas* was observed with the highest cell count and paint

degradation than *Micrococcus* and *Bacillus.* In each case, however, the marginal cell count and degradation was at pH 8.1 and 37^{o}C.

DISCUSSION

The study revealed the bacterial load, and diversity on paint as a substrate. Paint with a high DOC supported the contamination and colonization by a wide range of bacterial population. The isolation and percentage distribution of the isolates is in concert with the work reported by O'niell (1986), Realimi (1988), Okpokwasili and Ituen(1996) and Ogbulie and Obiajuru (2004). In their different reports, the authors opined that *Pseudomonas, Bacillus, Norcadia, Alcaligenes, Micrococcus, Streptomyces* and *Flavobacterium* are characteristic biodeteriogens of paints. The frequency of occurance indicated that the composite nature of paint with a wide range of organic and inorganic constituents provided different ecological niche for bacteria at favourable environmental conditions as those experienced in the tropic.

Bacterial colonization and deterioration of paint are consequent of environmental condition such as; pH change, temperature, moisture and substrate composition. In the humid tropical climate, bacterial contaminants are encouraged to grow, degrade and deteriorate paint. The degradation of the test bacterial cells: *Pseudomonas, Bacillus* and *Micrococcus* was a direct response to pH and temperature changes. Different cells exhibited different response to changes in temperature and pH change. In their report, Ogbulie and Obiajuru (2004) confirmed the role of water activity an environmental factors in the biodeterioration process of paint.

Therefore, in an industrial environment, where aerial industrial wastes contain compounds of sulphate (SO^{2-}_4), chlorine (Cl^{-}_2) and nitrate (NO^{-}_3) when in contact with high humid condition of the south, enhances the growth of these organisms thereby promoting microbial growth and degradation of paint on painted surfaces and paint film. Secondly, pH most obvious effect is on viscosity, pigment stability, stability of paint and hydrolytic stability of resins. Since most waterborne paints are formulated at pH 7.5 and 9 therefore any shift in pH will drastically alter the paint's properties. For instance, thickeners composed of carboxylic acid polymers will interact with the hydronium ion or the hydroxides along the polymer chain. This reaction either increases the length of the polymer chain and increases the viscosity or shortened the chain and decreases the viscosity (Bock and Sand1993, Hare,2001). The result showed that the test organisms had the marginal cell count and the paint degradation at pH 8.1 and 37^{o}C. This is an indication that, at this pH and temperature, the paint maintained a high level of stability and remained unstabled at pH 3.5, 4.2 and 6.3

Also *Pseudomonas, Bacillus* and *Micrococcus* species have been reported in many circumstances to degrade hydrocarbon and cellulotic compounds at various temperature range.(Antia,1980).Thus, higher temperature increases the rate of organic decay. However, for most organic materials, the rate of decay and degradation is unpredictably fast at temperatures humans find comfortable.

Table I: Some physicochemical properties of paint industrial waste and paint scrapings.

S/No	Parameter	Paint industrial waste(PIW)	Paint film scrapping(PFS)
1	pH	6.6 - 6.8	7.8 - 8.3
2	Temperature	30 - 33^{o}C	-
3	Optical density (OD)	2.50 - 3.3	2.71 - 3.9
4	Dry organic content (DOC)	28.2 - 34.8	31.6 - 37.3
5	Cations	Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}	Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}
6	Anions	CO_3^{-}, SO_4^{2-}, NO_3^{-}	CO_3^{-}, SO_4^{2-}, NO_3^{-}

Table 2: Number of bacterial isolates and heterotrophic count.

Source	Location or sites	No of isolates	Heterotrophic
PIW	A	6	8.7×10^{8}
PIW	B	7	9.4×10^{8}
PFS	C(24 building)	52	3.4×10^{6} - 6.8×10^{6}

KEY = PIW = paint industrial waste, PFS = paint film scraps

Table 3: Bacterial isolates and percentages abundance

Organisms	PIW	PFS
Pseudomonas	40	32
Bacillus	26	44
Nocardia	9	6
Streptomyces	-	6
Alcaligenes	11	3
Micrococcus	14	7
Flavobacterium	-	2

Table 4: Effect of pH on total variable count (TVC) of bacterial isolates from paint scrap on paint degradation

Ph	Bacterial isolates and cell count (TVC)		
	Pseudomonas	*Micrococcus*	*Bacillus*
3.5	1.57×10^8	2.7×10^8	3.3×10^7
4.2	1.3×10^{10}	3.2×10^8	8.3×10^7
6.3	1.13×10^8	1.4×10^9	3.4×10^7
7.4	7.9×10^7	1.9×10^8	7.9×10^7
8.1	1.43×10^8	1.6×10^{10}	5.6×10^7

Mean of 3 readings

Table 5: Effect of temperature on total viable count (TVC) of bacterial isolates from paint scrap on paint degradation.

Temperature	Bacterial isolates and cell count (TVC)		
	Pseudomonas	*Micrococcus*	*Bacillus*
20	5.0×10^6	1.2×10^7	4.8×10^7
28	2×10^8	2.7×10^8	3.0×10^8
37	4.5×10^6	7.0×10^6	7.8×10^7
40	1.4×10^{10}	1.9×10^{10}	1.0×10^6

Meaning of 3 readings

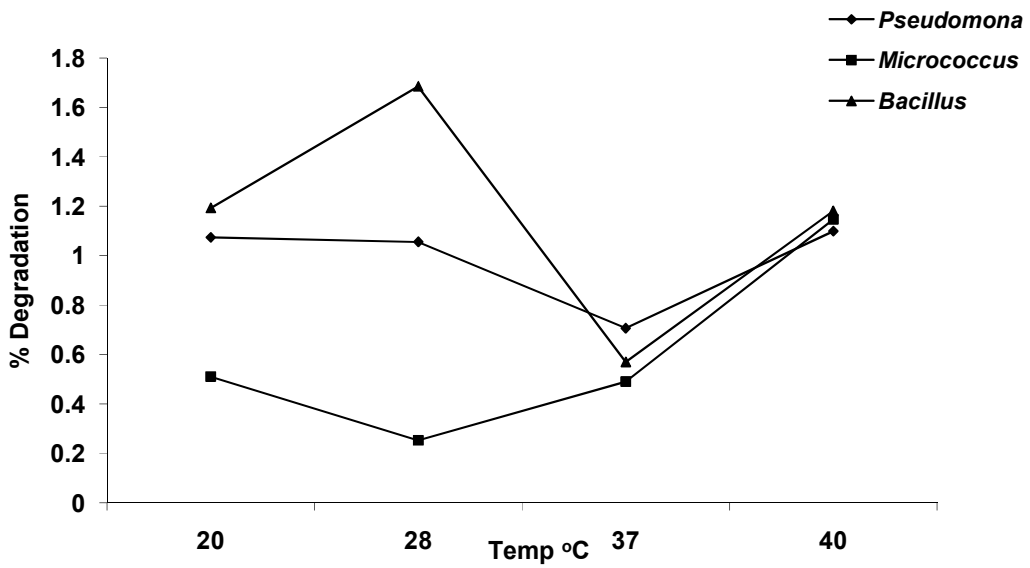

Fig.1: Effect of temperature on cell growth (optical density) of bacterial isolates from paint scrap on paint degradation. Mean of 3 readings

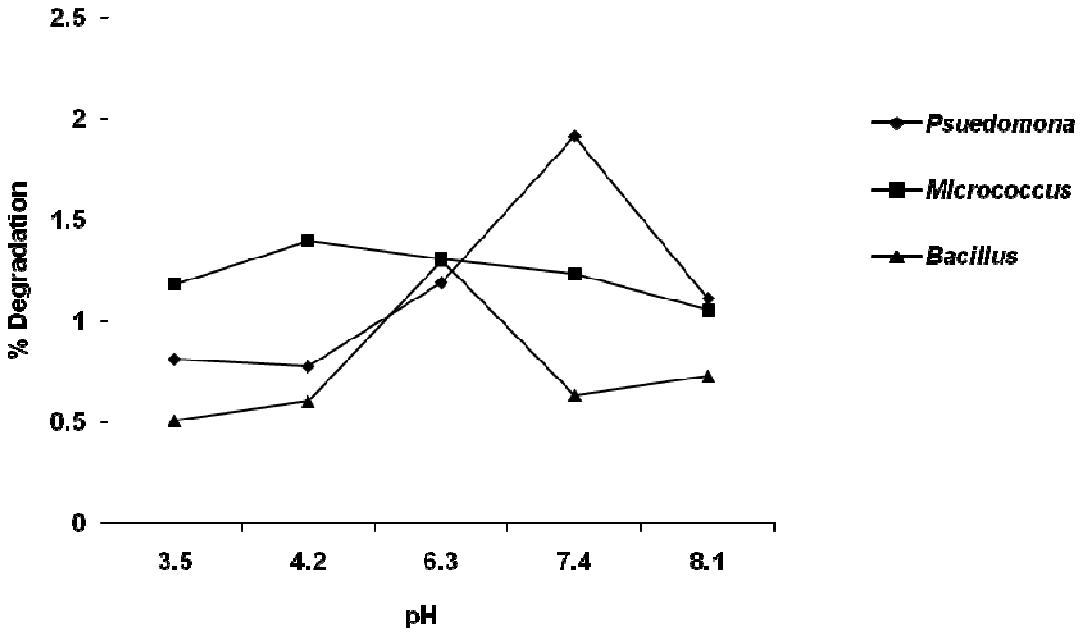

Fig.11: Effect of pH on cell growth (optical density) of bacterial isolates from PIW and PFS on paint degradation. Mean of 3 readings.

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