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Bio-kinetics of Acrylic-Based Paints Biodegradation

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Abstract

This study was carried out to estimate the bio-kinetic parameters involved in the acrylic based paints biodegradations. After biochemical analysis of the paint effluent contaminated soil samples, the total bacterial counts varied significantly ($P<0.05$) between 2.6×10^5 (20 m away) to 8.6×10^5 CFU/g (on site), and predominant bacterial isolates were *Bacillus* spp, *Pseudomonas* spp., *Micrococcus* spp., *Arthrobacter* spp., *Aeromonas* spp. and *Flavobacterium* spp. Growth kinetics of the isolates were significantly greater ($p < 0.05$) for 1,000 - 3,000 ppm, with rate constant values of 0.025-0.012/day, respectively. Cell viability was observed after 30 days biodegradation rates for 4,000 ppm (0.0051/day) and 5,000 ppm (0.0021/day). These findings suggest that the isolates can be used in the bioremediation of soils contaminated with acrylic paint at concentrations of 1,000 and 2,000 ppm, and the equation is useful in monitoring and predicting the performance of microorganisms in bioreactor and predicting the performance of microorganisms in bioreactor containing acrylic effluent.

1. Introduction

Paint is one of the oldest synthetic substances known to mankind. Paint has become one of the most essential items in the modern times. Paints are stable mechanical mixtures of one or more pigments [1] and of various chemical components [2]. Paints are used as protective coatings to prevent the environmental weathering of materials and to provide a decorative finish. Paint may be broadly categorized as solvent-borne products (e.g. Gloss paint) and water-borne coating (e.g. emulsion paint and text coat paints) [3, 4]. Acrylic paint (or "latex" paint) is a fast-drying paint containing pigments suspended in an acrylic polymer emulsion. Water is the main solvent for acrylic based paints [4, 5]

Generally, industrial effluents can potentially affect hydrological and environmental parameters of a catchment, as well as pose significant threats to man and natural ecology. In Nigeria, acrylic paint production utilises large volumes of water without adequate wastewater treatment facility. Hence, large quantities of both hazardous and non-hazardous wastes are inherently released to the environment, thus causing health problems, ecological imbalance and bioaccumulations [6]. The important environmental elements like soil, water and air are being continuously contaminated by toxic pollutants generated from these sources. The types of effluents produced in the paint industries are liquid effluents, solid wastes and volatile organic compounds. Nowadays they have grown in different zones of the country. Due to the increasing demand for paints, these industries are growing daily and have become a great burden to treatment plants. Consequently, the wastes generated are disposed into drains, canals and rivers without treatment and the solid wastes are dumped into surrounding land or water bodies which contaminate the soil or water with highly toxic inorganic or organic pollutants [6]. Paints, lacquers and varnishes are among the products that have distinct effects on environment and health [5, 6].

Exposure to acrylic paints may cause serious health hazards to human beings as well as to the wide array of animals and plants [7, 8]. For instance, continuous exposure to acrylic paints can exert mutagenic effects on cells derived of humans or other animals due to covalent bonding with deoxyribonucleic acids [9, 10]. It is also one of the well-known human neurotoxins and may cause leukoencephalopathy in humans at long exposure time [9]. Acrylics and their derivatives are of environmental concern because many are mutagenic and/or carcinogenic [11, 12], hence the need for their remediation. While bioremediation has been suggested as a feasible technology for remediating acrylic contaminated sites [13], their sparing solubility and tendency to form associations with organic matter greatly decrease the portion of acrylic paint available to microorganisms [14]. This means that successful remediation of acrylic paint contaminated soils may require special strategies. The potential for in situ bioremediation of acrylic paint may be further limited if conditions in soils are not optimal for the growth and metabolism of acrylic paint degrading microorganisms. These difficulties may potentially be overcome by physically manipulating conditions in soils or by adding organic and inorganic supplements. In general, supplements are used either to increase the bioavailability of the pollutant(s) of interest and/or to increase or stimulate populations of degrading microorganisms.

In the last two decades, an increasing interest has been observed for utilising microorganisms such as bacteria for removal of various aromatic compounds from the environment [5, 15, 16]. Acrylic was shown to be degraded by a variety of aerobic bacterial species and at least five different degradation pathways have been identified [5, 17]. Interest in the use of bacteria to get rid of acrylic paint and aromatic compounds from contaminated soil has continued. There are several studies on aerobic bacterial biodegradation of aromatic compounds at the international level, however, there is dearth of study on application of models in bioremediation of some pollutants in developing countries.

Kinetics modeling of acrylic paint contaminated soil remediation is very complex due to the number of factors involved. The kinetic studies in a natural environment are often experimental, reflecting only the basic knowledge about the microbial density and its activity in the given environment. But kinetic analysis is the key factor to understanding a biodegradation process. The extent of remediation offered by microbes is best articulated from the bioremediation kinetic model and described by first order kinetic equation. The kinetic information is essential in the estimation of the concentration of the contaminant at any time and permits the prediction of its level in the future. This study was therefore undertaken to estimate the bio-kinetic parameters involved in the acrylic based paints biodegradations using polluted soil samples collected from two main factories within Ondo State, Nigeria.

2. Materials and Methods

2.1. The study area

The study site is Akure, the capital city of Ondo State, Southwestern Nigeria. Geographically, Akure is situated on latitude 7^0 5' North of the Equator and longitude 5^0 15' East of the Greenwich Meridian with a population of about 340,021 [39] (Fig. 1).

2.2. Chemicals and Reagents

Acrylic paint and chemicals, used in the study, were of analytical grade; glucose and inorganic salts, used in preparing microbial growth media, were of reagent grade.

2.3. Collection of soil samples

Soil samples were collected from different locations of acrylic paint contaminated soil within Akure Ondo state. A total of five soil samples were collected from two different sites. The aerated surface material of spilled paints was scraped off to a depth of 3–10 mm from dried areas showing extensive contamination.

2.4. Preparation of soil samples and serial dilution (CFU/mL)

The collected soil samples were processed according to previous method [18] with slight modification. One gramme of each collected soil sample was dispensed in 50 ml of distilled water in shake flasks (200

rpm) at 37 ºC for 30 min. After settling of the larger soil particles at room temperature for 30 min., approximately 1ml of soil suspension was used for serial dilution. Exactly 1 ml of the suspended soil samples was added to 9 ml of sterile 0.85 % (w/v) saline solution to make a one in 10 dilution (10^{-1}), then one ml of this dilution was added to 9 ml 0.85 % (w/v) saline solution to make a one in 100 dilution (10^{-2}). This procedure was repeated until 10^{-8} dilution was reached. Triplicate samples of 100 µl of each dilution were spread onto agar plates for microbial counts.

Fig.1. Map of Nigeria showing Ondo State

Diluted samples were plated onto different bacterial media Plate Count Agar (PCA), Trypticase Soy Agar (TSA), Alkaline Buffered Medium 2 (ABM2) and Malt Extract Agar (MEA) agar plates for fungi, and incubated at 25 ºC, for up to 10 days. Colonies were monitored after 24 h and then daily for bacterial cultures, and fungal counts were observed between 2 and 10 days with recorded counts every 48 hrs. Colony forming units (CFU) per milliliter (ml) was calculated using the formula:

$$\text{Mean Count } (CFU/mL) = (\text{Dilution used} \times \text{amount plated [mL]})$$

2.5. Enrichment of soil samples and media conditions

The medium used was mineral salt medium (MSM) included (g/L): $MgSO_4.7H_2O$, 10; $CaCl_2.7H_2O$, 0.2; and, KH_2PO_4, 13.6; $(NH_4)_2.SO4$, 2.4; $FeSO_4.7H_2O$, 0.2; $Na_2HPO_4.12H_2O$, 15; and agar, 12 (in case of MSM agar only) [5]. All the salt solutions were separately prepared and autoclaved at 121 ºC for 15 min, and dissolved one by one into the main MSM medium (approximately 980 ml of distilled water). The pH of the medium was adjusted to 7.0 ± 0.2 using 6 N HCl and 1 M NaOH solutions. The soil suspension (0.1 %, v/v) was aseptically added to the 250-ml shake flasks containing 100 ml of MSM broth enriched with oil paint (ALBA synthetic enamel, white-32) at a final concentration of 100, 200, and 300 ppm as a sole source of carbon and incubated at 37 ºC and 160 rpm for 14 days in the Multi-tier environmental shaker (Innova, Germany). The growth absorbance (OD600 nm) of each sample was measured daily using a visible spectrophotometer (Jennway, Germany).

2.6. Isolation and screening of acrylic-based-paint-utilizing bacteria

Based on the growth absorbance (OD 600 nm), the samples showing maximum optical density were selected and streaked onto the surface of MSM agar plates enriched with (w/v) oil-based paint (final conc. 300 ppm). The MSM agar contained the same composition of salts as mentioned above in previous subsection of this manuscript and agar at a concentration of 1.2 % (w/v) as solidifying agent. Afterward, the bacterial colonies were purified by sub-culturing onto nutrient agar plates. The bacterial isolates obtained were studied for morphological characterization [19, 20]. The tests including Gram staining, motility test, spore staining, and capsule staining

were performed for the morphological characterization. Finally, the bacterial isolates, as pure cultures, were grown in nutrient broth and further investigated for their biochemical characteristics using standard procedures.

2.7. Acrylic paint biodegradation study

Different concentrations of acrylic paint were prepared (1,000 – 5,000 ppm soil) and filtered using Millipore filter 0.45 µm. Acrylic paint was added to 100 mL sterile MSM 250 mL capacity with a two-bored screw cap; one bore for air flow input (6.27 mL/h) to maintain aerobic conditions and the other bore for capturing CO_2. As the commonly recommended earlier [21], ammonium nitrate (NH_4NO_3) and potassium phosphate dibasic (K_2HPO_4) were added at a ratio of 100:15:1 (C: N: P). Previously standardized bacterial inoculum (over-night culture of each isolate) was added in a concentration of 3×10^6 CFU/mL. Viable counts were determined for all batch reactors as a function of time (0, 5, 10, 15, 20, 25, and 30 days). Controls without bacteria inoculations were used to monitor microbial activity and losses as abiotic control. Moisture in all treatments was maintained at 70 % of field capacity for the period of the experiment (30 days) at 28 °C in the dark. Acrylic paint extraction from samples was carried out using the modified EPA 3550B method to measure the loss of acrylic paint.

2.8. Calculation of biodegradation rate constant and half-life time

The biodegradation first-order rate constant (k) was estimated by evaluating the slope of the best fit line on a plot of concentration versus time, using equation below:

$$C = C_{o.}e^{(kt)} \text{ ------- ---------- (i)}$$

Where C_o is the initial concentration of acrylic paint (ppm soil), C is the concentration of acrylic paint at a time (t) [22].

The half-life time ($t_{1/2}$) was estimated from the derivative of equation used earlier [23].

$$t½ = \ln/K \text{ ---------- (ii)}$$

2.9. Estimation of bio-kinetic parameters

The equations to model the interaction between the use of acrylic paint as a substrate-carbon source-electron donor and the kinetics of cell growth of seven isolates were based on the mathematical model established by Haldane as shown on equation below:

$$U = U_{max}\, S/K_s + S + (S^2/K_i) \text{ ---------- (iii)}$$

Where U is the specific growth rate (1/day),
U_{max} is the maximum specific growth rate (1/day),
S is the amount of substrate (mg/kg),
K_s is the saturation constant (mg/kg), and
K_i is the growth inhibition constant (mg/kg).

Since high concentrations (1,000 - 5,000 ppm) of acrylic paint were tested, the equation was reduced to equation below; the model parameters, U_{max} and K_i, were fitted to the experimental data with Excel solver (Microsoft) using the Levenberg-Marquardt method [24].

$$U = U_{max}\, S/S + (S^2/K) \text{ ---------- (iv)}$$

The generation or doubling time (t_d) required for cell division during the exponential phase was calculated the equation below [25].

$$t_d = \text{In } 2/U \text{ ---------- (v)}$$

The maintenance energy coefficient (m) was estimated using equation below, according to the model proposed by Pirt; where Y is the observed yield coefficient and Y_G is the true growth yield coefficient [26].

$$1/Y = 1/Y_G + m/U \text{----------} \quad \text{(vi)}$$

Endogenous decay rate k_d (1/day) during the stationary phase was estimated using equation [27].

$$X = X_{S0}.e^{-kdt} \text{----------} \quad \text{(vii)}$$

X is the initial biomass concentration (CFU),

X_{So} is the biomass concentration at the beginning of stationary phase (CFU), and

t is time (day).

2.10. Data collection/ Statistical analysis

The data were presented as mean ± standard error using the analysis of variance (ANOVA) SPSS version 16. Values of $p < 0.05$ was considered statistically significant.

3. Results and discussion

The result of the total bacterial count of soil samples collected from three different locations in two paint factories were presented in Table 1. The result showed significant differences (p>0.05) among the total bacterial counts from the four soil samples with samples collected inside the factory having the highest bacterial counts (8.6×10^5 and 6.8×10^5) for site A and B respectively. This was followed by samples collected 10m away from the sites (5.0×10^5 and 2.1×10^5) for sites A and B respectively and samples 20m away (3.2×10^5 and 2.6×10^5) for sites A and B respectively. When compared with the control soil sample collected from pristine soil, the bacterial count of the control sample was significantly (p>0.05) lower than that of the contaminated site.

Table 1: Total bacterial count from various sampling locations

S/N	Sampling location	Total bacterial counts (CFU/mL)*			Control
		0 m	10 m	20 m	
1.	Factory A	$8.6 \times 10^5 \pm 0.117^a$	$5.0 \times 10^5 \pm 1.000^b$	$3.2 \times 10^5 \pm 0.010^b$	$0.1 \times 10^5 \pm 0.017^b$
2.	Factory B	$6.8 \times 10^5 \pm 0.003^b$	$2.1 \times 10^5 \pm 0.100^a$	$2.6 \times 10^5 \pm 0.002^a$	$0.1 \times 10^5 \pm 0.002^a$

*Values are mean scores ± Standard deviation of three (3) replicates

The morphological and biochemical characteristics of the isolates from the two sites showed that the most frequently isolated species were *Bacillus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Arthrobacter* spp., *Aeromonas* spp. and *Flavobacterium* spp. Table 3 shows the results for the biodegradation of acrylic paint at different acrylic paint concentrations by isolates for 30 days of experimentation. A greater significant reduction of hydrocarbons was reached in 1,000 and 2,000 ppm concentrations with higher constant rate values and significantly lower half-life time values, as compared to the other concentrations. It was observed that as acrylic paint concentration increased (3,000, 4,000, and 5,000ppm) the rate constant values decreased substantially to 0.012 0.0002, 0.005 0, and 0.002 7.07E-05 (1/day) respectively; resulting in a significantly low hydrocarbons biodegradation.

Table 2: Experimental results at different acrylic paint concentrations by isolates for 30 days of experimentation

Acrylic paint concentration (ppm)	Acrylic paint removal (%)	Biodegradation rate constants k (1/day)	R^2	$t_{1/2}$ (day)
1,000	90.08 ± 0.408	0.025 ± 0.0013	0.873	26.9 ± 1.41
2,000	87.68 ± 0.420	0.023 ± 0.0003	0.895	29.4 ± 0.44
3,000	69.87 ± 0.219	0.012 ± 0.0002	0.867	57.0 ± 0.99
4,000	36.58 ± 0.188	0.005 ± 0	0.885	135.9 ± 0
5,000	18.17 ± 0.368	0.002 ± 7.07E-05	0.948	308.2 ± 9.69
Control	2.97 ± 0 .075	0	0	0

The growth-associated models plotted using first order kinetics for biodegradation of acrylic based paint shows that the first-order kinetic can be applied with accuracy to describe the biodegradation process of acrylic paint contaminated soils. The first-order rate constants obtained were consistent and comparable with other published

data by other authors in previous studies concerning the degradation of hydrocarbon in soil. Table 3 showed the effect of substrate concentration on growth of microbial culture in the biodegradation of acrylic based paint. The result obtained from the six isolates at five acrylic paint concentrations (1,000 - 5,000 ppm) showed that as the Maximum Specific Growth (μ_{max}) decreases the Half Saturation Constant (Ks) increases and vice versa (Fig. 2).

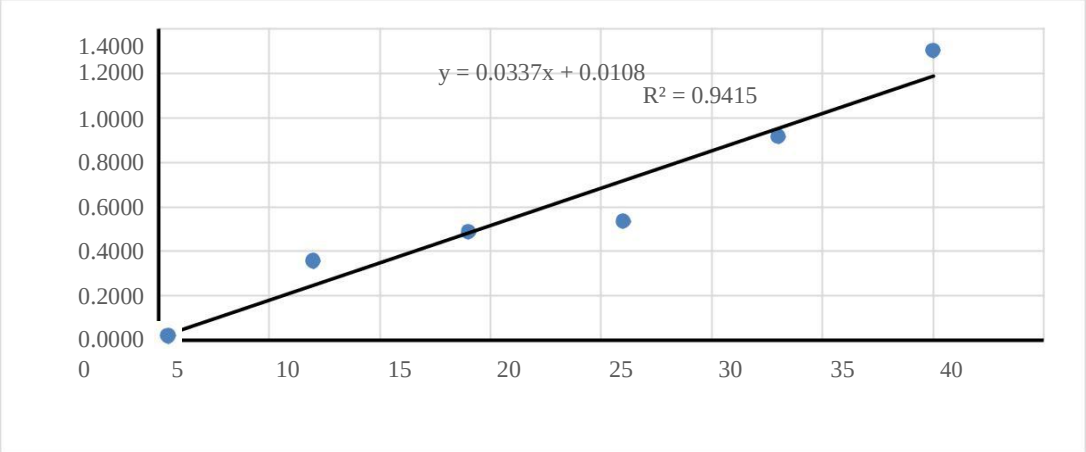

Fig 2: Growth-associated models using first order kinetics for biodegradation of acrylic based paint.

Table 3: Effect of substrate concentration on growth of microbial culture in the biodegradation of acrylic based paint

Isolate	Concentration(ppm)	Maximum specific Growth (/hr)	Half Saturation constant(K_s) (mg/Lhr)
Micrococcus sp.	1,000	0.060	4.22
	2,000	0.017	40.60
	3,000	0.006	53.77
	4,000	0.005	70.68
	5,000	0.005	70.70
Bacillus sp.	1,000	0.630	60.21
	2,000	0.056	180.70
	3,000	0.013	410.09
	4,000	0.003	506.00
	5,000	0.003	506.10
Pseudomonas sp.	1,000	0.050	9.78
	2,000	0.016	20.00
	3,000	0.012	75.45
	4,000	0.011	160.20
	5,000	0.010	160.00
Alcaligenes sp.	1,000	0.008	2.29
	2,000	0.005	10.00
	3,000	0.002	23.60
	4,000	0.001	30.98
	5,000	0.001	30.90
Aeromonas sp.	1,000	0.080	8.00
	2,000	0.052	44.60
	3,000	0.011	67.00
	4,000	0.005	85.80
	5,000	0.005	85.00
Klebsiella sp.	1,000	0.060	6.03
	2,000	0.010	27.80
	3,000	0.008	44.00
	4,000	0.004	63.00
	5,000	0.004	63.00

Table 4 shows the bio-kinetic parameters for isolates obtained in the biodegradation of acrylic paint at different concentrations. The Haldane's model inhibition by substrate was used to represent microbial cell growth kinetics of the six isolates. Higher growth values were obtained at 1,000 and 2,000 ppm concentrations, as compared to the other three concentrations. Time required for doubling the biomass concentration (t_d) was significantly greater for 3,000, 4,000, and 5,000 ppm concentrations; meaning that at low acrylic paint concentrations the isolates required less time to double cellular material indicating inhibition was caused by higher concentrations.

All the collected samples were thus processed for the isolation of bacteria by using soil enrichment technique under shake flask conditions. In this study, a total of seven acrylic paint tolerant bacteria were isolated from soil samples collected from two paint companies within Akure. Six of the isolates namely, *Bacillus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Arthrobacter* spp., *Aeromonas* spp. and *Flavobacterium* spp. have been shown to be among the most frequently isolated paint degrading species. Previous studies have reported species belonging to genera *Pseudomonas, Micrococcus, Sphingomonas, Thiobacillus, Mycobacterium, Clostridium, Alcaligenes, Bacillus, Flavobacterium, Arthrobacter, Gallionella, and Shewanella* as paint and painted surface-degrading bacteria [13, 28].

Table 4: Biokinetic parameters for isolates obtained in the biodegradation of acrylic paint at different concentrations

Acrylic paint (ppm)	max (/day)	K_i (mg/ml)	m (mg substrate /CFU-day)	k_d (/day)	d (day)
1000	0.76	26,598	0.001	0.117	0.912
2000	0.80	11,923	0.004	0.134	0.862
3000	0.43	19,567	0.010	0.189	1.612
4000	0.24	696	0.020	0.196	2.896
5000	0.18	609	0.022	0.217	3.707

Several negative effects on the cell are induced by high acrylic paint concentrations, as reported by Sikkema *et al*. [29]. Mechanical stress and increase in temperature caused by cellular accumulation of petroleum hydrocarbons in the area of the acrylic paint chains of the phospholipid monolayers of the cell membrane affect the structure of the cell membrane causing alterations as increased permeability with an increase in the flow of protons.

Time is a factor that had a significant effect on acrylic paint biodegradation by isolated bacteria, maximum biodegradation took place during the first 15 days; as the bioremediation process continued, the acrylic paint biodegradation gradually decreased. A possible explanation for this behavior might be that during biodegradation the incomplete acrylic paint metabolism tends to produce more toxic metabolites for microorganisms [30]; other studies conducted showed that during the biodegradation process the hydrocarbon bioavailability decreases due to formation of native toxins [31].

Previous studies concluded that the first-order rate constant for acrylic paint ranged from 0.016/day to as high as 0.38/day depending on the constituents of the acrylic paint in question [32]. Similarly, it was discovered that the k-value for the degradation of acrylic paint contaminated soil was 0.099/day [33]. Also it was shown that showed that the first-order kinetic constant at 38^{o}C was 0.013/day for hydrocarbon contaminated soil [34]. Endogenous decay coefficient k_d describes the capacity of the microorganisms to survive during some periods in the absence of nutrients [25]. In this study k_d coefficient was estimated from values compiled during the period of cell reduction considering death phase; values obtained for 3,000, 4,000, and 5,000 ppm were greater than those of 1,000 and 2,000 larger values in m coefficient so that the energy spent at these concentrations to maintain cell activity of the isolates were higher. In this study high K_i values were obtained at lower concentrations of acrylic paint indicating that it was less toxic for the isolates to metabolize.

The bio-kinetic parameters of the microbe has been estimated by fitting the growth data to Monod Kinetic Model. The maximum specific growth (U_{max}) and half saturation constant (*Ks*) has been determined for each initial concentration of acrylic paint. It has been observed that with increase in initial concentration of acrylic paint the *μmax* decreases owing to toxic nature of the substrate. In this study acrylic paint is used as the growth limiting

factor since it is the sole source of carbon and energy. But even if it is a sole source for the microbe its increasing concentration is toxic for the microorganism. Hence instead of increasing, the specific growth rate of the microbe decreases with each unit of increase in concentration. From the results it can be noted that with increasing concentration of the substrate the maximum specific growth rate decreases and the half saturation constant increases. After a certain point the half saturation constant for the microorganism increases drastically. As reported earlier, Agarry *et al*. [35] pointed out that increase in the concentrations of acrylic lead to a reduction in the specific growth rates, and thus could be the possible reason for the pattern of growth graph observed in this study. A similar submission was noted by Hinteregger *et al*. [36]. The maximum specific growth rates of the subjected microbes obtained in the present study does not falls within the range of the maximum specific growth rates reported by the literature available. The probable reason behind this difference in the obtained results may be attributed to the difference in the experimental method and conditions under evaluation.

Moreover sufficient data is not available regarding the bio-kinetic parameters of isolates from acrylic paint impacted soil. It can be observed that the difference in the maximum specific growth values obtained in the present study and the available literature is too high and the reason behind it is the use of additional carbon source along with acrylic paint. In the current study, acrylic paint is used as the sole source of carbon and energy and as mentioned earlier it is toxic in nature and confers certain toxicity to the microorganism. While in the available literature, it was observed that most of the experiments were carried out in the media containing additional carbon and nitrogen sources like glucose and peptone. Hence growth of microorganism in these medium will be more profuse as compared to the medium deficient of these additional sources resulting in a lower value of maximum specific growth exhibited by the microorganisms in the same concentration of acrylic paint. Kotturi *et al*. [37] had noted that the half saturation coefficient is influential on the growth kinetics in low concentration region. At the same time, the small changes in the biomass and substrate concentrations in batch reactors cannot be measured accurately. Therefore, this may be one of the possible reasons for the discrepancy in values of half saturation coefficient, *K*s. It may be mentioned that Hill and Robinson [38] determined the half saturation coefficient using continuous flow mixed reactor. The dilution rate and effluent substrate concentration data are required to calculate the value of *K*s. The dilution rate and effluent substrate concentrations can be relatively measured accurately.

Conclusion

Kinetic experimental research was conducted with bacterial species isolated from an acrylic paint contaminated soil from two paint industries in Akure. The isolates were tentatively identified as *Bacillus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Arthrobacter* spp., *Aeromonas* spp. and *Flavobacterium* spp. and they showed ability to degrade acrylic paint as sole carbon and electron donor source under aerobic conditions. The kinetic formulation of a correlation equation of particle size distribution and their interactive effect on the rate of acrylic paint reduction is essential and will give remediation consultants a simple, fast and effective tool to estimate the biodegradation rate constant for soils allowing reasonable predictions for contaminated lands when the soil texture is known. The microbial growth rate model equations derived in this work could be applied for the monitoring of the rate of degradation of the acrylic paint impacted sites, studying the mechanisms of biodegradation of acrylic paint, estimating the period of biodegradation for specific acrylic paint present in effluents or spills, predicting the performance of acrylic paint utilized, estimating the residence time for acrylic paint component in the design of bio-treatment reactors and as a guide in monitoring bioremediation process. The derived model equations for the biodegradation could be applied in solving various environmental and other technical problems in the petroleum and chemical industries since in practice, industrial effluents are composed of mixtures of different acrylic paint components.

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