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Optimization of biodegradation efficacy of acrylic-based paint contaminated soil by *Alcaligenes faecalis*

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Abstract

Acrylates/acrylic-containing chemicals are components of paints. During industrial production and applications, the acrylates and acrylic-containing compounds could contaminate/accumulate in water bodies and soil systems, hence the need for bioremediation. This study aimed to investigate the *in vitro* biodegradation of acrylic based paint; using an indigenous bacterial isolate namely; *Alcaligenes faecalis* and optimization of its activity in shake cultures. The bacterial isolate; *A. faecalis* (2 % v/v) was able to grow and effectively degrade 68 % of acrylic paints (1 %) amended mineral salt medium after 14 d of incubation. The rate of biodegradation was significantly ($p< 0.05$) increased with increasing the medium concentration, inoculum size, agitation speed and nitrogen sources. The most significant biodegradation efficiencies were obtained at a pH of 7.2, temperature of 37 °C, an agitation speed of 200 rpm, an inoculum concentration of 10 %, paint concentration of 2 %; when yeast extract (10 %) was used as a major nitrogen source. Accordingly, this work provides baseline data for optimum biodegradation of acrylate by *A. faecalis*, and thus could be possibly exploited as an effective bioremediation agent for acrylic paint polluted sites.

Keywords: Acrylates, Soil, Bioremediation, *Alcaligenes faecalis*, Biodegradation, Optimization

1. Introduction

There is a global pollution of the environment by the synthetic wastes of various chemicals, which have filtered into different layers of air, water and soil matter, and cover the immediate and extended ecosystems (Sethy *et al*., 2011; Orjiakor *et al*., 2020). The release of these pollutant chemicals has been rated as a direct factor related to the large variety of anthropogenic activities; mainly attributed to the industrial/technological exploits that occur in several specific areas (Soudi and Kolahchi, 2011). A great

deal of these pollutants has been identified in view of their public health impact. There have been several reports of carcinogenic; mutagenic, physiological alterations and other grades of medical conditions, which have been associated with these pollutants (Manisalidis *et al*., 2020). A previous study of Sethy *et al*., (2011) revealed that issues of bioaccumulation associated with the ingestion and uptake of these chemical pollutants within the vital cellular organs of the humans and animals have been equally identified. Good examples of these chemosynthetic agents that can act as pollutants are the synthetic paints.

Paints manufacturing process involves mixing of raw materials in large tanks at room temperature. This occurs in three major steps including; mixing and grinding of raw materials, tinting and thinning, and filling operations (Gaylarde and Gaylarde, 2005). Recently, Ashwini and Anchana, (2018) reported that latex paint, which is one of the most common paints, generally consists of organic and inorganic pigments; latexes, dyestuffs, cellulosic and non-cellulosic thickeners, preservatives, extenders, anti-foaming agents, emulsifying agents, solvents and coalescing agents. High volumes of waste water are generated on daily basis during the batch production processes; cleaning operations of the mixers, blenders, reactors, packing machines and floors, which eventually finds its way to the environment. Based on the effects of the chemical paints on the surrounding environment, Jolly *et al*., (2008) revealed that soil is an effective purifying medium that has a great capacity to receive and decompose wastes and pollutants of different kinds. However, Mahawar and Akhtar, (2015); Berihun and Solomon, (2017) reported that in the case of over-burdening of soil with extraneous pollutants, there arises a consequence that could impact adversely on the soil microbiological and physicochemical properties; with consequent effects on the growth and development of plants utilizing the soil as their habitats.

Previous works conducted by Ogu and Odo, (2015); Phulpoto *et al*., (2016) stated that bioremediation is the safest and most ecofriendly technique used for cleaning-up the environment from the various organic and inorganic pollutants. Bioremediation of acrylic-based paint contaminated ecosystems is based on the ability of microbial species to degrade the target paint pollutant. Previous reports of Stranger-Johannessen and Norgaard, (1991); Sethy *et al*., (2011); Orjiakor *et al*., (2019) have revealed the efficacy of microorganisms as biodegradation agents of different paints and paint-wastes. A variety of microbial genera have been reported to be associated with paint-laden environments including; *Pseudomonas*, *Micrococcus*, *Mycobacterium*, *Flavobacterium*, *Arthrobacter*, *Gracilibacillus*, *Salibacillus*, *Virgibacillus*, *Aspergillus*, *Trichoderma*, *Aureobasidium*, *Penicillium*, *Stemphyllium*, *Actinomyces* and *Gallionella* (Heyrman and Swings, (2001); Gorbushina *et al*., (2004); Imperi *et al*., (2007); Sethy *et al*., (2011); Ravikumar *et al*., (2012); Phulpoto *et al*., (2016); Orjiakor *et al*., (2019). The search for bacteria with potentials to degrade various environmental pollutants is still up to date. Previous study of Phulpoto *et al*., (2016) reported that *Bacilllus* species are potential agents for biodegradation of acrylic oil paints. Therefore, it has become pertinent to extend the study for the other indigenous bacterial isolates inhabiting paint based pollutant soils, to increase the array of potential bioremediation agents. Hence, the objectives of this study were to investigate the in vitro biodegradation of acrylic based paint using an indigenous bacterial isolate namely; *A. faecalis*, and optimization of its activity in shake cultures.

2. Materials and methods

2.1. Sampling location

The sampling locations are paint effluents contaminated sites around the production unit of two famous factories (A and B); both located in Ado Ekiti, the State capital of Ekiti, South Western of Nigeria. Geographically, Ado-Ekiti is situated between latitude 7.667° N and longitude 5.250° E, and is bounded in the north by Kwara State and Kogi State, Osun State occupies the west, while Ondo State lies in the south and extends to the eastern part (Fig. 1).

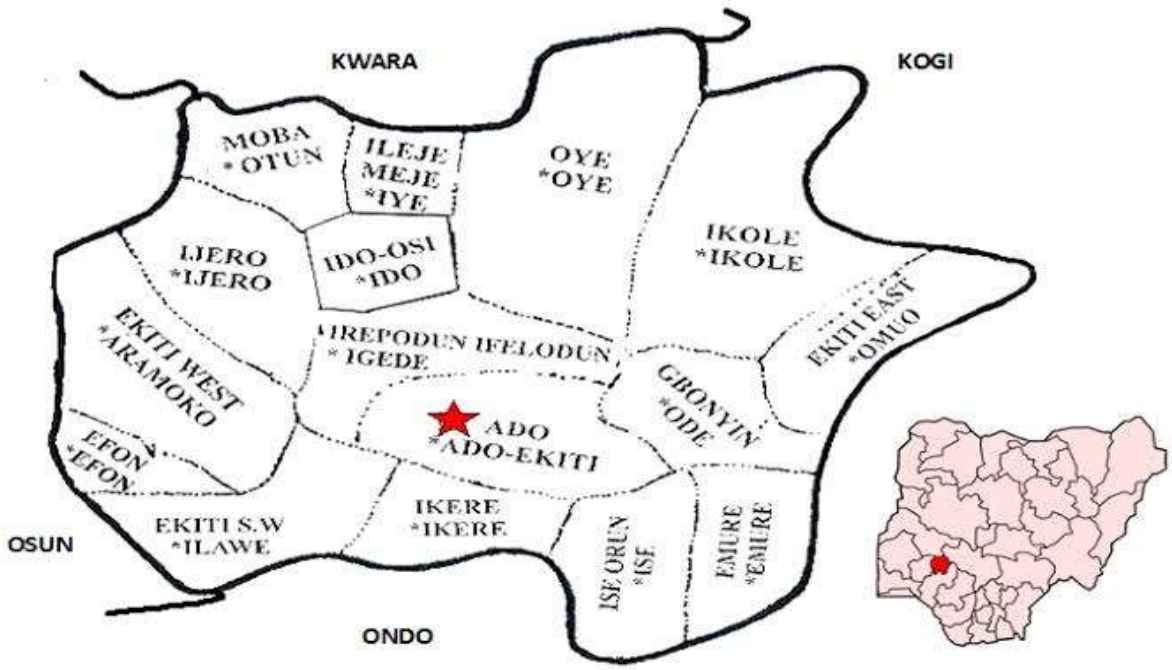

Fig. 1: The map of Nigeria with location of Ekiti State in red (Salau *et al*., 2016)

2.2. Samples collection

Pooled soil samples used during the study were collected randomly from acrylic-paint impacted soil around the paint production, and waste disposal units at a depth of 10 cm using soil auger. The soil samples were gently placed in clean plastic containers before transportation to the laboratory for further assays.

2.3. Isolation and identification of the bacteria

Soil samples were collected and analysed based on the previous assays conducted by Hassan *et al*., (2013); Phulpoto *et al*., (2016), with slight modifications. Approximately 1 g of each soil sample was dissolved in 50 ml of sterile dist. water, placed in 250 ml flasks, and then the flasks were incubated for 2 h at 37 ^{o}C. After incubation, 5 ml of each soil suspension was aseptically added into flasks containing 100 ml of prepared mineral salt media (MSM) broth, enriched with 1 % v/v acrylic paint (Finecoat® Acrylic Emulsion Paints) as a carbon source. The MSM was composed of; 0.5 g/ l $MgSO_4$, 0.2 g/ l $CaCl_2$, 13.6 g/ l KH_2PO_4, 5 g/ l $(NH_4)_2.SO_4$, 0.05 g/ l $FeSO_4.7H_2O$, 15 g/ l Na_2HPO_4 (Phulpoto *et al*., 2016). The experimental set-ups were incubated for 10 d at 37 ^{o}C under agitation (150 rpm). An aliquot of 0.1 ml of each enriched sample was cultured using spread plate technique on sterile mineral salt agar plates, and then incubated for 5 to 10 d for bacterial growth and colony development. The recovered colonies were purified and then sub-cultured on mineral salt agar slants for characterization. Phenotypic identification of the bacterial isolates was carried out using several assay methods including; Gram reaction, spore stain, motility, Hydrogen sulphide production, indole production, catalase production, citrate utilization, oxidase production, Methyl red and Voges Proskauer reactions, coagulase production, urease production, nitrate reduction and sugar fermentation tests, in reference to Bergey's

Manual of Determinative Bacteriology (Krieg and Holt, 1984).

2.4. Determination of growth rate and biodegradation efficiency of the promising bacterium

Investigation of the growth rate and biodegradation efficacy of the potent bacterium were carried out in a batch shake-flask assay using the MSM plus acrylic paint for 14 d, according to the modified method adopted by Phulpoto *et al*., (2016). The test bacterial isolate was standardized by serial dilution to 10^6 before inoculating 2 % (v/v) into three different shake-flasks containing 100 ml of the 1 % acrylic paint amended mineral salt medium. The flasks were then incubated at 37 ^oC on a shaking incubator (150 rpm). After incubation, absorbance's were estimated at 600 nm every 2 h to determine the growth rate of the test bacterium.

To evaluate the biodegradation efficacy of the bacterium, about 5 ml of the bacterial culture+ MSM+ acrylic paint samples were collected, dissolved in 5 ml petroleum ether (10 % v/v), and then their absorbance's were measured at 285 nm. In addition, 1 % (v/v) of acrylic paint was dissolved in petroleum ether and then its absorbance was measured spectrophotometrically at 285 nm as a control. Petroleum ether (10 % v/v) was used as a blank for the spectrophotometric readings. The biodegradation efficiency (%) was calculated using the formula of Phulpoto *et al*., (2016):

Biodegradation efficiency (%)= (Control absorbance (285 nm)-Test Sample absorbance (285 nm))/ (Control absorbance (285 nm))

2.5. Effects of growth conditions and acrylic concentration on acrylic paint biodegradation potential

The effects of different growth parameters including; temperature, initial media pH, inoculum size, agitation speed, nitrogen source and acrylic paint concentration, on acrylic paint biodegradation potency were tested. The tested temperatures were 27 ^oC, 37 ^oC, 47 ^oC, 57 ^oC; pH 5.2, 7.2, and 9.2 adjusted with 1M NaOH and 0.1 M HCl; inoculum concentrations tested include; 1 %, 2 %, 5 %, and 10 %; agitation speeds of 100, 150, and 200 rpm; different nitrogen sources used were ammonium sulphate, sodium nitrate, yeast extract and peptone; whereas the tested acrylic paint concentrations mainly were 1 %, 2 % and 4 %. The effects of these various factors on the rate of biodegradation acrylic paint in a shake flask were studied as described above.

2.6. Statistical analysis

Statistical methods documented by Paulson, (2008) were adopted throughout this study. Assays were carried out in triplicates and values were expressed as mean ± standard deviation. Where necessary, data obtained were statistically analysed using different Analysis of variance (ANOVA) adopting probability levels below 5%. Difference in means were analysed using the Duncan's Multiple Range Test.

3. Results

3.1. Isolation and characterization of the recovered bacteria

A total of 29 bacterial species belonging to 6 different genera were recovered from the soil samples identified biochemically as; *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Arthrobacter*, *Aeromonas* and *Alkaligenes*. The test bacterium *A. faecalis* was selected based on the preliminary acrylic paint biotolerance screening test carried out on all the recovered isolates. The biochemical characteristics of the selected bacterium, *A. faecalis* are shown Table (1).

3.2. Growth rate and biodegradation efficiency of selected *A. faecalis*

The selected bacterium *A. faecalis* was tested for *in vitro* biodegradation potential of the acrylic paint. Fig. (2) demonstrates the 14-d growth curve and

corresponding acrylic-paint biodegradation efficiency for each day based on analyses for the tested bacterial isolate. It was observed that the growth rate for the selected *A. faecalis* isolate showed significant ($p< 0.05$) increase between 0 d and the 4^{th} d of growth. Maximum peak of growth was observed at 6^{th} d of incubation, after which the rate significantly ($p< 0.05$) decreased between the 10^{th} to the 14^{th} d. The level of biodegradation efficiency also followed a similar trend to that of the growth peak; recording a peak biodegradation efficiency of 68 % at the 14^{th} d (Fig. 2).

Table 1. Biochemical characterization of the selected *A. faecalis* bacterial isolate based on Bergey's Manual of Determinative Bacteriology

Characteristics	Result
Gram reaction and Morphology	Gram (-) coccobacilli
H_2S production	(-)
Spore	(-)
Motility	(+)
Growth on MacConkey agar	(+)
Pigment on Nutrient agar	(-)
Growth in Cetrimide	(+)
Growth on MSA	(+)
Indole	(-)
Catalase	(+)
Citrate utilisation	(+)
Oxidase	(+)
Methyl red	(-)
Coagulase	(-)
VP	(-)
Urease	(-)
Nitrate reduction	(-)
Arabinose	(+)
Ribose	(-)
Glucose	(+)
Lactose	(-)
Mannitol	(+)
Xylose	(+)
Maltose	(-)
Sucrose	(-)

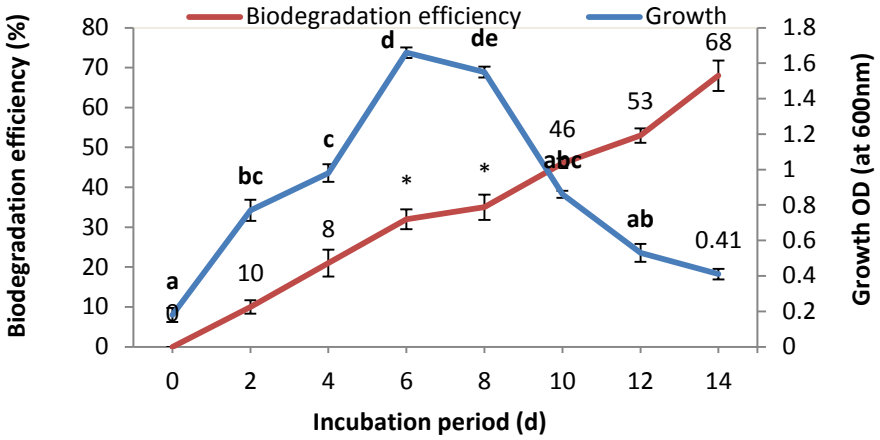

Fig. 2: Growth rate and acrylic paint biodegradation efficiency of *A. faecalis*. Where; data obtained are mean values of three replicates; data with similar alphabets and\ or asterisks were not significantly different at 95 % significance levels

3.3. Effects of variable growth parameters on biodegradation potency of *A. faecalis*

The effects of temperature, pH, inoculum size, agitation speed, nitrogen source and acrylic paint concentration on acrylic paint biodegradation efficiency are shown in Fig.'s (3-8). Under pH variations, the recorded biodegradation efficiency was 69 %, which was achieved on biodegradation of acrylic paint by *A. faecalis* at a pH of 7.2 (Fig. 3). The effects of pH at 7.2 and 9.2 were significantly different ($p< 0.05$) from those at 5.2. With respect to the different incubation temperatures (27 ^{o}C – 57 ^{o}C), optimum temperature was observed at 37 ^{o}C with a biodegradation efficiency of 67 % (Fig. 4). This was not significantly different ($p> 0.05$) from degradation efficiency at 27 and 47 ^{o}C. The increase in agitation speed from 100 to 200 rpm also affected the biodegradation potency as shown in Fig. (5), where significantly ($p< 0.05$) high biodegradation of 75 % was obtained at 200 rpm. Similarly, with respect to the paint concentration, maximum degradation efficiency of 73 % was recorded using 2 % (v/v) of acrylic paint concentration (Fig. 6). Concentration at 4 % (v/v) significantly ($p< 0.05$) reduced the biodegradation efficiency. Results of the effects of inoculum size variations on the biodegradation efficiencies of *A. faecalis* are presented in Fig. (7). Inoculum sizes ranging from 1-10 % were evaluated, and it was observed that increasing the inoculum up to 10 % yielded the best biodegradation efficacy; compared to the concentration of 1 %. The maximum recorded biodegradation activity of *A. faecalis* was 75 % at inoculum size of 10 %, and this was not significantly ($p> 0.05$) different from 2-8 % concentrations. Finally, the effects of exogenously amended nitrogen sources on the biodegradation potency of the tested *A. faecalis* are presented in Fig. (8). Results demonstrated that the highest biodegradation rate of 78 % recorded by *A. faecalis* ($p< 0.05$) was achieved on using yeast extract as a nitrogen source.

Generally, the highest biodegradation rate of acrylic paint by *A. faecalis* was recorded at pH of 7.2, temperature of 37 ^{o}C, paint concentration of 2 %, at an agitation speed of 200 rpm, and an inoculum concentration of 10 %; on using yeast extract as the major source of nitrogen.

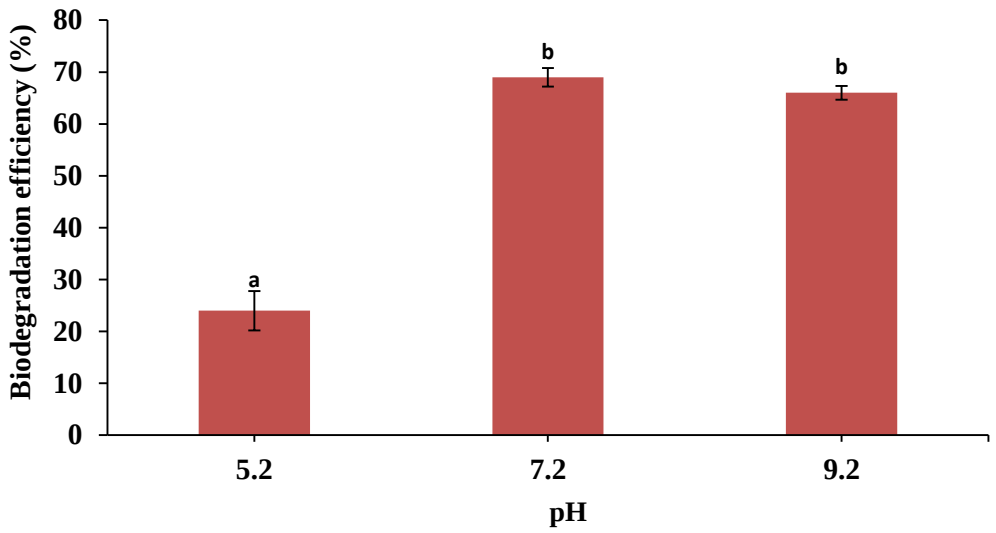

Fig. 3: Effect of initial pH variation on the biodegradation efficiency of *A. faecalis*. Where; data with similar alphabets are not significantly different at 95 % significance levels

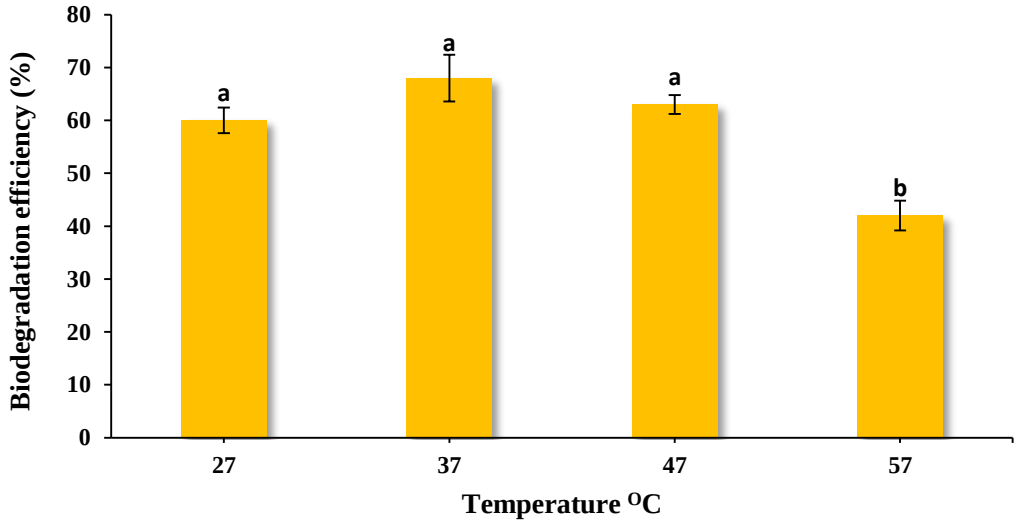

Fig. 4: Effect of variable incubation temperatures on the biodegradation potential of *A. faecalis*. Where; data with similar alphabets are not significantly different at 95% significance levels

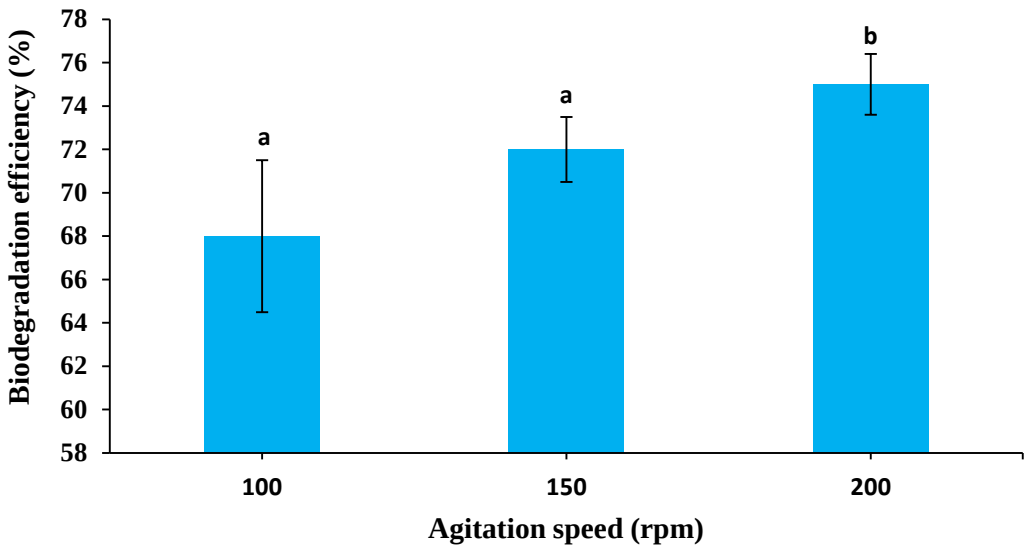

Fig. 5: Effect of agitation speed on the biodegradation potency of *A. faecalis*. Where; data with similar alphabets are not significantly different at 95 % significance levels

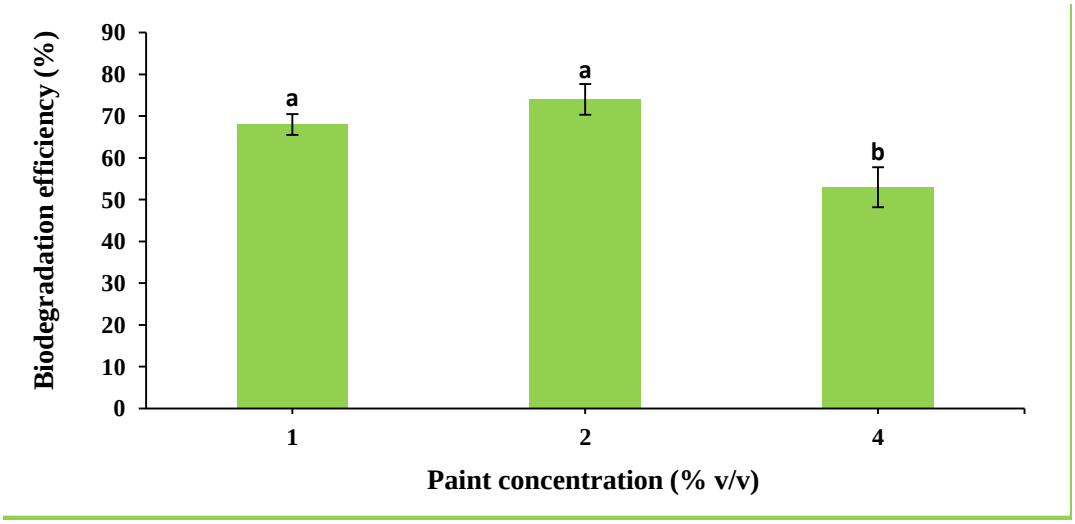

Fig. 6: Effect of acrylic paint concentration on the biodegradation efficacy of *A. faecalis*. Where; data with similar alphabets are not significantly different at 95 % significance levels

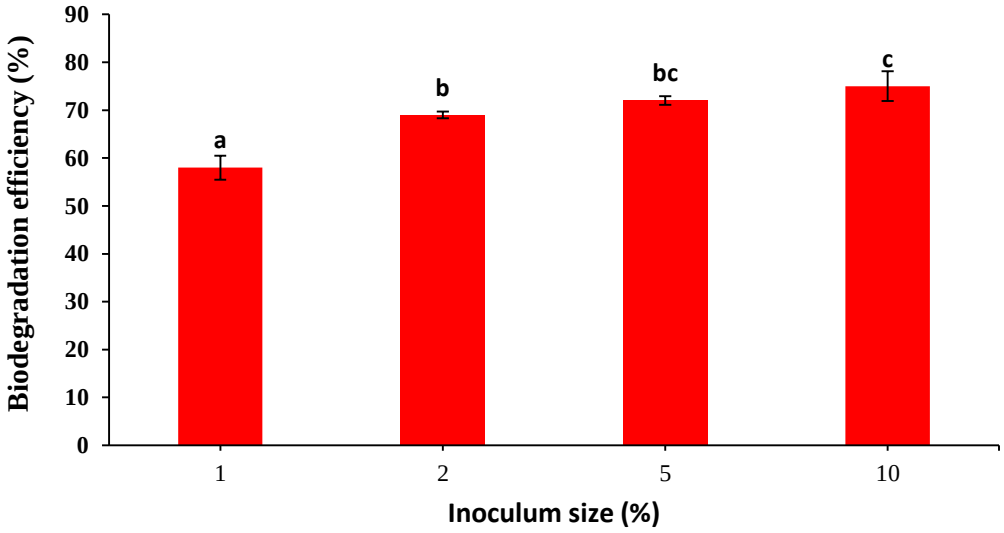

Fig. 7: Effect of inoculum size on the biodegradation activity of *A. faecalis*. Where; data with similar alphabets are not significantly different at 95 % significance levels

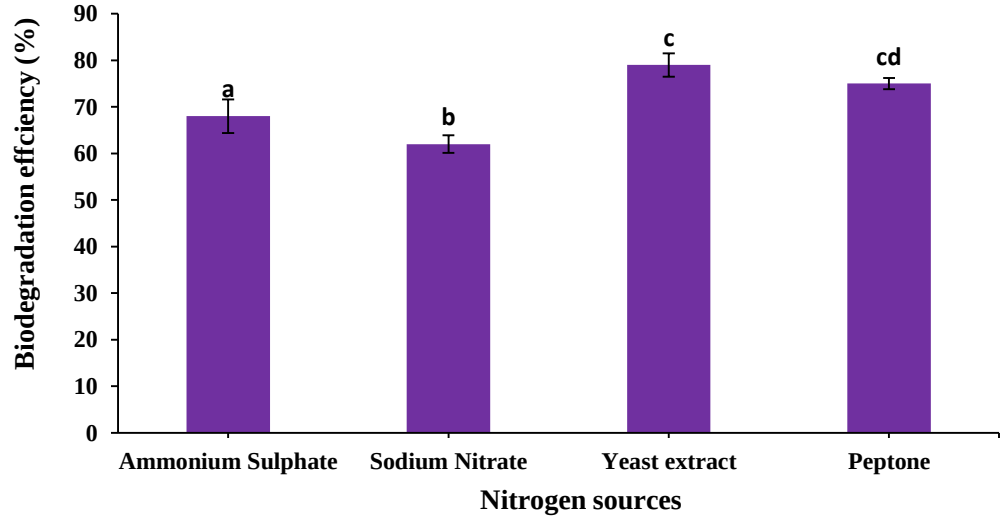

Fig. 8: Effect of different nitrogen sources on the biodegradation potential of *A. faecalis*. Where; data with similar alphabets are not significantly different at 95 % significance levels

4. Discussion

Acrylates and acrylic-containing chemicals are important industrial products used in the production of adhesives and printing inks, as paper colorants, thickening agents in automotive sprays, as emulsions in paints for buildings, and are used also in lubrication of crude oil drilling bits, as highlighted by Charoenpanich, (2013). During their industrial productions and applications, there is a large probability that acrylates and acrylic-containing compounds be released into the environment as wastes, thus contaminating the surface water bodies in addition to the soil systems. According to the report of the United States Environmental Protection Agency (USEPA. 1994) on toxic chemical, the risk of release of the acrylic-containing compounds led to the investigation of the impact of acrylic pollution on the surface water quality, underground sediments and different land sites. Moreover, Weideborg *et al*., (2001); Chang *et al*., (2002) added that acrylate concentrations of 0.3 - 5 ppm have been detected in different terrestrial and aquatic ecosystems as a result of the applications of these chemicals in sewer grouting. These acrylates remain stable in the water samples for more than two months. Accordingly, the dangers of these compounds necessitated the need for their urgent bioremediation.

Results from this study showed *A. faecalis* effectively metabolized the acrylic paint compounds with biodegradation rate of 68 % after 14 d of incubation. Similarly, Charoenpanich, (2013) previously reported that *Alcaligenes* spp. had been implicated as potential acrylate degraders, which prompted their applications in the biochemical breakdown of acrylates and related chemical compounds. A previous study of Hesham *et al*., (2012) attributed the efficacy of *A. faecalis* in biodegradation of these unique chemical compounds to its ability to withstand hard environments such as those requiring saprophytic tendencies, and the soil environments containing high doses of organics and inorganics. *A. faecalis* has been recently identified by Pangallo *et al*., (2015) to possess a positive trait with respect to its high growth rates in the presence of chemically unique substrates. The previous work conducted by Igwo-Ezikpe *et al*., (2009) also evaluated the effectiveness of *A. faecalis* in the biodegradation of diesel and chrysene oils, and according to their results; secretions of bio-surfactants by this bacterial species led to the increased production of carbohydrates and proteins, and consequently the increased biodegradation of both types of oils. Similarly, unique strains of *A. faecalis* have been earlier recognized to utilize phenols and phenol-containing compounds, thus expressing the importance of *A. faecalis* as a potent bioremediating agent (Rehfuss and Urban, 2005).

Generally, the physiological growth conditions that mostly affected the acrylic paint biodegradation efficiencies of the tested bacterium include; pH, temperature, agitation speed, substrate concentration, inoculum concentration and type of nitrogen source used. Currently, *A. faecalis* demonstrated the most efficient biodegradation efficacy of the acrylic paint at pH of 7.2. This probably could be attributed to the fact that acrylic paint hydrolytic enzymes are most active at the near neutral pH. Extreme pH has been reported by Jamwal *et al*., (2013); Pena-Montenegro *et al*., (2015) to affect the bacterial biodegradation potentials. Results of this study showed that incubation temperature of 37 ^{o}C favored the tested *A. faecalis*. An agitation speed of up to 200 rpm caused the highest biodegradation efficiency of *A. faecalis*. According to Sethy *et al*., (2011); Phulpoto *et al*., (2016), agitation represents a key role in the increase of the bacterial biomass, as it improves mixing and the contact rate between the substrate and enzymes of the fermenting bacterium. Abatenh *et al*., (2017) recently added that agitation helps in de-clumping of the un-dissolved components of the substrate to allow adequate entry of the bacterium, as there will be an increased surface area of the

dissolved substrate. In the present study, an increase in the inoculum load led to the increase in biodegradation efficiencies. This may be attributed to the elevated level of bacterial biomass will directly translates into improved cell numbers, which can hydrolyse the available molecules of the acrylic substrates. Biomass increase could also directly affect enzyme secretion, thereby leading to an increased rate of biodegradation. A previous study conducted by Hassan *et al*., (2013) also concluded that elevated inocula sizes of the microorganism led to improvements in the biodegradation of the azo dyes, and thus led to an improved bioremediation. Results of the current study revealed that the preference of yeast extract peptone in the fermentation medium resulted in improved biodegradation potencies. This could be attributed to the fact that peptone and yeast extracts are organic sources of nitrogen, which are complex compared to the simple inorganic sodium nitrate and ammonium sulphate. In accordance, Ravi *et al*., (2015) reported that organic substrates are most preferred by bacteria over inorganics, due to their broad chemical forms that can accommodate a wide range of metabolic activities. Similarly, Niu *et al*., (2009) previously documented that organic substrates such as yeast extract and peptone also contain high levels of trace elements; minerals and vitamins, which suits the bacteria as much better nutrients for growth and proliferation. Previous studies of Asira, (2013); Ogu and Odo, (2015); Phulpoto *et al*., (2016) have demonstrated the importance of growth parameters like incubation time and substrate dose on the rate of biodegradation. The current noticeable improvements in biodegradation efficiencies may be attributed to the fact that when favorable bacterial growth conditions are maintained, optimized enzymes bio-production are achieved.

Conclusion

This study showed that *A. faecalis* bacterium; isolated from paint waste impacted soil, possesses the capacity to grow aerobically on acrylic paint supplemented growth medium with efficient biodegradation potential. The best recorded conditions for biodegradation was at a pH of 7.2, temperature of 37 ^{o}C, paint concentration of 2 %, an agitation speed of 200 rpm and an inoculum concentration of 10 %; when yeast extract was the major source of nitrogen. This work provides baseline data for optimum biodegradation of acrylate by *A. faecalis*, and thus could be possibly exploited as a key bioremediation agent for acrylic paint polluted sites.

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Conflict of interest

The author has no conflict of interest.

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Ethical approval

Non-applicable.

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