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DECOLOURIZATION OF PAINT EFFLUENTS BY NITROGEN AND CARBON SOURCES

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ABSTRACT

This research investigate the effects of bacterial isolates Pseudomonas species from soil sample of Marshall paint effluent and their effects on the decolorization of paint. After Gram staining series of biochemical test and spectrophotometer reading showing the absorbance rate of the carbon source (lactose) and nitrogen source (urea) on the decolourization of paint. The result here indicates that for bacteria isolate Pseudomonas cruciviae carbon source (lactose) is optimal at 0hr and 48hr while at 24hrs and 72hrs urea is optimal. For bacteria isolate Pseudomonas putida, carbon source (lactose) is optimal at 0hr while nitrogen source (urea) is optimal at 24hrs, 48hrs, and 72hrs. it can therefore be concluded that nitrogen source (urea) is more favourable to the decolourization of paint for Pseudomonas putida while in bacteria isolate Pseudomonas cruciviae, lactose and urea as carbon and nitrogen sources have equal decolorization rate respectively.

KEYWORDS

Paint Effluent, Carbon and Nitrogen, Decolorization, Bacteria Isolates, Biodegradation

INTRODUCTION

Paint effluents from manufacturing company (PEMC) contains highly toxic compounds. It harms fish, wild life fish and contaminates the food chain if poured down a storm drain. Paint waste

waters have also adverse effects on human health occupants. If used in closed areas, its chemical components can irritate eye, skin and lungs and causes headaches and nausea. It can also contribute to respiratory problems; muscles weakness, liver and kidney damage. The pain manufacturing water (PMW) must be needed to discharge after treatment due to legal restriction in organized industries zone and environment conservation (Mackey et al., 1996).

Waste is a byproduct which is generated from production process and generated from waste treatment process both industrial scale and domestic scale. Chemically, waste is classified into two kinds; they are organic waste and inorganic waste. The presence of waste can give the negative effect to the environment and health if the concentration stays on the high level and handled improperly. One kind of so many kinds wastes are liquid waste containing colour agent which is released to the environment without any treatment. Even the dye concentration may be less than IPPM, lower than many other chemicals found in waste water, the colour is predominant and visible. Approximately, 10,000 different dyes and pigments are used industrially (Sukumar et al., 2007).

Large amount of chemically different dyes are used for various industries application and a significant proportion of these dyes enter the environment in waste water. These dyes designed to be resistant to the light, water , and oxidizing agents and are therefore difficult to be degraded once released into aquatic system. Conventionally waste water treatment system is often insufficient and existing physical and chemical technologies are expensive, time wasting and often methodologically demanding. Therefore, it may be economical to develop alternative means of dye decolorization, such as bioremediation due to its reputation as an environmentally friendly and publicly accepted treatment technology. The treatment systems having mixed microbial populations are effective due to concerted metabolic activities of microbial community. As the catabolic activities of microorganisms in mixed consortium offers considerable advantage over the use of pure cultures in the degradation synthetic dyes. The individual strains may attack the dye molecules at different position or may use decomposition products produced by another strains for further decomposition (Jadhav et al., 2008).

In decolourization process of effluents the use of bacterial constitute an alternative mode of treatment in aerobic conditions. Some specialized strains of aerobic bacteria have develop the ability to use azo dyes as sole source of carbon and nitrogen, others only reduce the azo group by special oxygen tolerant azo reductases (Sharma et al., 2009).

Studies carried out in the past have used undefined microbiological consortium or pure cultures for dye decolourization. In the present study, a defined microorganism culture of newly isolated Bacillus Species is identified (Jadhav et al., 2008) isolation of bacterial strain that highly decolourized the toxic azo dye methyl red MR can be enhanced by an enrichment culture under aerobic conditions. This bacteria strains is said to Bacillus Cereus group (Ooiet,et al., 2007).

Over the past decade, biological treatment have been investigated. Microbial decolourization is an environmental friendly and cost competitive alternative to chemical decomposition process. The microorganisms (i.e. bacterial) which include :(Bacillus species, pseudomonas species, acetogenic species) have the abilities to remove colour (Jiranuntipon et al., 2008).

The pure bacterial culture has been studied in order to develop bioprocess for melanoidins decolourization in molasses waste water. The bacterial decolourization is usually faster, but it may require a mixed community of Bacillus species exhibited through combined metabolic mode of individual Bacillus isolate. Hence the bacterial consortium seems to be more competent for treatment due to maintenance of microorganism and co metabolism to enhance the efficiency of decolourization (Jiranuntipon et al., 2008).

Special treatment or paints liquid waste is to enhance the ability of the bacteria to utilize chemical compound inside the paint as the carbon source. The fact that the composition inside the paint is not dominant, pigment application is along with thinner as the solvent. Then the component which have to be tested are soluble pigment in thinner, acrylic resin and melamine (Jiranuntipon et al., 2008).

Ancient coloured walls of Dendra, Egypt which were exposed for years to the elements, still possess their brilliant colours, as vivid as when they were painted about 2,000 ago. The Egyptians mixed their colours with a gummy substance, and applied them separate from each other without any blending or mixture. Paint was made the yolk of eggs and therefore the substance it is applied to paint. Pigments was made from different soils, sand and plants (Berendsen, 2009).

Paint effluent are those liquid waste discharged from sewage or other industrial plants. They include mainly of water and ingredients used in paint making. Hence, they are known as liquid waste (Redmond, 2008).

Paints effluent and other hazardous waste has effects on organisms, materials and the environments. Virtually all hazardous waste are poisonous to a degree, some to the extreme. In addition, hazardous waste can damage air by causing deterioration of air quality either directly or by formation of secondary pollutants. Hazardous waste compounds dissolved suspended in or floating as render it unsafe for use and for aquatic organisms (Manahan, 2009).

Bioremediation is the use of microorganism metabolism to remove pollutants. Technologies can be generally classified as in situ or exsitu, Insitu bioremediation involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediaton related technologies are phyto-remediation, bioventing, bioleaching, land farming, bio reactor, compositing, ilizo filtration and biostimulation. Bioremediation can occur on its own (natural attention or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioremediation are known as bioremediator (Terra, N. 2008). Not all contaminants however are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium and lead are not readily absorbed or captured by microorganism. The assimilation of metals such as mercury into the food chain may worsen matters. Phyto-remediation is useful in this circumstances because natural plants or transgenic plants are able to bioaccummulate these toxins in their above ground parts, which are then harvested for removal (Meagher, RB 2000). In every process that occurs in industry, not only main product but also produced by-product that either can still be used, it is known as industrial waste. One of industrial waste is paint waste (effluent) that contains colouring agent that can be harmful to the environment if not treated appropriately (Reynolds, 1998). One of the major advantages of bioremediation is that it is employed in the areas that are inaccessible without excavation. For example, hydrocarbon spills (specifically, petrol spills or certain chlorinated solvents may contaminate groundwater and introducing that appropriate electron acceptor or electron donor amendment, as appropriates may significantly reduce contaminant concentrations documented successful use of bioremediation in a large scale was the 1989 Exxon Valdez out spill in Alaska (Wu, 2001).

Biodegradation is the chemical dissolution of materials by bacteria or other biological means. Although often conflated, biodegradable is distinct in meaning from compostable while biodegradable simply means to be consumed by micro organisms and return to compounds found in nature (Diaz, E.2008). It is also the process of oxidation of organic compounds by microorganism in the soil, water or in installation of waste water treatment. Biodegradable is a

natural phenomenon indispensable to the maintenance of equilibrium in the biosphere since its helps to limit the accumulation of organic substances and ensure the recycling of the essential elements in the complex molecules formed by biosynthesis of animals, plants and microorganisms (Dennok, 2009). It is therefore, used in monitoring the composition of indigenous and added bacterial (Okabe and Kamagata 2010). Major methodological breakthrough in microbial biodegradation have enable detailed genomic, metagenomic, proteomic , bioinformatics and others high through put analysis of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathway and, the ability of microorganisms to adapt to changing environmental conditions (Cupples and Shaffer 2007). Temperature influences rates of biodegradation by controlling rate of Enzymatic reactions within microorganisms. Speed of enzymatic in the cell approximately doubles for each 10^0c rise in temperature (Nester et al., 2001).

MATERIALS AND METHOD

Our purpose here is to investigate the effects of bacterial isolates Pseudomonas species from soil sample of Marshall paint effluent and their effects on the decolorization of paint. After Gram staining series of biochemical test and spectrophotometer reading showing the absorbance rate of the carbon source (lactose) and nitrogen source (urea) on the decolourization of paint.

MATERIALS USED

Bunsen Burner, wireloop , Spatula, Cotton wool, test tubes, bijou bottles, alaminium foil, glass slides, measuring cylinder, breaker, conical flask.

Equipment Used

Incubator (Model DNP 9022), Autoc lave (Model YX 280B), Refrigerator (FK 330), Microscope (Model 210 2300/50 60Hz), Hot air Oven (Model DHG 9202), Spectrometer 752 W)

Reagents Used

Distilled water, Ethanol, crystal violent, Mythyl Red, Actone, Safranine, Lugol Iodine Oxidase reagent, Hydrogen Peroxide.

Sugars Used/Carbon Source

Glucose, Lactose, Galactose, Sucrose and Fractose

Nitrogen Source Used

Yeast extract, Ammonium Sulphate, Ammonium nitrate, Urea.

Agar Used

Nutrient Agar this medium was used for the enumeration of bacteria cells and to maintain pure cultures. Nutrients agar is a general medium and it was therefore used here for bacterial growth.

INGREDIENTS / COMPOSITION

Ingredients	gmlltr
Agar	15.00
Peptone	5.00
Sodium Chloride	5.00
Beef Extract	1.50
Yeast Extract	1.50
PH	7.4I 0.2 at $25^0$2
Diluted Water	1ltr

NUTRIENT BROTH

Formula	Grams/Litre
Peptic Digest of Animal Tissue	5.00
Beef Extract	1.50
Sodium Chloride	5.00
Yeast Extract	1.50
Final PH	7.4 + 0.2

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MINIMAL SALT MEDIA (MSM)

The minimal salt has the following composition $G^{L\ 1}$ Na_2HpO_4 (3.6g), $NH_4)_2$ SO_4 (1.0g), K_2HPO_4 (1.6g), MgSO4 (1.0g), $CaCl_2$. $2H_2O$ (0.01g) and 10ml of trace element was of following composition: $CoCl_2$. $6H_2O$ (1.0g), $NiCl_2.6H_2O$ (2.0g) H_3BO_3 (3.0g) and $CuCl_2$ (1.0g).

RESULTS

In this study (research) a total of five bacterial isolates were characterized as shown bellow. The isolates were identified using series of biochemical tests. The isolates were coded as thus; EFF I, EFF II, SS I, SS II and SS pet. Table 4.1 contains identification and characteristics of the isolates while table 4.2 contains result of the cell mass obtained with different carbon and nitrogen sources. Tables 4.3 showing absorbance of spectrophotometer reading for Pseudomonas cruciviae at 0hr, 24hrs, 48hrs, and 72hrs, 4.3.1 (fig. 1) showing histogram representation of lactose for table 4.3. Table 4.4 showing spectrophotometer for Pseudomonas cruciviae for urea

and 4.4.1 showing histogram representation for it. Table 4.5 showing lactose absorbance for Pseudomonas putida and 4.5.1 (fig. 3) showing its representation. Table 4.6 is the result of spectrophotometer reading for nitrogen source (urea) and 4.6.1 (fig. 4) is the histogram representation for Pseudomonas putida respectively.

Table 3.1 Biochemical Test for the Identification of Bacterial Isolates

Isolation Code	Morphology	Gram Reaction	Oxidase	Catalase	Indole	Methyl red	Sugar Fermentation					Most probable Organisms
							Glucose	Fructose	Sucrose	Lactose	Galactose	
EEF I	Yellow, Round, flat, Muciod, Translucent	- rod	+	+	-	-	-	-	-	-	-	Pseudomonas alcaligens
EEF II	Whitish yellow, Flat	- rod	+	+	-	-	-	+	+	-	-	Pseudomonas cruciviae
SS I	Cream, flat, opaque	- rod	+	+	-	-	-	+	+	+	-	Pseudomonas arvilla
SS II	Yellow, Flat, Opaque	- rod	+	+	-	-	-	+	+	+	+	Pseudomonas putida
SS pet	Yellow flat	-rod	+	+	-	-	-	+	-	-	-	Pseudomonas medrocina

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Table 3.2 RESULT OF CELL MASS OF THE ORGANISM

Isolated organism	Sucrose	Fructose	Lactose	Galactose	Glucose	Ammonium sulphate	Ammonium nitrate	Urea

Pseudomonas cruciviae	0.078	0.197	0.440	0.050	0.060	0.050	0.043	0.068
Pseudomonas putida	0.049	0.059	0.55	0.085	0.052	0.052	0.058	0.056

TABLE 3.3 RESULT OF SPECTROPHOTOMETER READING FOR *Pseudomonas cruciviae* AT 0, 24, 48 AND 72 HOURS WITH CARBON SOURCES. (Lactose)

Carbon source in grams	0 hour	24 hours	48 hours	78 hours
2	1.961	1.937	1.952	1.924
4	1.699	1.919	1.959	1.924
6	1.718	1.931	1.945	1.915
8	1.329	1.975	1.937	1.942
10	1.485	1.925	1.926	1.924

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3.3.1 HISTOGRAM

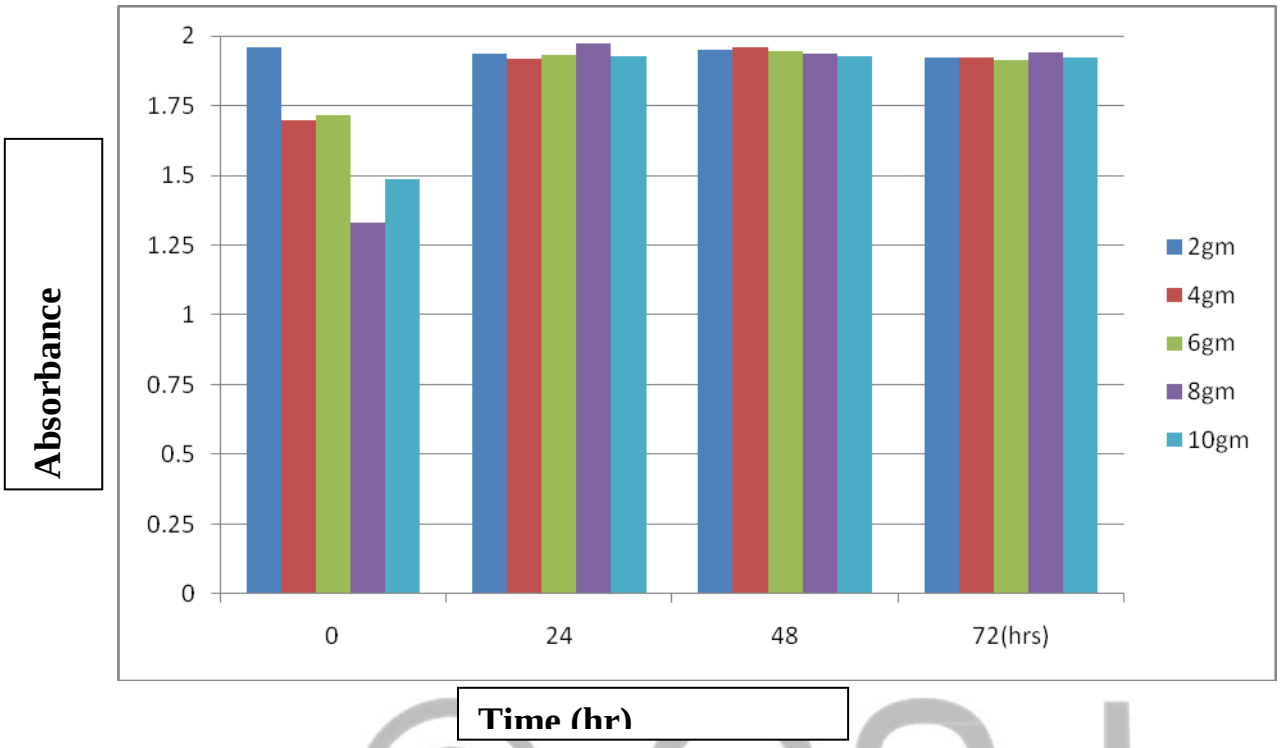

FIG. 1 HISTOGRAM SHOWING DECOLOURIZATION ABSORBANCE OF CARBON (I.E. LACTOSE IN GRAM AGAINST TIME (HOUR) FOR *Pseudomonas cruciviae*.

TABLE 3.4 RESULT OF SPECTROPHOTOMETER READING FOR *Pseudomonas cruciviae* FOR 0, 24, 48 AND 72 HOURS WITH NITROGEN SOURCE (UREA).

Nitrogen source in grams	0	24	48	72
2	1.961	1.931	1.945	1.957
4	1.966	1.937	1.939	0.150
6	1.961	1.1937	1.936	1.050
8	1.329	1.931	1.932	0.196
10	1.961	1.932	1.930	1.953

3.4.1 HISTOGRAM

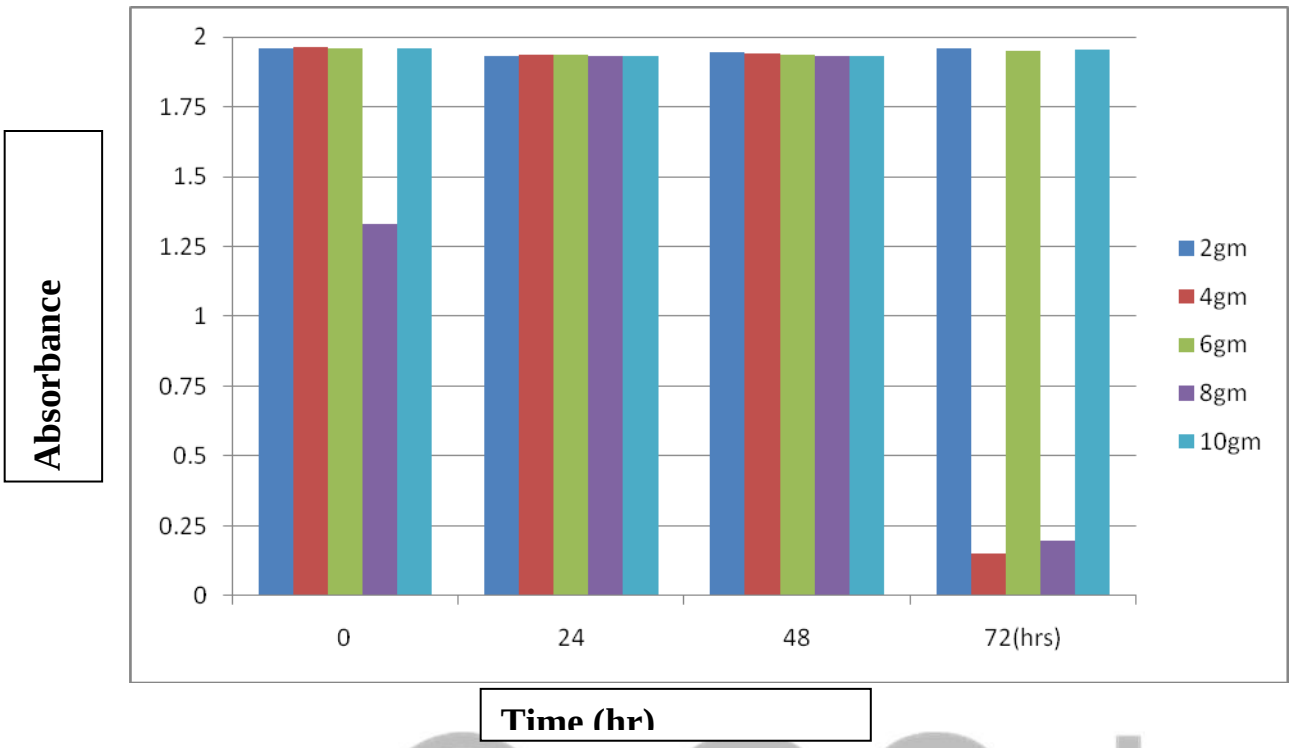

FIG. 2 HISTOGRAM SHOWING DECOLOURIZATION ABSORBANCE OF UREA IN GRAMS AGAINST TIME (HOUR). FOR *Pseudomonas cruciviae*.

TABLE 3.5 RESULT OF SPECTROPHOTOMETER READING FOR *Pseudomonas Putida AT 0, 24, 48 AND 72 HOURS WITH CARBON SOURCE LACTOSE.*

Carbon source in grams	0	24	48	72
2	1.882	1.905	1.944	1.950
4	1.812	1.686	1.926	1.937
6	1.924	1.911	1.914	1.937
8	1.940	1.905	1.932	1.937
10	1.944	1.598	1.944	1.937

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3.5.1 HISTOGRAM

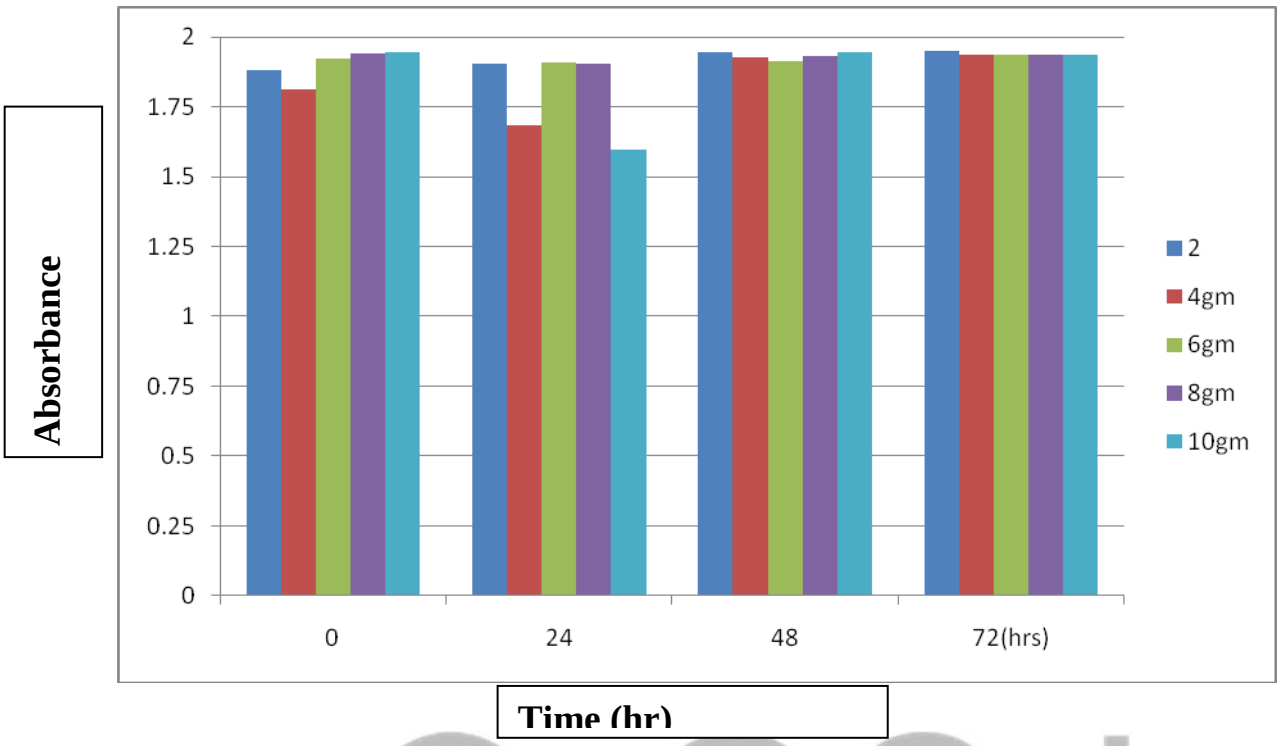

FIG. 3 HISTOGRAM SHOWING DELOURIZATION ABSORBANCE OF CARBON SOURCE LACTOSE IN GRAMS AGAINS TIME (HOUR) FOR *Pseudomonas putida*

TABLE 3.6 RESULT OF SPECTROPHOTOMETER READING FOR *Pseudomonas putida* AT 0, 24, 32, AND 72HRS AND NITROGEN SOURCE UREA.

Nitrogen source in grams	Ohr	24hrs	48hrs	72hrs
2	**1.842**	**1.899**	**1.944**	**1.956**
4	**1.913**	**1.936**	**1.956**	**1.962**
6	**1.924**	**1.936**	**1.934**	**1.952**
8	**1.924**	**1.917**	**1.935**	**1.956**
10	**1.918**	**1.893**	**1.919**	**1.956**

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3.6.1

HISTOGRAM

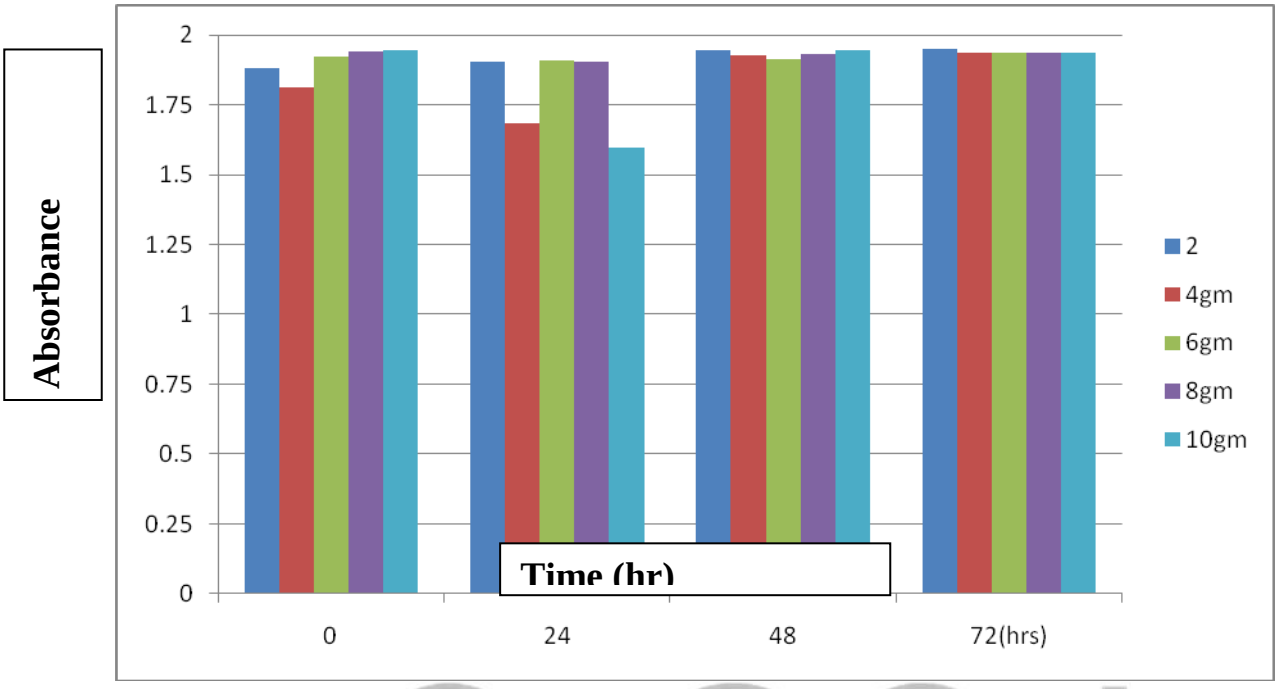

FIG. 4 HISTOGRAM SHOWING DECOLOURIZATION ABSORBANCE OF NITROGEN SOURCE (UREA) IN GRAM AGAINST TIME (HOUR) FOR *Pseudomonas putida*

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1. DISCUSSION

The result of this research, after gram staining and necessary biochemical tests for the identification of bacterial isolate, the following pseudomonas species were identified. They are; *Pseudomonas alcaligenes, Pseudomonas cruciviae, Pseudomonas arvilla, Pseudomonas putida and Pseudomonas medicocina.* For Pseudomonas cruciviae have equal decolourization of paint with carbon and nitrogen sources (i.e. lactose and urea) respectively, which is in conformity / harmony with findings Kirchma (1990). For *Pseudomonas putida*, nitrogen source (urea) have high decolourization for paint than carbon source (lactose) which is in harmony with findings Laird *et al.*, (1990). The low / decrease in the decolourization in carbon source with *Pseudomonas putida* could be as a result of accumulation of simple carbon compound acting as co metallic substrate in media. Similar trend has been reported by others (Adosinda *et al*., 2001).

2. CONCLUSION

The toxic effects of hazardous waste on the environment cannot be neglected. This is due to the presence of volatile organic compounds found in paint. It is therefore necessary to state clearly here, that from the study/research carried out the microbes isolated from the paint effluent and the soil where the effluent is deposited are majorly Gram negative organisms that are capable of degrading volatile organic compounds. *Pseudomonasspecies*are the most suitable for

bioremediation include; *Pseudomonas alcaligens* which degrade polycyclic aromatic hydro carbons, *Psudonomasputida*has the ability to degrade organic solvents such as toluene.

Carbon and nitrogen sources help the microbes that degrade paint effluent effectively and efficiently. It can be said from the result of my study/ research that in isolate EFF II from zero (0) hour to 72 hours, lactose and Urea are of 50% at piece while in isolate SS II urea (nitrogen source) is 75% to 25% of lactose. Therefore, it can conclude that nitrogen source (urea) enhance degradation/bioremediation of paint effluent isolate SSII than EFF II. It can also be deduced from my study/research that nitrogen source (urea)enhance bioremediation of paints effluent effectively which makes it less harmful to human lives and aquatic environment.

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