Bacteriological Removal of Toxic Phenol

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Abstract : Phenols, widely used in Industries, are of growing concern owing to their high toxicity, carcinogenicity and wide distribution in Industrial wastes. In the present study, one *Pseudomonas* isolate, identified as *Pseudomonas spp.* was obtained using the enrichment process with 2,4,6- Trichlorophenol (2,4,6-TCP) as a sole carbon source. This isolate was found to be able to degrade phenol. The ability of *Pseudomonas spp.* isolate to remove phenol from a Industrial effluent was tested by a UV-VIS spectrophotometer. The results indicated that this isolate metabolized phenol in the meta-pathway. The optimal phenol degradation conditions of *Pseudomonas spp.* isolate were at pH 7.2 and 30oC. At the 160 mg/l of phenol concentration, the highest specific degradation rate was observed. Further increase in phenol concentration slowed down the degradation ability of the isolate. However, the supplementation of 1% glucose stimulated the growth of *Pseudomonas spp.* isolate and enhanced the ability to utilize phenol from the sample. Visible spectrophotometer results show that 70.70% of phenol in the effluent sample was metabolized after 50 days. In conclusion, *pseudomonas spp.* isolated in this study has ability of utilizing phenol compounds and demonstrates its potentials of degrading high concentration of phenol in Industrial effluents.

I. INTRODUCTION

Phenol or its derivatives are important chemicals widely used in Industries for the manufacture of products such as dyes, Insecticides, disinfectants, wood preservatives, chemical products in buildings, agriculture and Hospital. Because of its broad application in Industrial and medical settings, it has become one of environmental contaminants especially in the underground water. Phenol or its derivatives stick to soil and to sediments at the bottom of the Lakes, rivers or streams and rapidly enter the body through the skin and the gastrointestinal tract. Owing to their high toxicity, carcinogenicity and wide distribution in Industrial wastes leading to great harm to human being and marine organisms.

Although there has been many studies regarding the environment fate of chlorinated phenols, including photochemical degradation and sequential aerobic and anaerobic degradation. Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds. The organic pollutants are used as sole source of carbon and energy. Growth process results in a complete degradation (mineralization) of pollutants. Generally chlorinated phenols are transformed via oxidative dichlorination while in anaerobic conditions via reductive de-chlorination.

II. Methodology

Soil sample were collected from the Botany Nursery (Garden Soil). 10gm of Garden soil was added into 100ml of Bushnell Haas (BH) medium at pH of 7.2, containing NH4NO3 (1 g/L), K2HPO4 (1g/L), CaCl2.2H2O (0.02 g/L), MgSO4.7H20 (0.2 g/L), FeCl3 (0.05 g/L). 200 muM of 2,4,6-TCP) was used as sole carbon source.

These flasks were shaken at 30oC and 160 rpm for four weeks with 1ml of enriched media transformed into freshly prepared enrichment media each week. Serial dilutions (1/10) of final enriched media were spread-plated on BH agar supplemented with 2,4,6-TCP incubated at 30oC.

The single colonies were streaked onto nutrient agar plates, incubated at 30oC overnight and the pure isolates were stored at 4oC until further use.

The isolate that was capable of degrading chlorophenolic compounds were identified by gram stain, biochemical tests.

III. Substrate Preferences for *Pseudomonas* isolate

The substrate preferences of the isolate studied by using the optical various chlorophenolic compounds as carbon sources (Table-1) at 30oC and 160 rpm. The cell growth at density of 600nm.

IV. Degradation of phenolic compounds in three different samples

The phenolic sample containing with known concentration, used as the sole carbon source to test the degradation potentials of the bacterial isolate.

Degradation studies were carried out with the addition of 10ml normalized inocula which is prepared in distilled water which is inoculate in 100ml of BH broth that containing 160 mg/l of phenol and containing different pH such as 6.0, 7.2, 9.0 from each sample flasks were incubate at 30oC.

Also prepared 100ml flasks with different concentration such 120 mg/L, 160mg/L and 180mg/L.

The reaction containing all components but avoid of bacterial inoculums were used as controls.

To enhance the phenol-degradation ability of bacterial isolate at high phenol concentration, additional 1% glucose was supplemented in the growth media.

Table 1. Standard of Substrate

Sr. No.	Substrate	Pseudomonas spp.
1	Phenol (200 micro molar)	+++
2	2,4-dibromophenol (200 micro molar)	++++
3	2,4-dichlorophenol (200micro molar)	++++
4	4-chloro-3-nitrophenol (200 micro molar)	++++
5	2,6-dichlorophenol (200 micro molar)	+++++
6	2,4,6-trichlorophenol (200 micro molar)	+++++
7	2,4,5-trichlorophenol (200 micro molar)	++
8	Pentachlorophenol (200 micro molar)	++

+++++ : OD 600>0.700
+++ : 0.700>OD600>0.400
++ : 0.400>OD600>0.200
+ : OD600<0.200

Table 2. Result of Substrate preferences

Sr. No.	Substrate	OD	Pseudomonas spp.
1	Phenol (200 micro molar)	0.505	+++
2	2,4,6-trichlorophenol (200 micro molar)	0.819	+++++

Table 3. Phenol degradation at different pH

Sr. No.	Sample description	Different pH	% degradation after 25 days	% degradation after 50 days
1	Sample 1 (160 ppm)	6.0	16.44%	45.16%
		7.2	21.38%	64.20%
		9.0	14.16%	32.54%
2	Sample 2 (160 ppm)	6.0	17.00%	51.36%
		7.2	23.12%	69.48%
		9.0	13.43%	28.74%

Table 4. Phenol degradation at different concentration

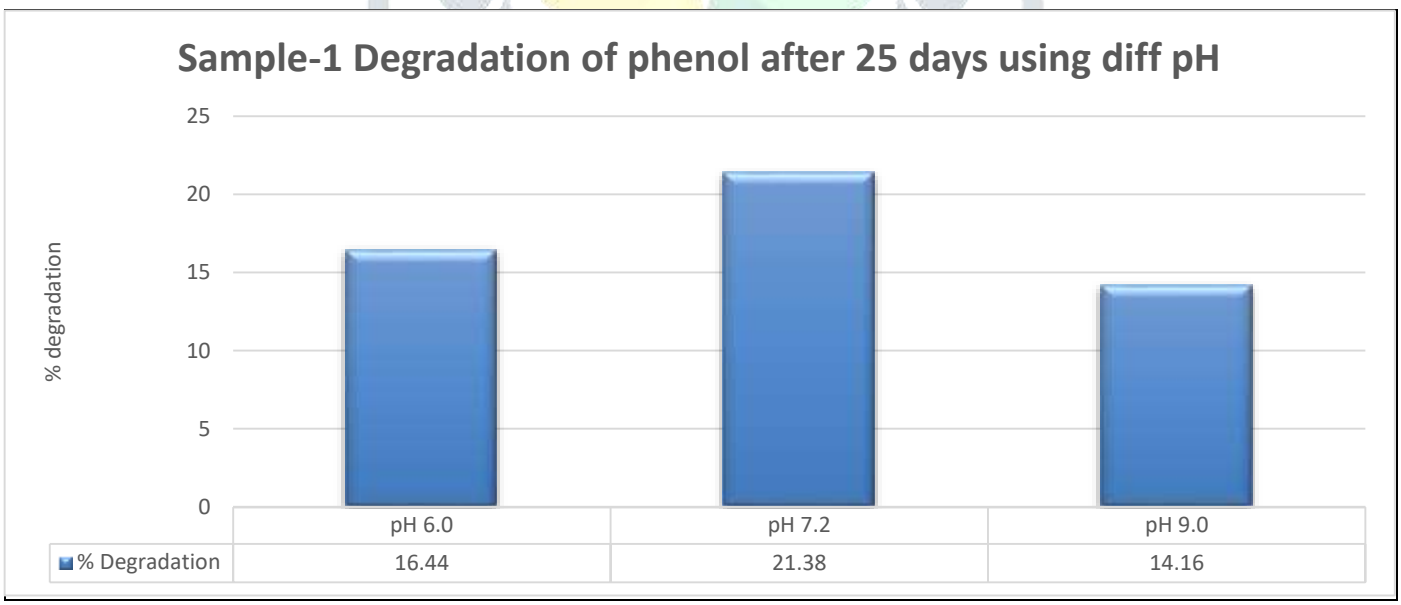
Sr. No.	Sample description	Different concentration	% Degradation after 25 days	% Degradation after 50 days
1	Sample 1 (pH 7.2)	120	18.06%	50.28%
		160	20.11%	70.70%
		180	15.20%	38.78%
2	Sample 2 (pH 7.2)	120	16.21%	36.12%
		160	17.38%	62.53%
		180	12.52%	26.66%

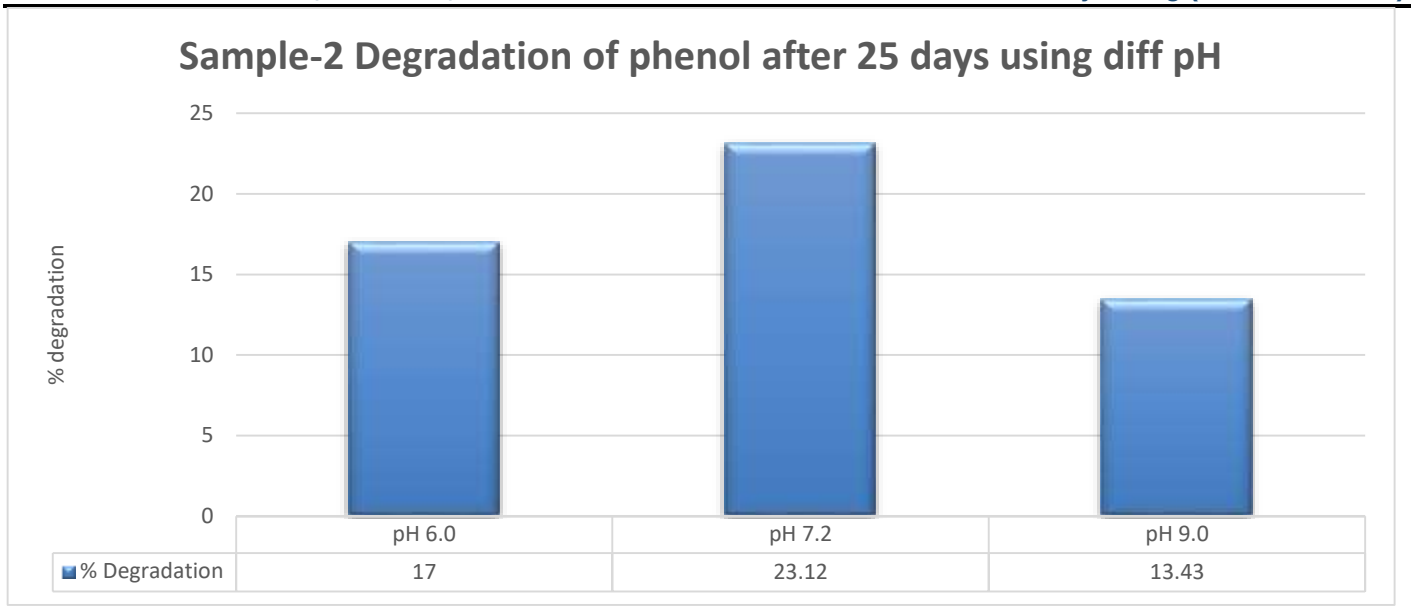
The bacterial isolate was tested for the abilities to utilize various chlorophenolic compounds as the sole carbon source.

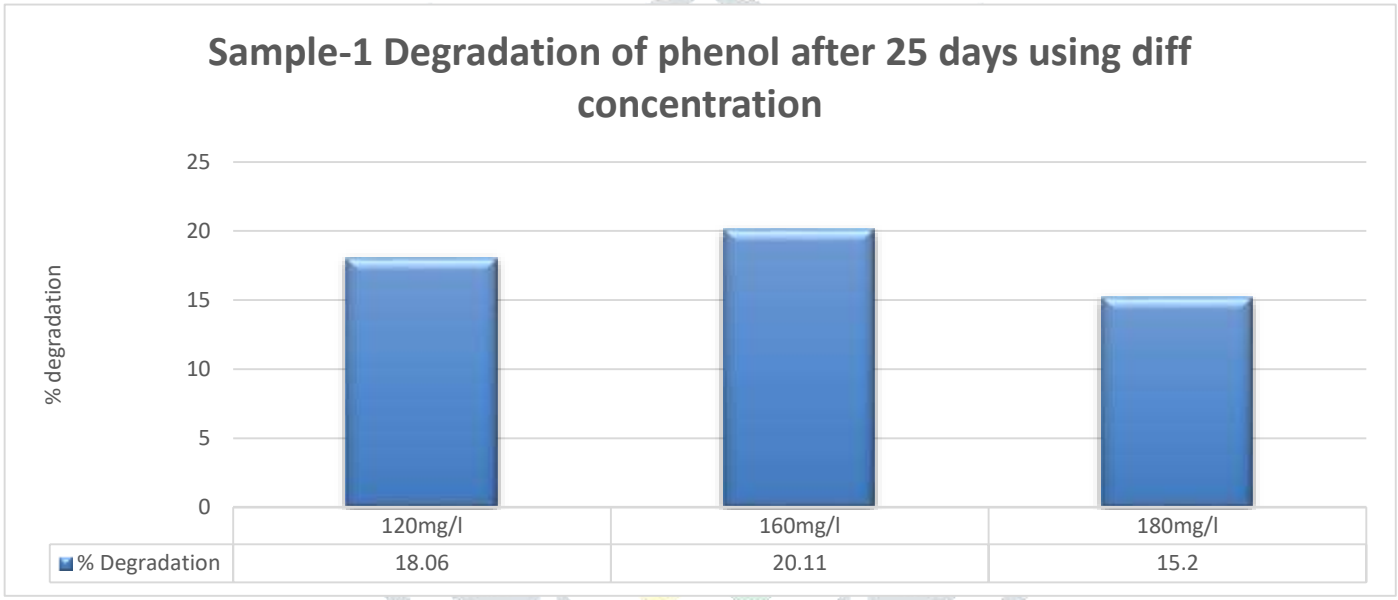
pH 7.2 showed best pH parameter for degradation of phenol under 50 days incubation, it showed 70.70% degradation. Degree of ionization of phenol and the surface properties of the biomass were affected by the pH that an increase in pH value led to lower electrostatic attraction between the substrate and the binding sites of biomass surface showed best result at 30oC.

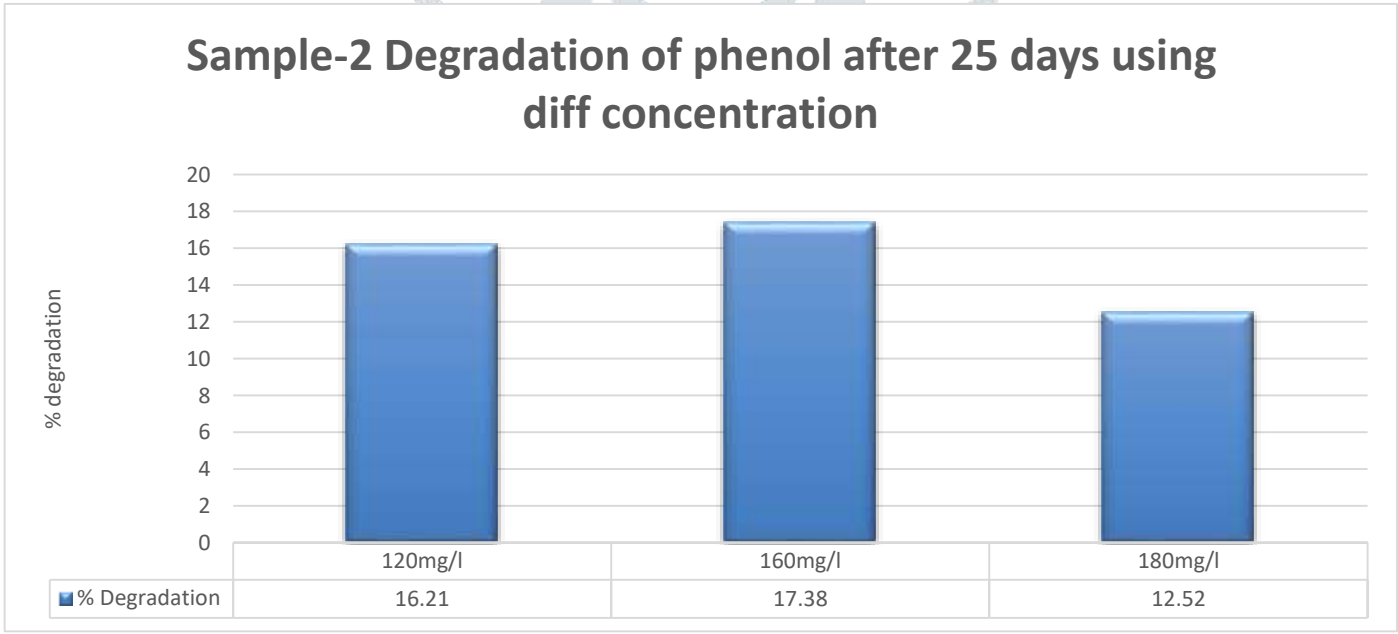
Pseudomonas species area regarded as one of the most common species of bacteria degrading phenol isolated from contaminated sites.

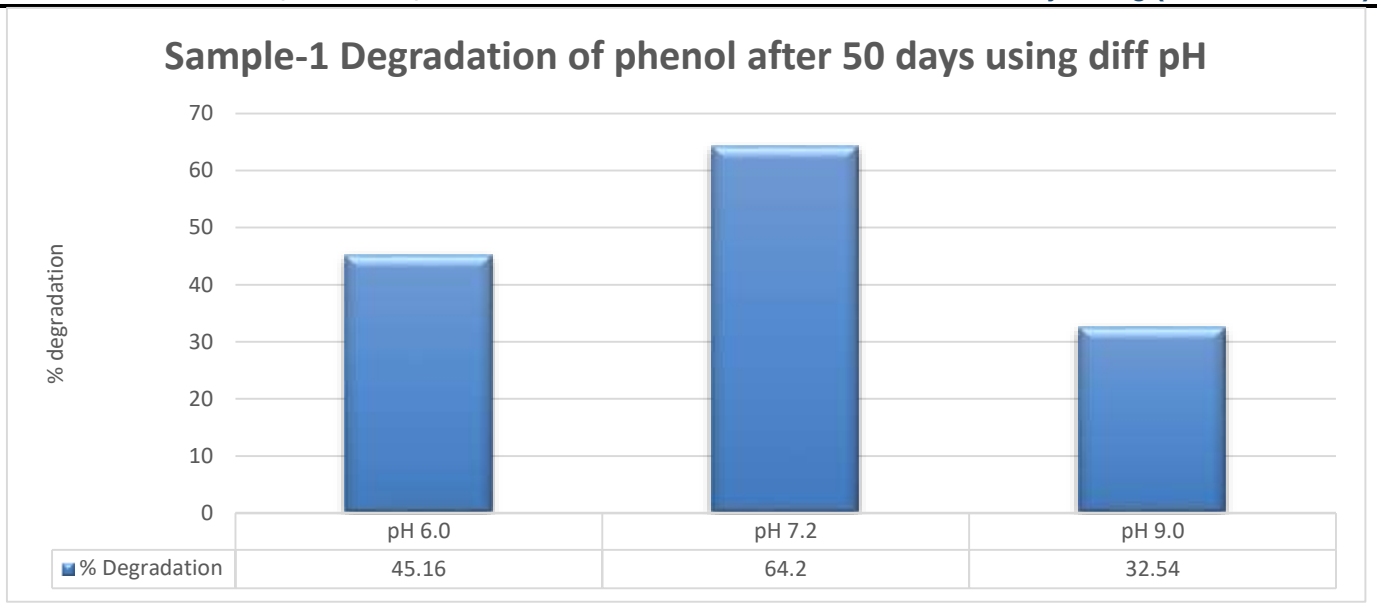
A cream white gram-negative rod was obtained using 2,4,6-trichlorophenol (2,4,6-TCP) as the sole carbon source using the enrichment process. The bacteria isolate was identified as *pseudomonas spp.*

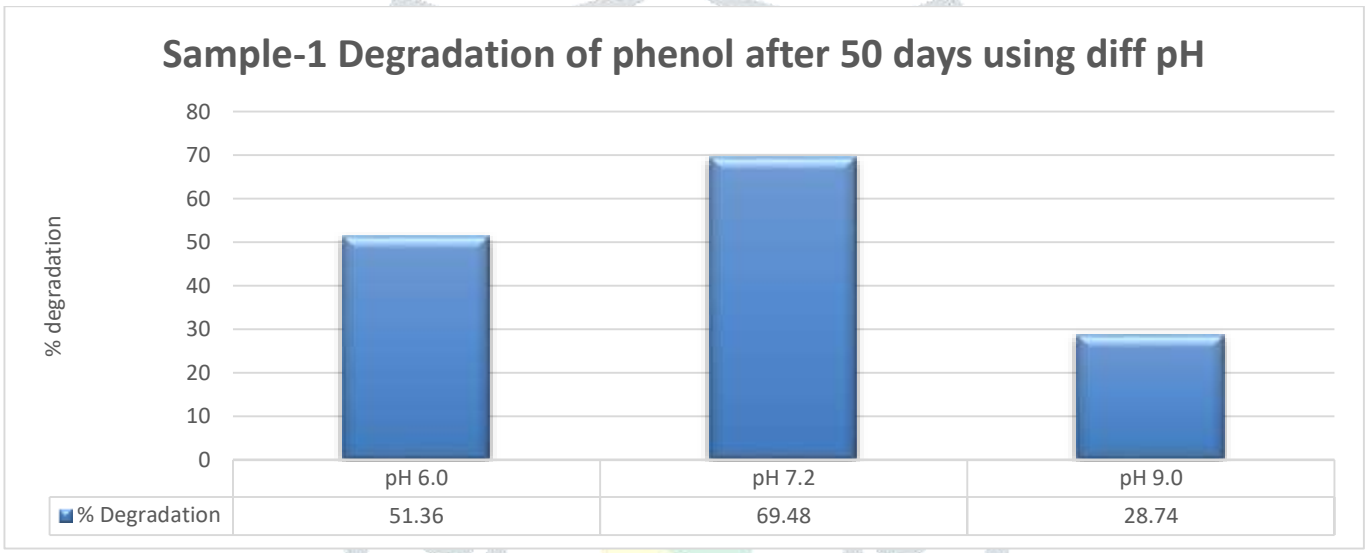


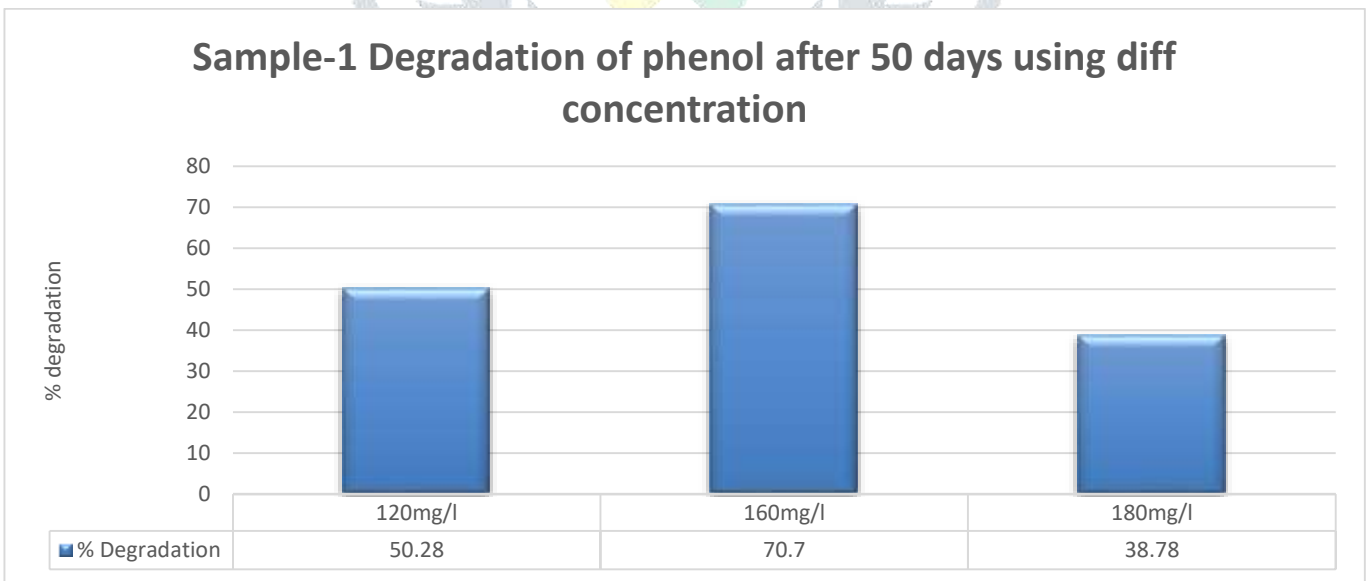

Sample-2 Degradation of phenol after 25 days using diff pH
% degradation
25
20
15
10
5
0
pH 6.0
pH 7.2
pH 9.0
% Degradation
17
23.12
13.43

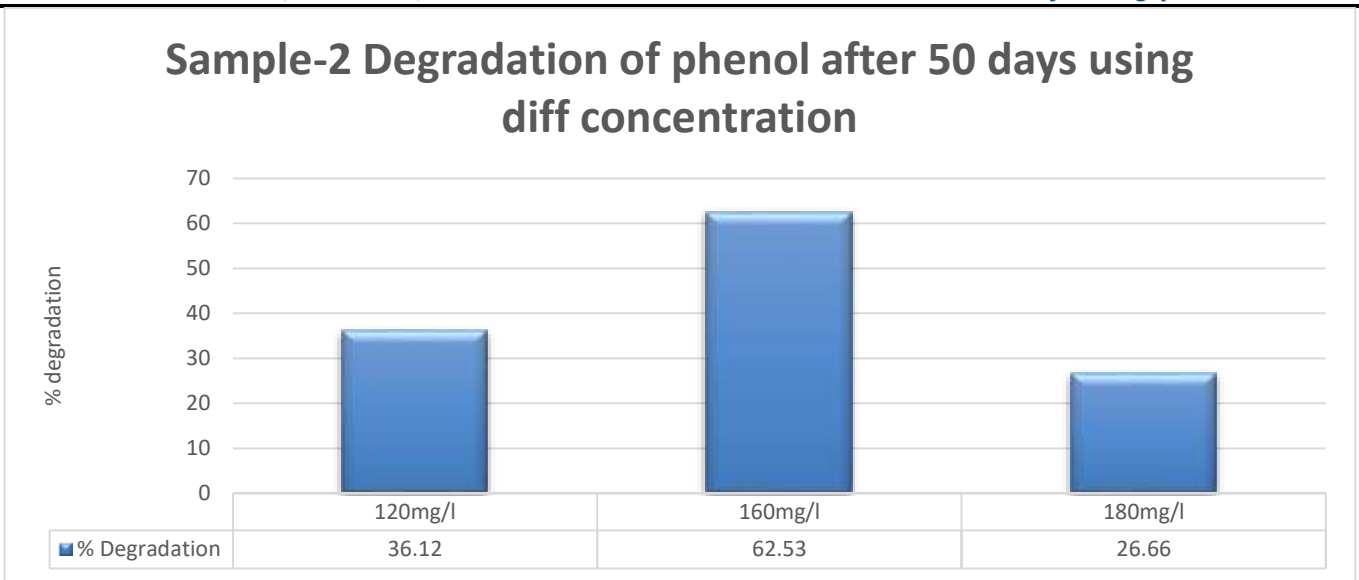

Sample-1 Degradation of phenol after 25 days using diff concentration
% degradation
25
20
15
10
5
0
120mg/l
160mg/l
180mg/l
% Degradation
18.06
20.11
15.2


Sample-2 Degradation of phenol after 25 days using diff concentration
% degradation
20
18
16
14
12
10
8
6
4
2
0
120mg/l
160mg/l
180mg/l
% Degradation
16.21
17.38
12.52


Sample-1 Degradation of phenol after 50 days using diff pH
% degradation
70
60
50
40
30
20
10
0
pH 6.0
pH 7.2
pH 9.0
% Degradation
45.16
64.2
32.54


Sample-1 Degradation of phenol after 50 days using diff pH
% degradation
80
70
60
50
40
30
20
10
0
pH 6.0
pH 7.2
pH 9.0
% Degradation
51.36
69.48
28.74


Sample-1 Degradation of phenol after 50 days using diff concentration
% degradation
80
70
60
50
40
30
20
10
0
120mg/l
160mg/l
180mg/l
% Degradation
50.28
70.7
38.78



Increasing substrate concentration enhance the growth of *pseudomonas* isolate but further increase in the concentration retarded the cell growth of the same isolate.
Phenol have the abilities to partition into membranes, disrupted membrane functions and caused cell death.

V. CONCLUSION

The bacterial *spp.* of *pseudomonas* used can degrade phenol compound. The bacterial *spp.* can significantly remove phenol at different pH but suitable for degradation is found to be 7.2. Increasing substrate concentration enhanced the growth of *pseudomonas* isolate but further increases in the concentration retarded the cell growth of some isolate. 160mg/L concentration showed the best degradation of phenol, it showed 70.70% degradation. Degradation by pseudomonas spp. is enhanced by the presence of glucose.

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