IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 5 Ver. II (May. 2015), PP 55-60
www.iosrjournals.org

Degradative Capacity of Indigenous Phenol Degraders Isolated From Phenolic Pesticide Contaminated Soil

Vedika M[1] And Sunita S[2]*
[1, 2](School of Biotechnology and Bioinformatics, D. Y. Patil University, Sector 15, Navi-Mumbai, 400614- India.)

Abstract: *Among the diverse toxic environmental pollutants routinely eluted from industrial effluents, phenol and its derivatives encompasses the most common eluents. Phenol even at minimal concentration, have been documented as recalcitrant and hazardous compounds. The present study was aimed to isolate indigenous phenol degraders from different agricultural locales of Mumbai. The isolates were characterized using phenotypic and molecular methods. The extent of phenol degradation was checked in both mineral and complex medium supplemented with phenol as a source of carbon and energy. Phenol biodegradation in the range of 24 – 70 % was achieved after 87 hr of incubation. 16s rRNA sequencing revealed Pseudomonas and Serratia spps. as predominant phenol degraders present in the sample under analysis.*

Keywords*: Phenol, Biodegradation, Pseudomonas sps., Serratia sps., 16s rRNA*

I. Introduction

Phenols and its derivatives are common starting materials and often major waste byproducts of industrial and agricultural products. Phenolic wastes present in water even at insignificant concentration are noxious for the aquatic organisms and also change the taste and odour of drinking water [1]. High risk of cardiovascular diseases, muscle tremors, skin damage are some of the health disorders associated with inhalation and dermal contact of phenol. Further oral administration can lead to chronic gastrointestinal damage [2]. Hence elimination of such hazardous pollutants becomes mandatory. Methods in vogue for purging phenols from wastewater include hybrid process, electrocatalytic degradation, adsorption on to different matrices, chemical oxidation, solvent extraction and irradiation [3]. The major predicament of these methodologies is cost per treatment and synthesis of hazardous byproducts.

"Bioremediation" or "Mineralization", using microbial cells is one of the low-cost alternatives that ensure near to complete mineralization of phenolics and related organic wastes [4]. Phenol degrading microbes have been isolated from different sources. Amongst the array of these microbes, bacteria such as *Pseudomonas* spp., *Acinetobacter* spp., yeast such as *Pleurotutus ostreatus*, *Candida tropicalis, Trichosporon cutaneum* and *Phanerochaete chrysosporiumn* and fungi like *Fusarium flucciferum* and *Aspergillus fumigates* are of specific importance ([5],[6], [7]). However till date the biodegradative potential of phenol degrading microbes may vary on the basis of adaptability of these on different substrates and metabolic aptitude. Thus isolation of efficient indigenous phenol degrading microorganisms from different phenolic wastes or soils and their cultivation and reuse for bioaugmentation has become the need of hour.

The present research endeavors study of native isolates from different locales of Mumbai proficient in phenol bioremediation. The isolates obtained were characterized using standard morphological, biochemical techniques for microbes as well using 16s rRNA.

II. Materials And Method

2.1Soil samples

Agricultural soil samples that were sprayed with different pesticide containing phenolics compounds were obtained from five farms of Mumbai. The microbes degrading phenol were enriched by mixing 10 gms of soil samples with 100 ml NB containing 0.01 % Phenol and incubated at RT with aeration for 1-2 weeks. One ml of growth from the first flask was inoculated to 100 ml NB containing 0.05 % Phenol followed by similar incubation conditions. This process was repeated by increasing the concentration of phenol to 5%. A total of six isolates was further used for morphological, physiological, biochemical and molecular characterization.

2.2Isolation and identification of bacteria from soil

The morphological characteristics of the isolated bacterium were studied by Gram and endospore staining. The physiological parameters like pH and temperature were optimized by growing the isolates in Nutrient broth at different pH range - 4.5, 6, 7.2, 8.5, 10 at room temperature for 24hours, while for optimization of temperature the isolates were incubated in static conditions at 16^0C, room temperature, 37^0C and 45^0C

respectively followed by measurement of optical density. The selected isolates were characterized using standard biochemical test.

2.3Evaluation of microbial competence with phenol

Isolated strains were tested for their ability to tolerate different concentrations of phenol in a mineral medium supplemented with phenol in the range of 100-1000 ppm. Growth was determined as absorbance at 600 nm. Phenol concentration was determined according toYang and Humphrey [8]. Phenol biodegradation was assayed using 1% each of Peptone, Tryptone, Beef, Yeast extract, Mineral salt medium (MSM) and Basal salt Medium (BSM) broth supplemented with phenol at a final concentration of 500ppm. Also NB and minimal media broth with 100 ppm of phenol were used. The medium was inoculated with the culture and incubated at room temperature under static conditions for 87-111 hrs. The average degradation rate of phenol was measured by dividing the net amount of phenol degraded for different hours of incubation.

2.4 Molecular characterization

Extraction of DNA was by Eurofins Genomics India Pvt Ltd, Bangalore, India and visualized in ethidium bromide stained 0.8% agarose gel. The 16S rRNA gene sequences were amplified and the amplicon obtained were further sequenced. The raw sequence data obtained were analyzed with Megablast of nBLAST. The hits obtained showed maximum hits of SSU (Small Subunit Sequences). The isolates were identified by taking into consideration the BLAST parameters of E-value, Maximum Identity, Query Coverage and total score. Complete alignment, Lineage report and Distance tree results of the BLAST "Hits" were analysed and compared to conclude the identity of the isolates.

III. Results & Discussion

Five soil samples contaminated with pesticides with phenolics groups were used for isolation of potential phenol degraders after the enrichment process (subsequent increases from 0.01% to 0.05% to 1% and 5% phenol concentration). Enriched organisms were subsequently purified and maintained. For further analysis, six microorganisms with variable colony characteristics were used. Most of the organisms were gram negative. The biochemical characteristics of all the isolates are as indicated in Table 1. The growth curve of all the isolates did not show any extended lag phase.

The optimum pH for all the isolates was found to be 7.2 with an exception of 3' which showed maximum growth at a pH of 8.5 (Fig. 1). The effect of temperature on growth pattern of the six isolates was studied at 16^0C, room temperature, 37^0C and 45^0C in NB at pH 7.2 under static condition. The optimum growth for all the isolates was at room temperature (Fig. 2). As the tolerance limit for all the six isolate was found to be above 500 ppm (Mona and Ashish unpublished data), all the media used in the study were supplemented with 500 ppm of phenol. Fig 3 shows the observed growth pattern for all the six isolates in NB – Phenol and Yeast extract – Phenol. In NB-phenol broth, isolates 3', 11' and 1a showed more than 50% of phenol degraded after 87 hours; however the rate of phenol degradation was highest (40%) for isolate 11' after 48 hrs (Fig. 4a). The isolates 1a, 9a, and 11' showed a decrease in cell mass at an interval of 72 hours of incubation, however the percentage phenol degradation was found to be highest in this period. This may be because of the enzymes or secondary metabolites being secreted in the stationary phase of the microbes which may be responsible for the degradation of phenol or its derivative.

The percentage of phenol degraded in 1% Peptone – Phenol broth was 50% only for isolate 11'(Fig. 4e), while, for 1% Beef extract- Phenol broth more than 60% phenol degradation was shown by isolate 2a only (Fig. 4b). The isolates showed less than 40% phenol degradation in 1% Tryptone - Phenol broth (Fig. 4c, d) and about 20% in Yeast extract- Phenol broth. Although a higher cell volume was observed in Yeast extract, the rate of phenol degradation was seen to be comparatively poor. The rate of phenol degradation was varying at different interval of time in different medium for all the six isolates. Unlike Singh et al [9], in the present study no growth and hence no phenol degradation was observed for the mineral medium as they were only supplemented with 500ppm phenol. This was attributed to non-acclimatization of culture to phenol due to non availability of any assimiliable sugar thus giving negative regulation of growth. Reports suggest that the time of addition of phenol influences the rate of phenol degradation in both revived enriched culture, non- revived enriched [10]. Hence it is possible that in absence of carbon source other than phenol, the phenol degradation was not observed. Reports also suggest that these conventional carbon sources are important as they may provide reducing power to phenol degradation or may act as inducing agents for the biodegradative enzymes [9].

Both the conventional carbon source and the effect of inoculums size also effects the growth pattern and hence the rate of phenol degradation. In the present study a lag of 15-20 hours was observed in growth pattern for medium other than NB which showed a lag of 9-10 hours. The necessity of easily assemble, carbon source for microbial growth and phenol degradation has been studied by Tambekar [11], Mohite [12]. Annandurai [13], Suhaila [14], Bhattacharya [15] and Singh [9] also studied the effect of different nitrogen

source (Organic/ Inorganic) on rate of phenol degradation. The present study has utilized both non acclimatized culture in mineral medium like M9, MSM, BSM and acclimatized culture in complex medium like NB, TWB, YEB, BEB supplemented with phenol as a source of carbon and energy. Like Hank [10] and Das [16] this study also manifest that the acclimatization of organism helps in higher or complete degradation of phenol. However optimization for medium variables, minerals and phenol screened by Plackett-Burman (PB) method utilizing Central composite design (CCD) and Response Surface Methodology (RSM) [17] could give a better insight on the biodegradability of phenol by these cultures.

Of all the six isolates, two high phenol degrading isolates (11' and 3') and one negligiable / low phenol degrading isolate (5a) were further characterized by 16S rRNA analysis. The Lineage Tree analysis and dendogram based on Maximum Fast Minimum Evolution Tree Methods keeping a maximum sequence difference of 0.75 was performed for all the three isolates. As these isolates were obtained from farms sprayed with pesticide, the likelihood of isolating a phenol degrading *Pseudomonas* was high. The strain 3′ was closely related to *Serratia marcescens* with 39 'hits' and 95% sequence identity, while 11′ was closely related to *Pseudomonas aeruginosa* with 99 hits and 94% sequence identity, 5a was possibly related to *Serratia* spps with 21 hits and 92% identity.

IV Figures and Tables

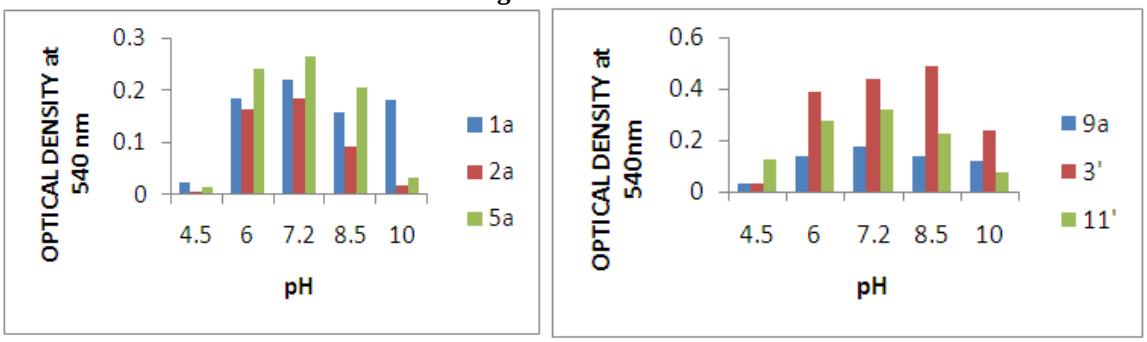


Fig-1: Effect of pH on isolates 1a, 2a, 5a, 9a, 3'and 11'

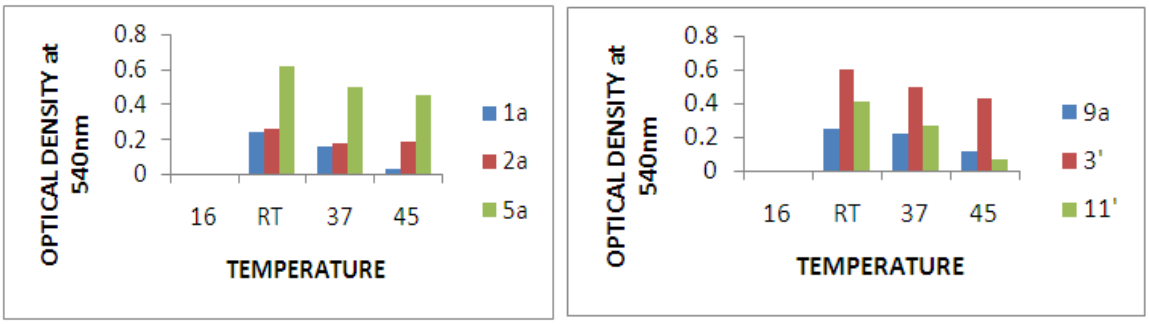


Fig-2: Effect of temperature on isolate 1a, 2a, 5a, 9a, 3'and 11

Table 2: Biochemical Characterization of the six isolates

Characteristics	Isolate					
	1a	2a	5a	9a	3'	11'
Gram Nature	-	+	-	-	-	-
Citrate Utilization	-	+	+	-	+	+
Methyl Red	-	+	+	-	+	+
Voges Proskauer's	-	-	-	-	-	-
Indole	-	+	+	-	+	-
Glucoronidase	-	-	-	-	-	-
Nitrate Reductase	+	+	+	+	+	+
ONPG	-	-	-	-	-	-
Lysine Utilization	-	+	+	+	+	+
Lactose	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	-
Oxidase	+	-	-	-	-	+
Catalase	+	+	+	+	+	+
Alkaline Phosphatase	-	-	-	-	-	-
Urease	-	-	-	-	-	-

Arginine Utilization	+	+	+	+	+	+
Mannitol	+	-	-	+	-	-
Arabinose	+	+	+	+	+	+
Raffinose	+	-	-	+	-	-
Trehalose	+	-	-	+	-	+
Maltose	+	-	-	+	-	+

Key: + indicates positive results - indicates negative results

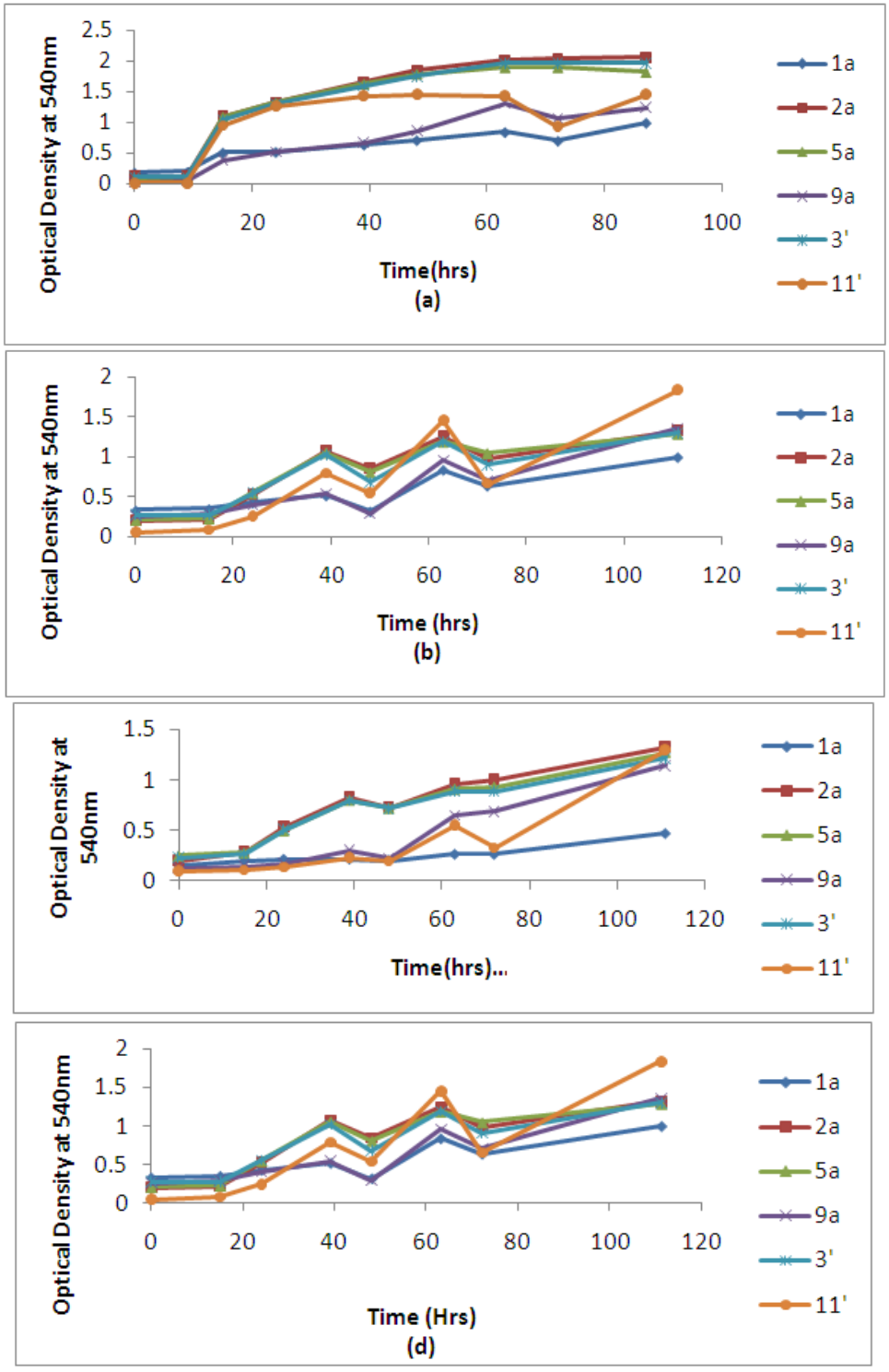
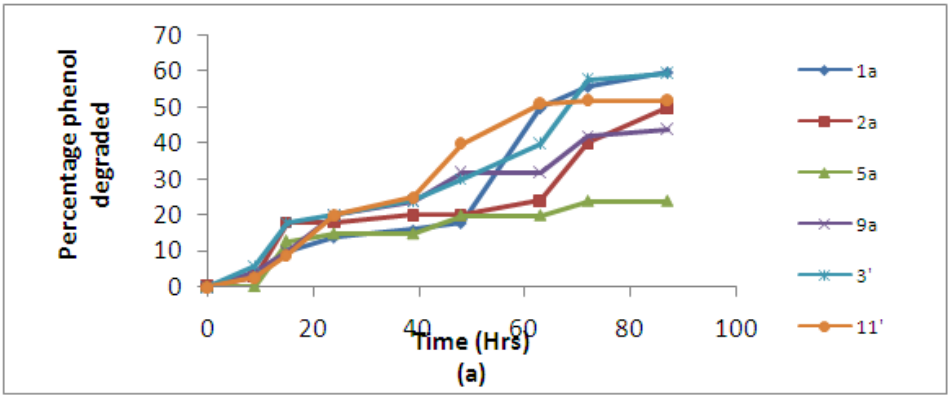
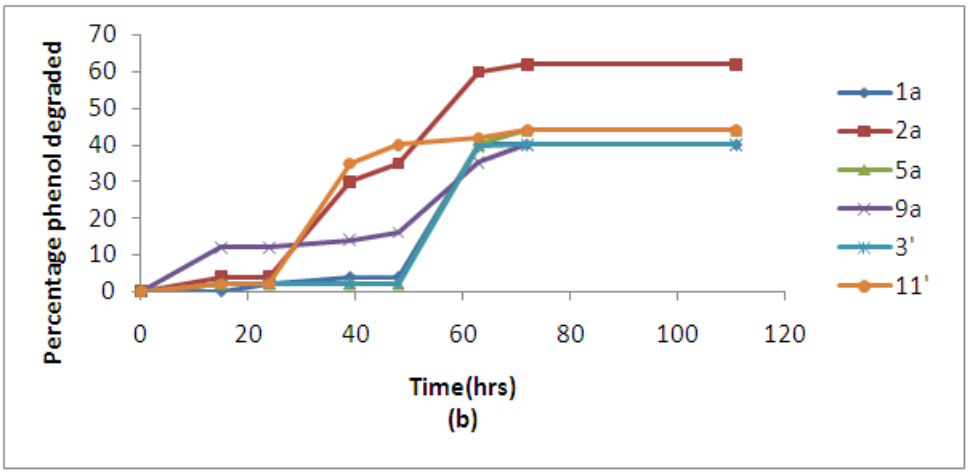


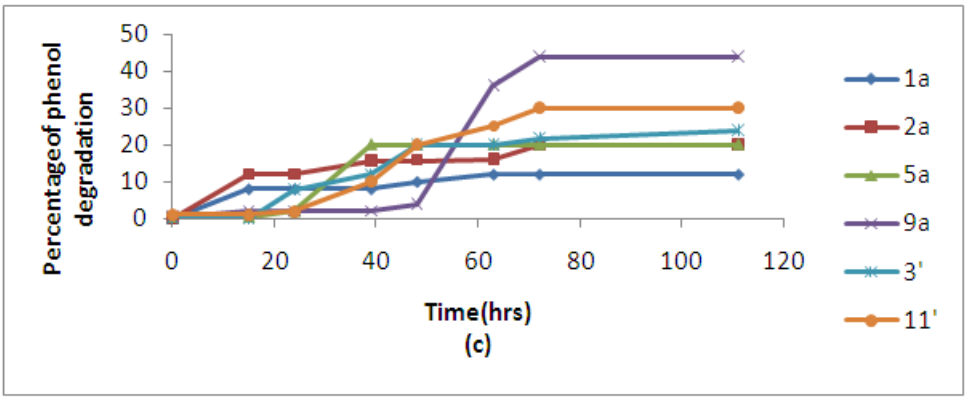
Fig-3: Growth pattern of all isolates in Nutrient-Phenol (a), Beef extract- Phenol (b), Typtone –Phenol broth (c) and Yeast Extract-Phenol broth (d).


Percentage phenol degraded
70
60
50
40
30
20
10
0
0
20
40
60
80
100
Time (Hrs)
1a
2a
5a
9a
3'
11'

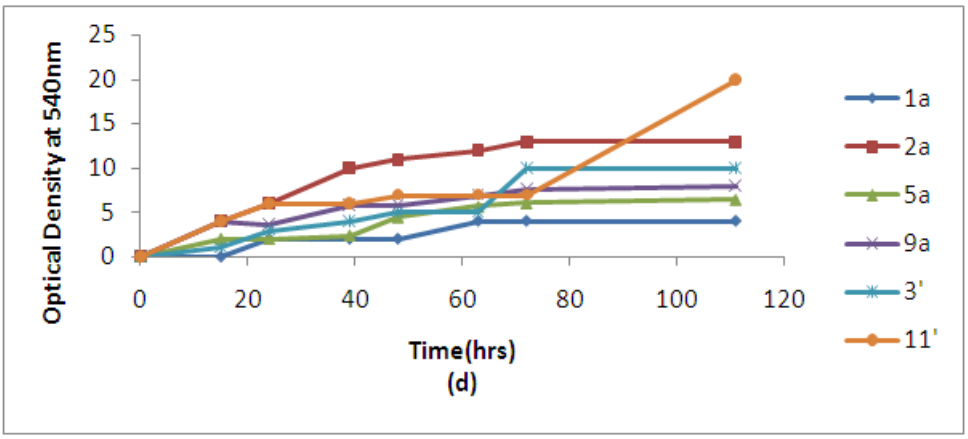
(a)


Percentage phenol degraded
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Time(hrs)
1a
2a
5a
9a
3'
11'

(b)


Percentageof phenol degradation
50
40
30
20
10
0
0
20
40
60
80
100
120
Time(hrs)
1a
2a
5a
9a
3'
11'

(c)


Optical Density at 540nm
25
20
15
10
5
0
0
20
40
60
80
100
120
Time(hrs)
1a
2a
5a
9a
3'
11'

(d)

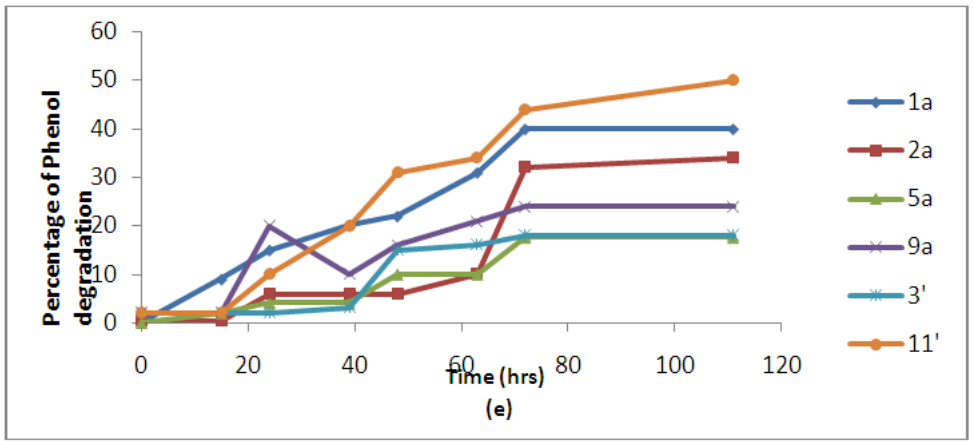


Fig 4: Percentage phenol degraded in Nutrient-Phenol Broth (a), Beef extract-Phenol broth (b), Tryptone-Phenol broth (c), Yeast extract-Phenol broth (d) and in Peptone-Phenol broth (e).

IV. Conclusion

Based on the morpho-physio-molecular characterization of the isolates it can be concluded that presence of good carbon and energy source augment the process of phenol degradation if the isolates are enriched / acclimatized. However further studies on influence of temperature, with the isolate, and different mineral medium needs to be characterized for utilizing these organisms for phenolics pesticide bioremediation.

References

[1]. B E Rittmann, And P L Mccarty, Environmental Biotechnology: Principles And Applications (New York: Mcgraw-Hill, 2001).
[2]. F Kafilzadeh, M S Farhangdoost, And Y Tahery,. Isolation And Identification Of Phenol Degrading Bacteria From Lake Parishan And Their Growth Kinetic Assay. African Journal Biotechnology 9(40), 2010, 6721- 6726.
[3]. S Chakraborty, T Bhattacharya, T N Patel And K K Tiwari. Biodegradation Of Phenol By Native Microorganisms Isolated From Coke Processing Wastewater. Journal Of Environmental Biology 31, 2010, 293-296.
[4]. S Wasi, S Tabrez And M Ahmad, Use Of Pseudomonas Spp. For The Bioremediation Of Environmental Pollutants: A Review. Environ Monit Assess, DOI 10.1007/S10661-013-3163-X
[5]. F Ariana, B Elke And B Thomas. Rapid Monitoring Of The Biodegradation Of Phenol-Like Compounds By The Candida Maltosa Using BOD Measurements. Int. Biodeterior. Biodegrad. 54, 2004, 69-76.
[6]. Q Xiangchun, S Hanchang And Z Yongming. Biodegradation Of 2, 4- Dichlorophenol And Phenol In Airlift Inner-Loop Bioreactor Immobilized With Achromobacter Sp. Purif. Technol. 34, 2004, 97-103.
[7]. T Godjevargova, D Ivanova, Alexieva Z, And Dimova N. Biodegradation Of Toxic Organic Components From Industrial Phenol Production Wastewater By Free And Immobilized Trichosporon Cutaneum R57. Process Biochem. 38, 2003, 915-920.
[8]. R D Yang And A E Humphrey, Dynamic And Steady State Studies Of Phenol Biodegradation In Pure And Mixed Cultures. Biotechnol Bioeng. 17(8), 1975, 1211-35.
[9]. R K Singh, A Agarwal, A Kumar, S Kumar And S Kumar, Studies On Biodegradation Of P-Nitrophenol By Arthrobacter Chlorophenolics And Its Metabolites. Research Journal Of Pharmaceutical, Biological And Chemical Sciences 3 (4). 2012, 695-704.
[10]. D Hank, N Saidani, A Namane And A Hellal, Batch Phenol Biodegradation Study And Application Of Factorial Experimental Design. J. Eng. Sci. Technol. Rev. 3(1). 2010, 123-127.
[11]. D H Tambekar, P S Bhorse And P V Gadakh, Biodegradation Of Phenol By Native Microorganisms Isolated From Lonar Lake In Maharashtra State (India). International Journal Of Life Science And Pharma Research 2(4), 2012, 434 – 441
[12]. B V Mohite, R E Jalgaonwala, S Pawar, A Morankar. Isolation And Characterization Of Phenol Degrading Bacteria From Oil Contaminated Soil. Innovative Romanian Food Biotechnology. 7, 2010, 61-65.
[13]. G Annadurai, R S Juang And D J Lee. Microbiological Degradation Of Phenol Using Mixed Liquors Of Pseudomonas Putida And Activated Sludge. Waste Manage, 22, 2002, 703-710.
[14]. Suhaila, Y A Ariff, M Rosfariza, I Latif, S A Ahmad, M N Norazah., M Y A Sukor, . Optimization Of Parameters For Phenol Degradation By Rhodococcus UKM-P In Shake Flask Culture. Proceedings Of The World Congress On Engineering, 1, 2010.
[15]. S Bhattacharya, A Das. And P Nalini. Ex Situ Biodegradation Of Phenol By Native Bacterial Flora Isolated From Industrial Effluent, Journal Of Chemical, Biological And Physical Sciences, 2(2), 2012, 1091-1101
[16]. N R Das And N Kumar, Assessment Of Phenol Biodegradation Capacity Of Indigenous Bacteria Isolated From Sewage Treatment Plant. African Journal Of Microbiology. 9(16). 2015, 1150-1161.
[17]. M Pandimadevi, P M Venkatesh And V Kumar, Optimization Of Phenol Degradation Using Pseudomonas Aeruginosa (Mtcc 7814) By Plackett-Burman Design And Response Surface Methodology, Bioremediation And Biodegradation. 2014 5: 261. Doi:10.4172/2155-6199.1000261