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Microbial Degradation of Phenols by "Bacillus Brevis"

T. M. Usha Marya[1] and M. Swaminathanb[2]
Department of Chemistry, Panimalar Engineering College, Chennai, India[1]
Nanomaterials Laboratory (IRC), Department of Chemistry, Kalasalingam Academy of Research and Education, India[2]
ushamarydr11@gmail.com and m.swaminathan@klu.ac.in

Abstract: *Industrial wastewater containing phenols causes significant environmental and ecological problems. Various methods such as chlorination, flocculation, adsorption etc. have been used for the degradation of phenol. But microbial degradation methods have proved to be the most effective and economical approach for the mineralization of toxic chemicals. A soil microbial strain* ***Bacillus brevis****, capable of utilizing phenol as a sole carbon source was isolated from the phenol bearing soil suspension of Briquetting and Carbonization Plant of NeyveliLignite Corporation Limited, (Tamil Nadu) and tested for its capacity to grow and degrade phenol. Based on it's morphological, physiological and biochemical characteristics, the organism was found to be a Gram-positive, motile, mesophilic and rod-shaped endospore bacterium. The results indicate that the growth of the organism decreases at very high concentration of phenol. The efficiency of the organism in the degradation of substituted phenols such as o & p chlorophenols and o & p nitrophenols were compared and discussed. The degradation was highly efficient in the pH range 8 – 10. The biocatalyst obtained by immobilizing the* ***Bacillus brevis*** *cells on alginate beads and lignite carbon are more effective in degrading phenols.*

Keywords: Bacillus Brevis, Immobilization, Phenol Degradation, Bacterial Growth and Degradation.

I. INTRODUCTION

Phenolic compounds are toxic to fish, plants and many organisms. The wastewater containing phenols are from variety of industries like Briquetting and Carbonization plant, Coking plant, Coal Conversion plant, etc., Several physico-chemical methods for treatment of these phenolic waste like hydrogenperoxide oxidation method[1](DongLi etal., 2018), [2](Jie Sun etal., 2020) photocatalytic degradation[3](MuhammadZulfiqar et al., 2019),[4](XiaohuiFeng etal., 2014) and adsorption of phenols by activated carbon[5](WeiweiLi etal., 2018) have been used for the removal of phenols. All the physico-chemical methods have it's own difficulties. Therefore microbial degradation is an alternative method proved to be more advantageous due to its eco-friendly cost-effective nature. The microbial degradation of phenols by pure and mixed culture of various pseudomonas species has been reported by several authors like [6](EyalKurzbaum etal., 2017),[7](Marwa Youssef etal., 2019), [8](SounakBera etal., 2017), [9](Fatimah Alshehrei, 2017)

The Briquetting and Carbonization plant effluent contains mainly phenols. Earlier in our laboratory two soil microbes, one from the soil suspension of cyanide effluent and another from (Briquetting and Carbonization plant) thiocyanate effluent have been isolated. So this prompted us to study the efficiency of this isolated bacterium ***Bacillus brevis*** in the degradation of phenols. Immobilization of microbial cells have received increasing interest in recent years. It increases the efficiency of bioprocesses. Compared with free cells, immobilized cells have several advantages. Due to high adsorption capacity, alginate beads and lignite carbon finds wider applications in phase adsorption system[10] (FaissalAziz etal., 2020), [11](YingQi etal., 2011). Alginate beads and Lignite carbon have beenused as an industrial catalyst and as a carrier for cells in biochemical reactions[12](Muhammad Bilal etal., 2017) and [13](JianxiuHao etal., 2019). In this paper we report the efficiency of free and immobilized bacterium, ***Bacillus brevis***, on the microbial degradation of phenols and substituted phenols.

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II. Materials and Methods:

2.1 Media

1. The nutrient agar medium was used for the isolation and substitution of the bacterium. This contains peptone 1%, beef extract 1%, sodium chloride 0.5% and agar 2% (pH7.0).
2. Peptone 1%, Beef extract 1% and NaCl 0.5%(pH 7.0)
3. Dilution water: Phosphate buffered water was prepared by adding 0.01N NaOH to 0.01N H_3PO_4 to adjust the pH to 7.0

III. Isolation of the Bacterium

The Carbonization wastewater soil suspension was collected fromBriquetting and Carbonization plant, Neyveli Lignite Corporation, Neyveli, TamilNadu. One gram of the soil suspension was inoculated into sterile test tubes containing sterilized water. The contents were streaked out on the plates containing medium for isolation. Colonies that grew on plates were selected for identification. The Isolate was identified as ***Bacillus brevis (MTCC3136)*** from the physiological and morphological test results.

IV. Preparation of the Culture

The organism was grown in nutrient broth for 48 hours at 35^0C under stationary conditions. The culture was then harvested by centrifugation at 10000 rpm for 20 minutes, washed twice with sterilized water and resuspended in sterile buffered water. The centrifuged biomass was used to study the degradation of phenols, by the bacterium. The pH was adjusted by using 0.01N phosphoric acid and 0.01N sodium hydroxide.

V. Immobilisation

Alginate Beads and Lignite Carbon were used as matrices for immobilization. The bacterial suspension used for immobilization, contained 48 hours grown cells of ***Bacillus brevis*** incubated at 35^0C in the nutrient broth. The immobilization was carried out by passing the suspension through the washed and sterilized lignite carbon and alginate beads.

VI. Results and Discussion

6.1 Growth of *Bacillus brevis* and Degradation of Phenol

The inoculums of ***Bacillus brevis*** was added to the solution with different concentration of phenol. The samples were taken at regular intervals of time and the cell growth of the organism was determined with different concentration of phenol as shown in **Fig.1**. The lag period of the bacterium increases and the growth rate decreases with increase in concentration of phenol, which reveals that the bacterium is not tolerant at higher concentrations of phenol.

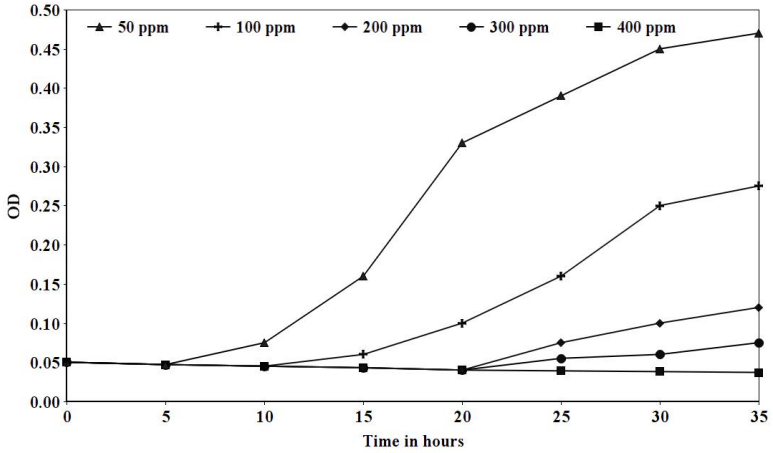

Figure 1: Growth of *Bacillus Brevis* with Different Concentrations of Phenol

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The influence of pH on phenol degradation and growth of the bacterium had been carried out. The bacterium showed maximum growth in pH 8-10. The degradation is also found to be efficient in the same pH range and therefore pH10 is taken as optimum pH **(Fig.2)**.

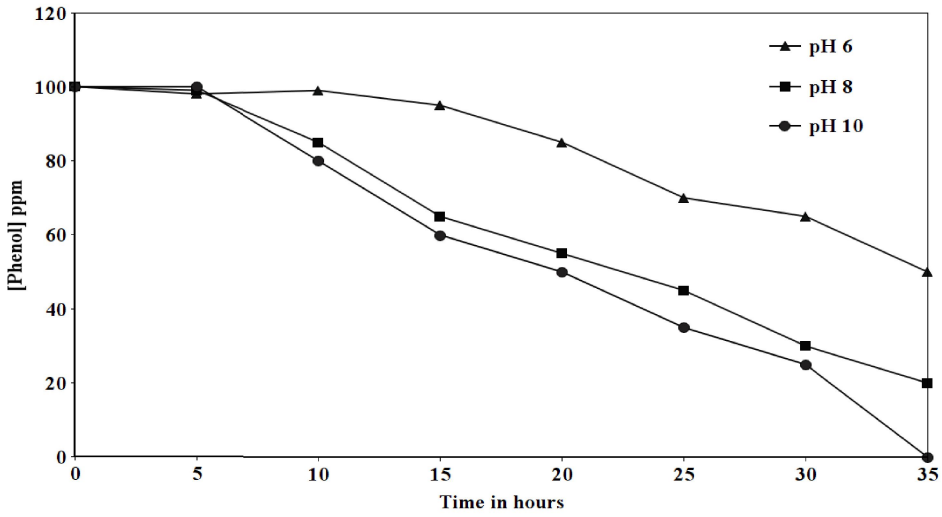

Figure 2: Effect of PH on Degradation of Phenol

Phenol degradation by *Bacillus brevis* (free) cells is shown in **Fig.3**. The time required for the complete degradation of phenol increases with increase in concentration of phenol. The bacterium *Bacillus brevis* (free cells) is capable of completely degrading 100 ppm of phenol in 35 hours.

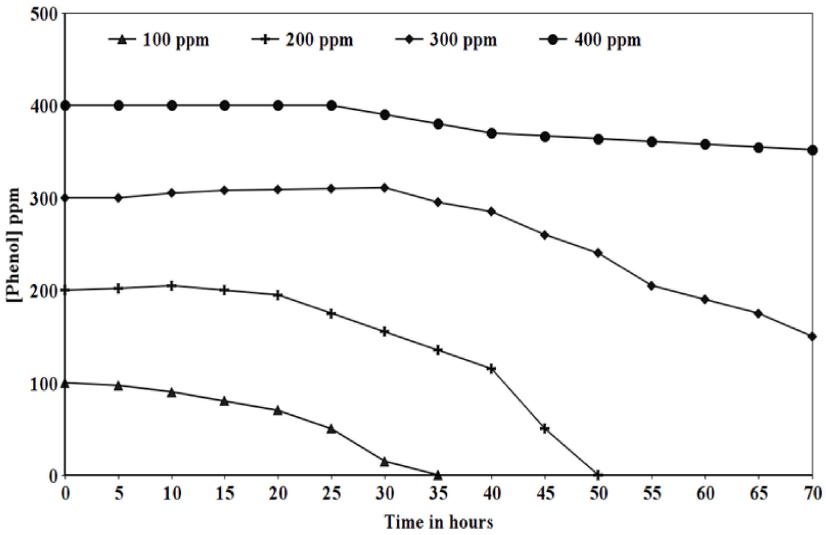

Figure 3: Phenol Degradation by *Bacillus Brevis* (Free) Cells

Phenol degradation by ***Bacillus brevis*** (Immobilized cells) on lignite carbon is shown in **Fig.4**. The bacterium ***Bacillus brevis*** is capable of completely degrading 100 ppm of phenol within 20 hours. In lignite carbon as the mass of the carbon is increased, the available adsorbent surface area and pore surface area increases. This shows that the adsorbed cells also increase. In immobilization, maximum adsorption of cells is on to lignite carbon due to its higher adsorption capacity. Therefore efficient degradation of phenols occurs.

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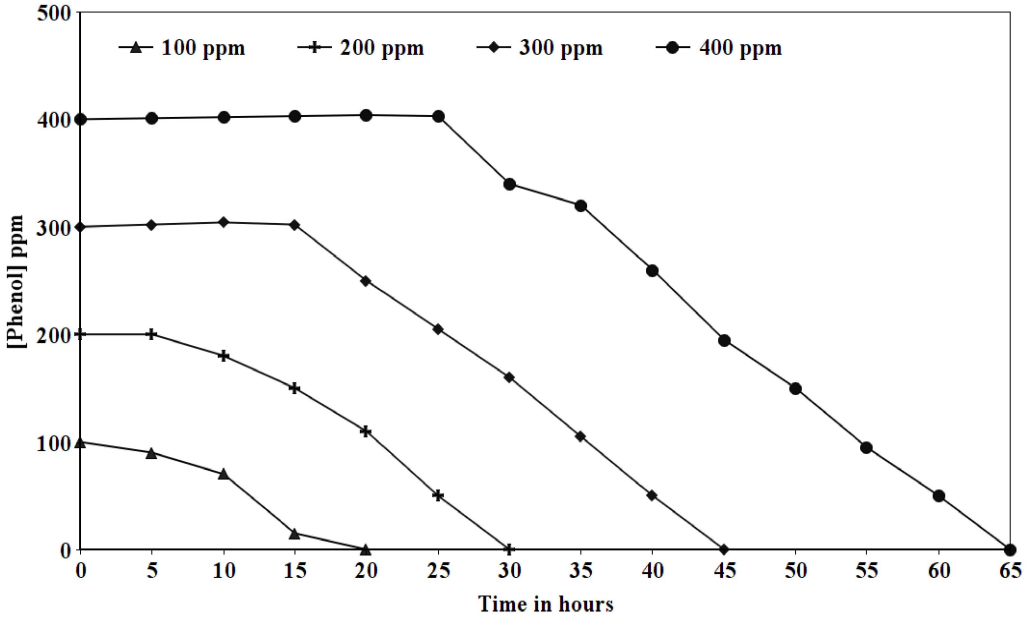

Figure 4: Phenol Degradation by *Bacillus Brevis*
(Immobilised Cells on Lignite Carbon)

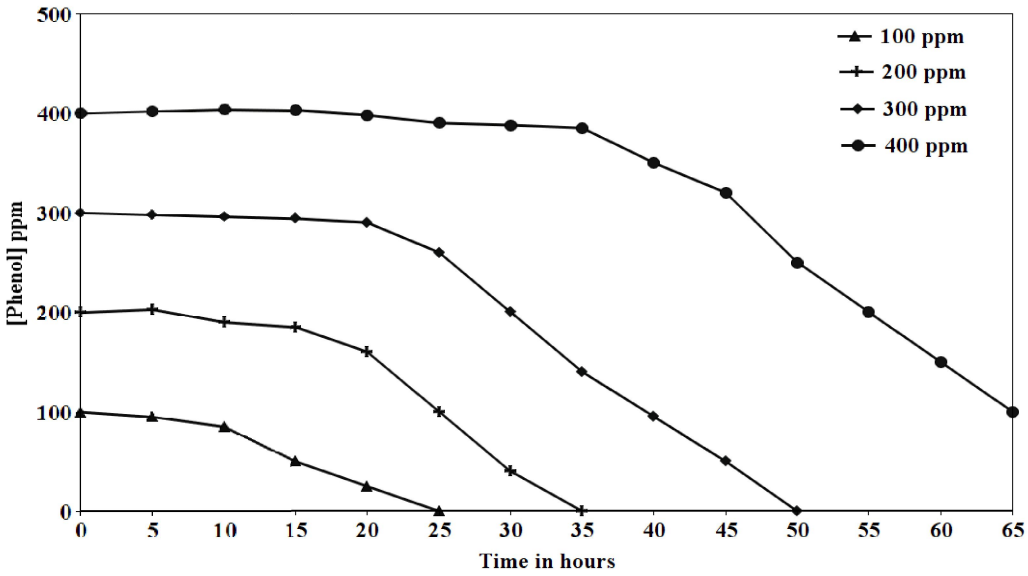

Figure 5: Phenol Degradation by *Bacillus Brevis*
(Immobilised Cells on Alginate Beads)

Phenol degradation by *Bacillus brevis* (Immobilized cells) on alginate beads is shown in above **Fig.5.** The concentration of 100, 200, 300, 400 ppm of phenol were used for the degradation by cells immobilized on Alginate beads. A complete degradation of 100 ppm of phenol was observed in 25 hours and the percentage of removal decreases with increase in phenol concentration. As the phenol concentration is less, the degradation is fast. As the number of beads increases, the degradation of phenol increases. This is due to the increase in number of adsorbed cells. Therefore,the immobilized cells on alginate beads are found to be more efficient than free cells in phenol degradation. The results indicate that the cells when immobilized are not only shielded from direct contact with toxic chemical but their efficiency is also increased. For degradation of phenol by ***Bacillus brevis*** by free and immobilized cells, phenol degradation was found to be more by immobilized cells than free cells [14](QianKe etal., 2018).

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The percentage of phenol degradation with immobilized cells(lignite carbon) is more (ie. 100 ppm degrades within 20 hours), but with free cells (100 ppm degrades within 35 hours), i.e.it takes more time. This shows that free cells are affected whereas immobilized cells are tolerant to this concentration of phenol. The degradation of phenols at 100 ppm by free cells in 20 hours is 62%, by immobilized cells (alginate beads) is 80% and by immobilized cells (lignite carbon) is 100%. **Table1**.

Table 1: Percentage degradation of phenols at 100 ppm by free and immobilized cells in 20 hours:

Substrate / Concentration in ppm	% Degradation by Free cells	% Degradation by Immobilized cells(Alginate Beads)	% Degradation by Immobilized cells (Lignite Carbon)
Phenol / 100	62	80	100

Degradation of Substituted Phenols using *Bacillus brevis*:

The degradation efficiencies with different concentrations of substituted phenols such as (o & p -chlorophenols, o & p - nitrophenols) are compared and discussed in **Table2.**

Table 2: Percentage degradation of substituted phenols by free and immobilized cells in 20 hours

Substrate	% Degradation By Free cells	% Degradation by Immobilized cells (Alginate Beads)	% Degradation by Immobilized cells (Lignite Carbon)
o - chlorophenol	32	60	64
p - chlorophenol	40	68	70
o - nitrophenol	24	57	60
p - nitrophenol	20	55	58

As observed in phenol, the lag period increases, the rate of growth decreases and degradation also decreases with increasing concentration ofsubstituted phenols. The toxicity of nitrophenols is more than chlorophenols. The difference in toxicity is due to the acidity of substituted phenols. Acidity of p - chlorophenol is close to that of phenol. When acidity increases, toxicity also increases and consequently degradation is affected. It was found out that the electron withdrawing effect of the substituent could delay the degradation. Since nitro group is more electronegative than chlorine the degradation is less efficient in nitrophenols when compared to chlorophenols. It is clear that, phenols undergo maximum degradation and p-nitrophenol undergoes minimum degradation. Therefore substituted phenols are toxic to the organism.

The percentage of degradation is high for phenols and substituted phenols using immobilized cells. This indicates that immobilized cells are more efficient than free cells.

VII. Conclusion

The microbe isolated from the carbonization wastewater soil of Briquetting and Carbonization plant, Neyveli was identified as ***Bacillus brevis***. The growth of the bacterium and phenol degradation efficiencies are optimum at pH 10. It is evident therefore that phenols undergo maximum degradation than substituted phenols.

The toxicity of phenols to micro-organisms increases with the increase in acidity of the phenols, therefore degradation decreases with increase in concentration of phenol. The immobilized cells are more efficient than free cells, which will be very useful in microbial degradation of phenol in wastewater.

References

[1]. Dong Li,Tianyi Sun,Lu Wang and Na Wang, "Enhanced electro-catalytic generation of hydrogen peroxide and hydroxyl radical fordegradation of phenol wastewater using MnO_2/Nano-G|Foam-Ni/Pd composite cathode," Electrochimica Acta, vol. 282, Aug., pp. 416–426, 2018.

IJARSCT
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[2]. Jie Sun, Guotong Xia, Wenjin Yang, Yue Hu and Weibo Shen, "Microwave-assisted method to degrade phenol using persulfate or hydrogen peroxide catalyzed by Cu-bearing silicon carbide, "Water Sci. Technol., vol. 82, no. 4,Aug., pp. 704 – 714, 2020.

[3]. Muhammad Zulfiqar, Mohamad Fakhrul Ridhwan Samsudin and Suriati Sufian, "Modelling and optimization of photocatalytic degradation of phenol via TiO_2 nanoparticles: An insight into response surface methodology and artificial neural network," Journal of Photochemistry and Photobiology A: Chemistry, vol. 384, Nov., pp. 1-15, 2019.

[4]. Xiaohui Feng, Haijuan Guo, Kunal Patel, Hong Zhou and XiaLou, "High performance, recoverable Fe_3O_4single bond ZnO nanoparticles for enhanced photocatalytic degradation of phenol," Chemical Engineering Journal, vol. 244, May, pp. 327 – 334, 2014.

[5]. Weiwei Li, Junjuan Yan, Zhifeng Yan, Yuncai Song, Weizhou Jiao, Guisheng Qi and Youzhi Liu, "Adsorption of phenol by activated carbon in rotating packed bed: Experiment and modeling," Applied Thermal Engineering, vol. 142, Sep., pp. 760 – 766, 2018.

[6]. Eyal Kurzbaum, Yasmin Raizner, Oded Cohen, Ran Y. Suckeveriene, Anatoly Kulikov, Ben Hakimi, Lilach Iasur Kruh, Robert Armon, Yair Farber and Ofir Menashe, "Encapsulated Pseudomonas putida for phenol biodegradation: Use of a structural membrane for construction of a well-organized confined particle," Water Research, vol. 121, Sept., pp. 37 – 45, 2017.

[7]. Marwa Youssef, Einas H. El-Shatoury, Sahar S. Ali and Gamila E. El-Taweel, "Enhancement of phenol degradation by free and immobilized mixed culture of Providenciastuartii PL4 and Pseudomonas aeruginosa PDM isolated from activatedsludge," Bioremediation Journal, vol. 23, no. 2, Apr., pp. 53 – 71, 2019.

[8]. SounakBera,AbhijitSarmaRoy,KaustubhaMohanty, "Biodegradation of phenol by a native mixed bacterial culture isolated from crude oil contaminated site," International Biodeterioration & Biodegradation, vol. 121, Jul., pp. 107 - 113, 2017.

[9]. Fatimah Alshehrei, "Effect of physicochemical factors on the biodegradation of phenol by Pseudomonas putida ATCC 12842 and Pseudomonas fluorescens ATCC 948," African Journal of Biotechnology, vol.16, no. 39,Sep., pp. 1962- 1968, 2017.

[10]. FaissalAziz, MounirElAchaby, AminaLissaneddine, Khalid Aziz, NaailaOuazzani, Rachid Mamouni andLaila Mandi, "Composites with alginate beads: A novel design of nano- adsorbents impregnation for large-scale continuous flow wastewater treatment pilots," Saudi Journal of Biological Sciences, vol. 27, no. 10, Oct., pp. 2499 - 2508, 2020.

[11]. YingQi, Andrew F.A.Hoadley,Alan L.Chaffee and GilGarnier, "Characterization of lignite as an industrial adsorbent," Fuel, vol. 90, no. 4, Apr., pp. 1567 – 1574, 2011.

[12]. Muhammad Bilal, Tahir Rasheed, Hafiz M. N. Iqbal, Hongbo Hu, Wei Wang and Xuehong Zhang, "Novel characteristics of horseradish peroxidase immobilized onto the polyvinyl alcohol-alginate beads and its methyl orange degradation potential," International Journal of Biological Macromolecules, vol. 105, Dec., pp. 328 – 335, 2017.

[13]. JianxiuHao, LiminHan, YufeiSha, XinxinYu, Haiying Liu, Xinyi Ma, Yezhao Yang, HuacongZhou andQuanshengLiu, "Facile use of lignite as robust organic ligands to construct Zr-based catalysts for the conversion of biomass derived carbonyl platforms into alcohols," Fuel, vol. 239, Mar., pp. 1304 – 1314, 2019.

[14]. Qian Ke,Yunge Zhang, Xilin Wu, Xiaomei Su, Yuyang Wang, Hongjun Lin, Rongwu Mei, Yu Zhang, Muhammad Zaffar Hashmi, Chongjun Chen and JianrongChen, "Sustainable biodegradation of phenol by immobilized Bacillus sp. SAS19 with porous carbonaceous gels as carriers,"Journal of Environmental Management, vol. 222, Sep., pp. 185 – 189, 2018.