j. port. sci. res.
ISSN: 2616-7441 (Online)
ISSN: 2616-6232 (Print)
ISSN: 2616-7220 (USB)
INTERNATIONAL IDENTIFIER FOR SERIALS

Journal port Science Research
Available online www.jport.co
Volume 7, special issue 2024

Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization

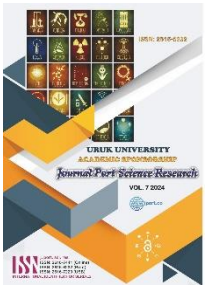
Mahammed E J. Al-Defiery*[1], Gopal Reddy[2]

[1]*Department of Biology, College of Science for Women, University of Babylon, Iraq*

[2]*Department of Microbiology - College of Science- Osmania University, India*

ubs.md@uobabylon.edu.iq

Abstract One of the primary concerns of the environment is the increment of the xenobiotics levels, which are released in the natural ecosystem. Phenol has been documented as a pollutant because it has a significant role in water contamination; this will, therefore have an impact on the health of humans. Phenol degradation studies were carried out using a mineral salts medium containing various percentages (v/v) of Ca-alginate beads, polyurethane foam, agar-agar and agarose in batches of culture for 1.5 g/L phenol degradation by immobilized cells of Rhodococcus pyridinivorans GM3 during 24 hours of incubation at 32ºC, 200 rpm and pH 8.5 .The results showed that a typical concentration of 3% (w/v) of the sodium alginate to form synthetic Ca-alginate beads was supporting phenol degradation which also emphasizes the structural stability of Ca-alginate beads. The concentration of 1.5 g/L phenol was completely degraded observed within 24 hours at 8% of the Ca-alginate beads immobilized cell and 10% of size cubes 0.125 cm3 of the polyurethane foam immobilized cell. Whilst, the degradation of 1.5 g/L of phenol concentration within 24 hours on both agar and agarose was 16% and 24% at cubes of size 0.125 cm3 and 1.0 cm3 respectively. However, the study of immobilization showed that Ca-alginate immobilized R. pyridinivorans GM3 was more efficient than polyurethane foam, agar and agarose.



Crossref 10.36371/port.2024.special.12

Keywords: *Phenol, Rhodococcus pyridinivorans, Immobilization, Degradation*

1. INTRODUCTION

The stresses of industrial development and an ever-increasing population have caused the accumulation of variants, resulting in great deterioration in the quality of the environment, contaminating food, water, air and soil [1]. Phenol is considered a toxic chemical compound and can threaten human health because of its hematotoxic and hepatotoxic characteristics [2]. Since phenol is highly soluble in water, it appears as the major pollutant in wastewater and industrial water that has a hazardous influence on human and environmental health [3]. Phenol characterizes a critical environmental problem due to its widespread use, occurrence and toxicity within the ambiance; hence, it is compulsory to improve efficient approaches for its waste control [4]. Among various methods available, biodegradation of phenol is eco-friendly and cost active technique. Many materials of polymeric have been measured by encapsulating various microorganisms as matrices for immobilization such as Ca-alginate beads, agarose cubes, polyacrylamide blocks, k-carrageenan blocks, chitin, bio-ceramic and sponge [5,6,7]. Immobilization protects microorganisms against phenol and can strengthen their respiratory system [8]. The damage of the membrane was decreased in cells grown in immobilized form for extended times conforming to the excess in tolerance and immobilization of the microbes is advantage more than the freely suspended [9,10,11]. Furthermore, a microbial immobilization method is safe for removing toxic pollutants from environments[12] and the consortium of bacteria can be considered very potential in the treatment of phenol[13].

Thus, immobilization of microbial cells has many advantages such as the reuse of biomass and phenol is confirmed as a cheap method for oxidizing the effluents [14]. Unique characteristics of Polyurethane deserve attractiveness as Polyurethane offers rubber elasticity, and resistance to fats, oil and solvents. Polyurethane was found to be applicable in the fields of biotechnological and biochemical as a proper assist for enzyme particle surface, the performance of immobilization by *Rhodococcus* sp F92 on the foam of Polyurethane can be used for the petroleum hydrocarbons bioremediation [15]. The efficiency of immobilization methods of used phenol to degrade many living organisms and no one found regarding *Rhodococcus opacus* 1G but able to live in phenol media with consolations of 0.75 g/L [16]. While the maximum degradation rate of immobilized *Acinetobacter* sp was 0.03125 g/L per hour [17].

The efficiency of xenobiotic remediation by microorganisms remains a main challenge to microbial environmentalists [18]. Among the diverse matrices applied for immobilization Ca-

Mahammed E J. Al-Defiery*1, Gopal Reddy, 2024. Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization. *Journal port Science Research*, 7(special), pp.121-128. https://doi.org/10.36371/port.2024.special.12

j. port. sci. res.
ISSN: 2616-7441 (Online)
ISSN: 2616-6232 (Print)
ISSN: 2616-7220 (USB)
INTERNATIONAL IDENTIFIER FOR SERIALS

Journal port Science Research
Available online www.jport.co
Volume 7, special issue 2024

alginate, polyurethane foam, agar and agarose with microbial degradation could be most effective and may be employed for removing phenols from wastewater. Therefore, the objective of the study is investigate the characterize immobilized bacterium for phenol degradation by *Rhodococcus pyridinivorans*.

2. MATERIALS AND METHODS

2.1. Growth Medium

The mineral salts medium (MSM) contains of 1.25 g/L of yeast extract, 0.35 g/L of K2HPO4, 0.35 g/L of MgCl2.6H2O, 0.2 of Ca(NO3)2, 0.12 g/L of FeCl2 and trace elements (0.2 mg/L of MnSO4. 2H2O, 0.2 mg/L of CuSO4.5H2O, 0.1 mg/L of Na2MoO4 and 0.1 mg/L of ZnSO4.7H2O) with phenol as only carbon source in MSM.

2.2. Inoculum Preparation For Immobilization

One bacterium was isolated from soil samples that showed high phenol degradation by enrichment of phenol degrading and it has been identified as Rhodococcus pyridinivorans GM3 (Figure 1).

A conical flask (250 mL) containing 50 mL MSM (adjusted at pH 8.5) was inoculated utilizing a new slant culture of R. pyridinivorans GM3 and was grown at 200 rpm and 32ºC. After 16 hours as exponentially grown, the culture is harvested by centrifuging for 5 minutes at 4000 rpm and 4ºC. The pellets of cells were washed with sterile normal saline twice and the pellets were suspended in 0.1 M buffer of phosphate at proportion 1:1 (w/v) [19]. The obtained vegetative cells were used directly for immobilization using Ca-alginate, polyurethane foam, agar-agar and agarose entrapment methods.

Immobilization of R. pyridinivorans GM3 for phenol degradation

Alginate entrapment of cells

Calcium chloride and sodium alginate were used to make the alginate beads including the bacterial cell. Sodium alginate solutions on concentrations 2%, 3 % and 4% (w/v). The cell suspension of each 1.0 mL having 45×109 cells of bacteria and alginate slurry was blended for 8 minutes at a percentage of 10% (v/v) to become a uniform mixture. The slurry was pulled into a syringe and then dripped into 0.2 M CaCl2. 2H2O solution at an ice-cold temperature from 5 cm height to form uniform-sized beads and reserved at 4ºC for one hour. The beads were transferred into MSM consisting of 1.5 g/L phenol to study the best concentration of sodium alginate for phenol degradation.

Phenol degradation studies were carried out in triplicates using 50 mL of MSM containing different concentrations of beads 4%, 6% and 8% (v/v) to study the effect of various percentages of Ca-alginate immobilized cells on complete phenol degradation at a concentration of 1.5 g/L phenol during 24 hours of incubation at 32ºC, 200 rpm and pH 8.5, each one mL of alginate-cells mixture product approximately 26-28 beads.

Polyurethane foam entrapment of cells

Polyurethane foam (PUF) of two types of densities i. e 0.018 g/cm3 (PUF1) and 0.03 g/cm3 (PUF 2) and each PUF with two cubes of sizes (1.0 cm ×1.0 cm ×1.0 cm=1.0 cm3) and (0.5 cm ×0.5 cm ×0.5 cm=0.125 cm3) were used for immobilized cells investigation. The PUF was rinsed with distilled water and then desiccated at 30ºC overnight .

A PUF (1.0 cm3 and 0.125 cm3 sizes) after sterilizing at 121ºC for 20 minutes was put in the flask (250 mL) containing 50 mL of MSM. Inoculum of R. pyridinivorans GM3 (2.0 mL) was placed into the flask and incubated at 75 rpm and 32ºC for 6 days. The immobilized cells of PUF were washed with sterile MSM, and then the PUF was moved by sterile forceps. Different concentrations of two cubes of sizes of PUF1 and PUF2 were used at percent 8%, 10%, 12% (v/v) and 10%, 12%, 14% and 16% (v/v) respectively for full degradation of 1.5 g/L of phenol concentration during 24 hours of incubation. Phenol degradation studies were carried out in triplicates using MSM (50 mL) and incubated at 32ºC, 200 rpm and pH 8.5 .

2.3. Agar-Agar Entrapment Of Cells

Agar-agar was dissolved in 90 mL of 0.9 % NaCl solution to get the concentration of 6% agar in a flask and sterilization (less than 6% of agar was not suitable for the experiment as the agar beads are unstable). Ten milliliters of cell suspension was added (10% ratio) into a dissolved agar flask that was maintained at 40ºC and mixed for several seconds without creating foam and then poured into Petri plates (9 cm diameter) to allow for solidification. The agar was cut after solidification into cubes of equal sizes 1.0 cm3 and 0.125 cm3. Then transferred to a sterile 0.2 M buffer of phosphate (pH 7.0) and saved in a refrigerator for one hour. The cubes were rinsed three times with sterile distilled water and transferred into MSM in an Erlenmeyer flask. Different concentrations of agar-agar entrapment of cells with each two cubes size were used at percent 8, 16 and 24% (v/v). Phenol degradation studies were carried out in triplicates using MSM (50 mL) and incubated at 32ºC, 200 rpm and pH 8.5 .

2.4. Agarose Entrapment Of Cells

Agarose was dissolved in 90 mL of 0.9 % NaCl solution to get the concentration of 6% in a flask and sterilization (less than 6% of agarose was not suitable for the experiment as the agar beads are unstable) and sterilized by autoclaving. Ten milliliters of cell suspension was added (10% ratio) into a dissolved agarose flask that was maintained at 40ºC and mixed for several seconds without creating foam and then poured into Petri plates (9 cm diameter) to allow for solidification. The agarose was cut after solidification into cubes of equal sizes 1.0 cm3 and 0.125 cm3. Then transferred to a sterile 0.2 M buffer of phosphate (pH 7.0) and saved in a refrigerator for one hour.

Mahammed E J. Al-Defiery*1, Gopal Reddy, 2024. Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization. *Journal port Science Research*, 7(special), pp.121-128. https://doi.org/10.36371/port.2024.special.12

j. port. sci. res.
ISSN: 2616-7441 (Online)
ISSN: 2616-6232 (Print)
ISSN: 2616-7220 (USB)
INTERNATIONAL IDENTIFIER FOR SERIALS

Journal port Science Research
Available online www.jport.co
Volume 7, special issue 2024

The cubes were rinsed three times with sterile distilled water and transferred into MSM in an Erlenmeyer flask. Different concentrations of agarose entrapment of cells with each two cubes size were used at percent 8, 16 and 24% (v/v). Phenol degradation studies were carried out in triplicates using MSM (50 mL) and incubated at 32ºC, 200 rpm and pH 8.5.

3. RESULTS

3.1. Calcium Alginate

Immobilization of bacterial cells is the most common method being used till now. Further, Ca-alginate gel is inexpensive, versatile and easily available. Immobilized bacterial cells were examined for degradation of phenol.

The concentration of the sodium alginate solution varied from 2% to 4% w/v for the preparation of alginate beads to establish the optimum concentration that has shown encouraging results with consideration of both structural strength and porosity of the beads for phenol degradation and 3% sodium alginate was investigated as the better matrix for immobilization (Figure 2).

Further, experiments were carried out using 3% of w/v of sodium alginate and various concentration of Ca-alginate immobilized cells (v/v). Encapsulated R. pyridinivorans GM3 was used to study phenol degradation at 1.5 g/L within 24 hours of incubation . The concentration of Ca-alginate immobilized cells (v/v) used for phenol degradation was 4, 6 and 8% at a phenol concentration of 1.5 g/L, phenol was completely degraded within 24 hours in the immobilized cell system with a concentration of 8% (v/v) Ca-alginate immobilized R. pyridinivorans GM3 (Figure 3).

3.2. Polyurethane Foam

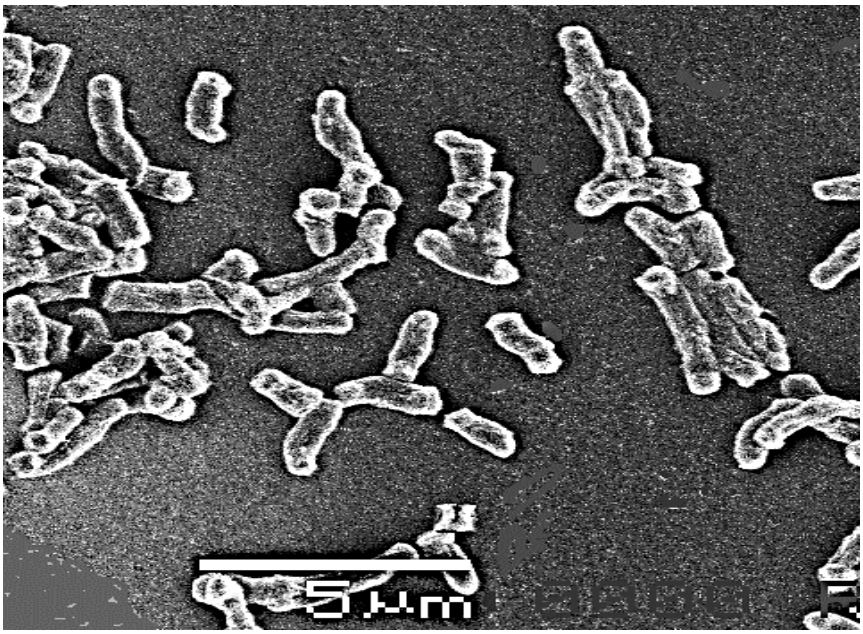
Soft PUF is an insoluble structured entrapment, nontoxic and inexpensive material. *R. pyridinivorans* GM3 was immobilized on PUF which is the most often used method for immobilization. Microorganisms could play an important role when immobilizing cells onto PUF and maintain the ability efficiently for degradation in some aromatic and products of petroleum. *R. pyridinivorans* GM3 immobilized in PUF was used in two types PUF1 and PUF2 and two cubes of size 0.125 and 1.0 cm^3. The results obtained on phenol degradation using immobilized cells in the PUF matrix are present in Figures 4 and 5. The data indicates that there was an incremental increase in phenol degradation with an increase in the PUF concentration (v/v). The degradation of 1.5 g/L phenol was observed within 24 hours with 10% (v/v) of PUF1 or PUF2 immobilized cell at cubes of size 0.125 cm^3 and it was observed that degradation of 1.5 g/L phenol within 24 hours with 14% and 16% (v/v) of PUF1 and PUF2 immobilized cell respectively at cubes of size 1.0 cm^3. Inference from these results, that the phenol degradation by immobilized cells of PUF in batch culture at cubes of size 0.125 cm^3 was more than at size 1.0 cm^3 with PUF1 more than PUF2.

3.3. Agar-Agar and Agarose Entrapment Method

Agar and agarose are natural polymers consisting of polysaccharide linear chains that are cross-related to supply mechanical stability. Small particles of agarose and agar act as porous gel filters or they can separate a mixture of molecules according to their sizes.

Agar and agarose were employed for the immobilization of bacterial cells and were tested several times under batch culture (Figures 6 and 7). The results showed that the phenol (1.5 g/L) degradation within 24 hours was 16% and 24% (v/v) on both agar or agarose at cubes of size 0.125 and 1.0 cm^3 respectively.

Agarose and agar beads were observed to be deformed after 24 hours during incubation at 200 rpm for phenol degradation; therefore, they are not tested in further experiments.



Mahammed E J. Al-Defiery*1, Gopal Reddy, 2024. Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization. *Journal port Science Research*, 7(special), pp.121-128. https://doi.org/10.36371/port.2024.special.12

j. port. sci. res.
ISSN: 2616-7441 (Online)
ISSN: 2616-6232 (Print)
ISSN: 2616-7220 (USB)
INTERNATIONAL IDENTIFIER FOR SERIALS

Journal port Science Research
Available online www.jport.co
Volume 7, special issue 2024

Figure 1*: SEM micrograph of R. pyridinivorans GM3 showing groups of cells*

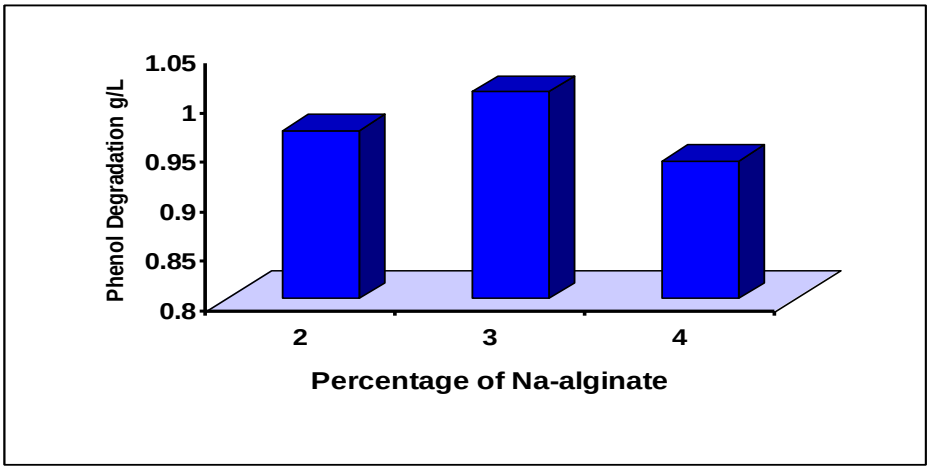


Figure 2: *Effect of different percentages of sodium alginate (w/v) in the preparation of immobilized cell beads on phenol degradation by R. pyridinivorans GM3 after 24 hours of incubation*

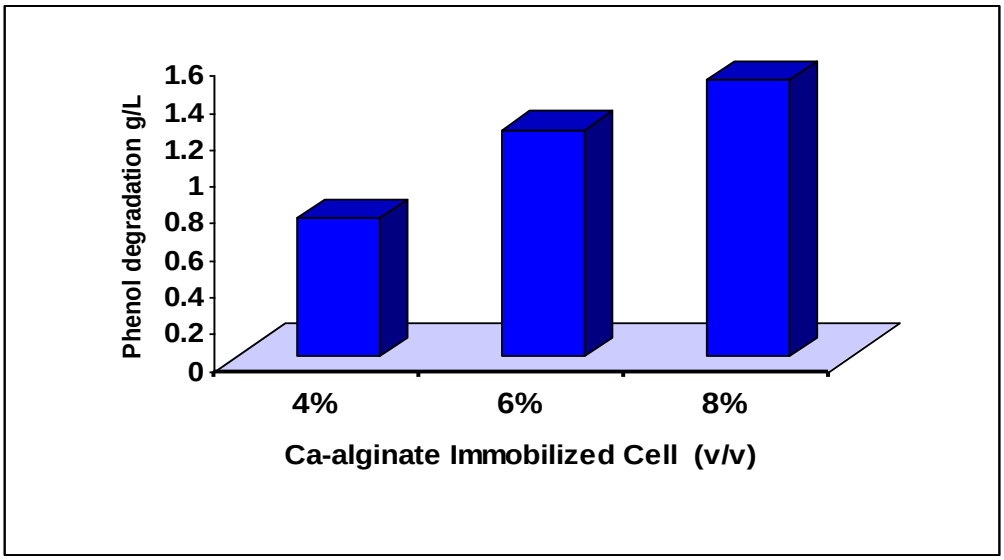


Figure 3: *Effect of different percentages of Ca-alginate immobilized cells (v/v) on phenol degradation by R. pyridinivorans GM3 after 24 hours of incubation.*

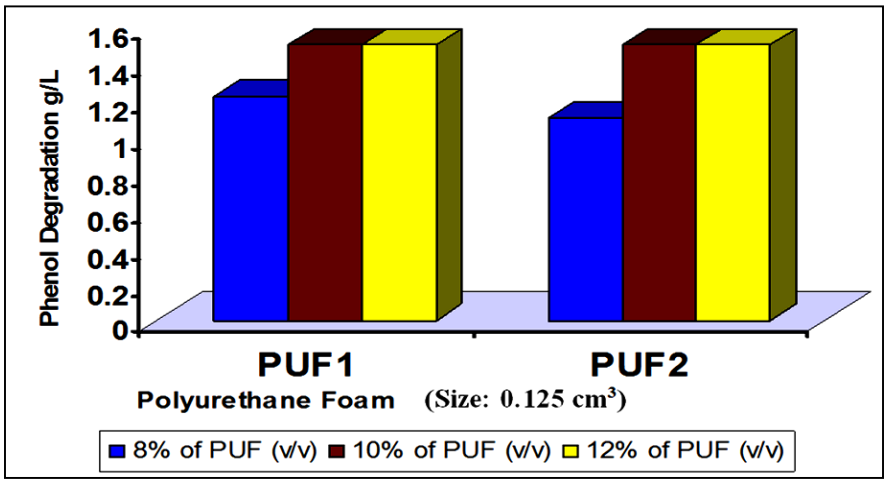


Figure 4: *Effect of different percentages (v/v) of PUF1 and PUF2 immobilized cells (cubes size 0.125 cm3) on phenol degradation by R. pyridinivorans GM3 after 24 hours of incubation*

Mahammed E J. Al-Defiery*1, Gopal Reddy, 2024. Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization. *Journal port Science Research*, 7(special), pp.121-128. https://doi.org/10.36371/port.2024.special.12

j. port. sci. res.
ISSN: 2616-7441 (Online)
ISSN: 2616-6232 (Print)
ISSN: 2616-7220 (USB)
INTERNATIONAL IDENTIFIER FOR SERIALS

Journal port Science Research
Available online www.jport.co
Volume 7, special issue 2024

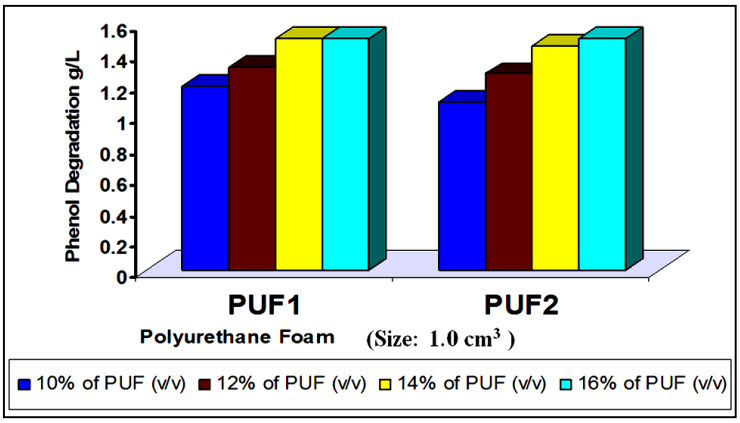


***Figure 5**: Effect of different percentages (v/v) of PUF1 and PUF2 immobilized cells (cubes size 1.0 cm3) on phenol degradation by R. pyridinivorans GM3 after 24 hours of incubation*

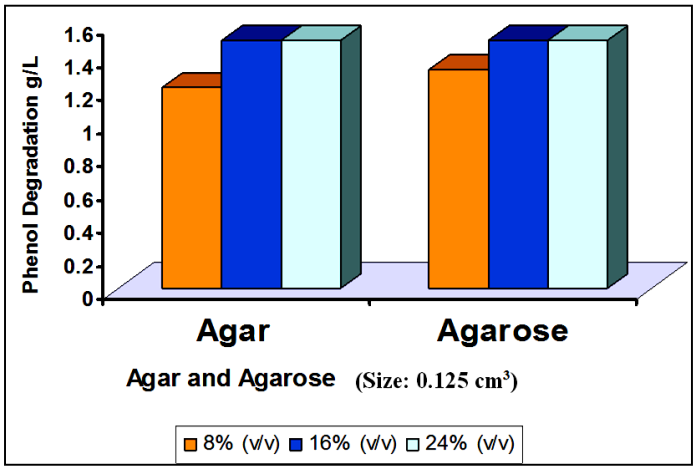


***Figure 6:** Effect of different percentages (v/v) of agar and agarose immobilized cells (cubes size 0.125 cm^3) on phenol degradation by R. pyridinivorans GM3 after 24 hours of incubation.*

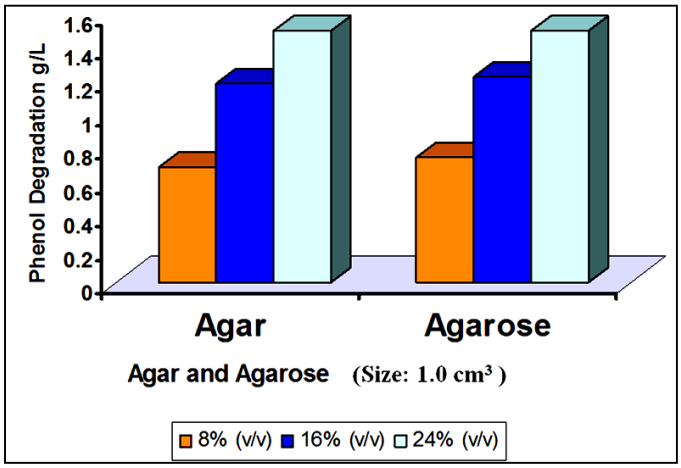


***Figure 7:** Effect of different percentages (v/v) of agar and agarose immobilized cells (cubes size 1.0 cm^3) on phenol degradation by R. pyridinivorans GM3 after 24 hours of incubation*

4. DISCUSSION

The best technique is immobilization which can embed the target microorganisms. Generally, the important features for immobilized cells are high surface area, simple techniques, cheap support materials and low loss of activity during immobilization [20]. As the high immobilization rate put in the experiments noticed higher degradation and major affinity among the hydrophobic these can increase the interaction among both the cells and substrates. Among the different matrices, Ca-alginate, polyurethane foam (PUF), agar and

Mahammed E J. Al-Defiery*1, Gopal Reddy, 2024. Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization. *Journal port Science Research*, 7(special), pp.121-128. https://doi.org/10.36371/port.2024.special.12

j. port. sci. res.
ISSN: 2616-7441 (Online)
ISSN: 2616-6232 (Print)
ISSN: 2616-7220 (USB)
INTERNATIONAL IDENTIFIER FOR SERIALS

Journal port Science Research
Available online www.jport.co
Volume 7, special issue 2024

agarose were used in immobilization studies that offered immobilization cells of the strain GM3 for phenol degradation on several consecutive batches of culture. A typical concentration of 3% w/v of sodium alginate with 0.2 M $CaCl_2$ $2H_2O$ solution to create synthetic Ca-alginate beads was supporting phenol degradation which also emphasizes the structural stability of Ca-alginate beads (Figure 2). The permeability of beads is impacted by the concentration of Na and resulting in much porosity when diluting with Na sodium alginate is used to form the beads, whilst increased sodium alginate lead to the structure stability of beads [21]. Thus, the encapsulated *Pseudomonas putida* in Ca-alginate beads that high mechanical strength produced decreased efficacy for biodegradation of phenol, hence, the best way as the author sought is immobilization as it could close the polluted cells going to the environment [22]. Because the biodegradation was increased several folds by immobilization[23].

The phenol biodegradation by *P. putida* was immobilized up to 0.3 g/L in batch experiments by using pellets of polyvinyl alcohol gel at different conditions in a bubble column bioreactor[24]. Observation of the immobilization study showed that Ca-alginate immobilized *R. pyridinivorans* GM3 was more efficient than PUF, agar and agarose immobilized for phenol degradation (Figures 3, 4, 5, 6 and 7). The beads of alginate and PUF are better than beads of agar in resistance to damage[25]. In general, the beads protect cells against environmental stress and provide a surrounding for cell growth and metabolism and prevent cell contact with media, whereby immobilization protected *Rhodococcus erythropolis* UPV-1 against phenol yielded in a significant improvement of their respiratory activity and a lower lag phase[8]. Besides, the alginate was a nontoxic compound, easily available and cheap, hence it was selected as the matrix for experiments of immobilization [5]. Though, beads of alginate gel should perfectly have chemical stability, high mechanical, controllable properties of swelling, a fine pore size distribution and a defined size of the pore [26]. Similarly, they were observed that operational constancy and permanence of cells immobilized in PUF is importantly better than with the other materials examined, polyurethane is very multipurpose and low cost with this approach, and also PUF was recycled 45 times over 90 days without losing degrading activity of naphthalene [27]. Bioremediation is a choice that proposes the opportunity to reduce various pollutants using the activity of natural biological [28]. However, the main problem still is the remediation of xenobiotics by used cells [18]. Many industries concerned with heath, chemical, energy as well as environmental sector are looking for the diversity of rhodococci organisms. In addition, they utilized bacterial strains of the genus *Rhodococcus* to get fantastic results to rescue the pollution incorporate with these cells [29]. Microbial species and degradation methods are factors that impact the degradation of phenols [30].

Furthermore, without immobilization, the ability of microorganisms in the degradation process will decrease. As *Stenotrophomonas* can degrade phenol concentration of 1.0 g/L in 18 hours [31] while the bacteria *Klebsiella variicola* can degrade 1.0 g/L phenol in the mineral salt medium [32]. In addition, the lag phase length is increase when used high concentrations of the phenol which affects on degradation rate [33]. However, the phenol-degrading ability of *Rhodococcus aetherivorans* has been quantitatively distinguished for high tolerance and assimilation of phenol as well as various hydrocarbons [34]. Nevertheless, the ability of bacteria to degrade phenol varies. *Bacillus cereus* can degrade a concentration of 1.4 g/L for 40 hours [35] while other alginate-immobilized *Pseudarthrobacter phenanthrenivorans* cells degraded 1.0 g/L phenol for 192 hours [36]. Remarkably, the immobilized bacteria have higher phenol degradation compared with free bacteria [37, 38] and immobilization in alginate beads increased the many-fold of degradation and tolerance to the toxic of phenol[39] by adaption of cell immobilized can be more resistant to higher concentrations of phenol [40].

5. CONCLUSION

Nowadays, the earth witness increased pollutants and environmental deterioration, among these pollutants, phenols are extremely toxic even at low concentrations. The study observations exhibited the Ca-alginate immobilized with strain GM3 was more efficient than PUF1, agar and agarose on phenol degradation and the high degradation was on 8% of Ca-alginate. The cubes of size 0.125 cm^3 of PUF1, agar and agarose were degraded maximum phenol at 10%, 16% and 16% (v/v) respectively. The increment in degradation of phenol showed when was increased in the immobilized cells concentration. *R. pyridinivorans* GM3 has the potential for phenol degradation and, hence, can be used for an in situ bioremediation approach by immobilization which can considered as a tool for acceptable cleanup to eliminate phenol.

Acknowledgments

The authors thank the Indian Council for Cultural Relations (ICCR), Govt. of India for providing the fellowship to carry out this research work.

REFERENCES

[1] P. Bhatt, M. S. Kumar, S. Mudliar and T. Chakrabarti. Biodegradation of chlorinated compounds-A Review. Critical Reviews in Environmental Science and Technology. vol. 37, pp. 165–198, 2007.

[2] J. Michałowicz and W. Duda. Phenols–Sources and toxicity. Polish Journal of Environmental Studies. vol. 16, pp. 347–362, 2007.

Mahammed E J. Al-Defiery*1, Gopal Reddy, 2024. Degradation of phenol by Rhodococcus pyridinivorans GM3 immobilization. *Journal port Science Research*, 7(special), pp.121-128. https://doi.org/10.36371/port.2024.special.12

[3] I. S. Almajali, A. Al-Tarawneh, H. Qaralleh, M. Al-limoun, M. M. Al-Sarayrah, M. Alqaraleh, W. A. Rayyan, K. M. Khleifat and S. M Dmour. Biodegradation of Phenol by *Curtobacterium flaccumfaciens*: Optimization of Growth Conditions. Pol. J. Environ. Stud. vol. 30, no. 6, pp. 5435-5442, 2021.

[4] C. I. Nair, K. Jayachandran and S. Shashidhar. Biodegradation of phenol. African Journal of Biotechnology. vol. 7, pp. 4951–4958, 2008.

[5] Y. Fatima, H. Kansal, P. Soni and U. C. Banerjee. Enantioselective reduction of aryl ketones using immobilized cells of *Candida viswanathii*. Process Biochemistry. vol. 42, pp. 1412–1418, 2007.

[6] G. Annadurai, L. Y. Ling and J. F. Lee. Biodegradation of phenol by *Pseudomonas pictorum* on immobilized with chitin. African Journal of Biotechnology. vol. 6, pp. 296–303, 2007.

[7] M. Mailin and R. Firdausi. Immobilization of phenol degrader *Pseudomonas* sp. in repeated batch culture using bioceramic and sponge as support materials. Jurnal Teknologi. vol. 46, pp. 51–59, 2007.

[8] M. B. Prieto, A. Hidalgo, C. Rodríguez-Fernández, J. L. Serra and M. J. Llama. Biodegradation of phenol in synthetic and industrial wastewater by *Rhodococcus erythropolis* UPV-1 immobilized in an air-stirred reactor with clarifier. Applied Microbial Biotechnology. vol. 58, pp. 853–859, 2002.

[9] H. J. Heipieper, H. Keweloh and H. J. Rehm. Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. Applied and Environmental Microbiology. vol. 57, pp. 1213–1217, 1991.

[10] S. S. Mohanty and H. M. Jena Biodegradation of Phenol by Free and Immobilized Cells of a Novel *Pseudomonas* sp. NBM11. Brazilian Journal of Chemical Engineering vol. 34, no. 01, pp. 75 - 84, 2017.

[11] J. Liang, S. Gong, Y. Sun, J. Zhang and J. Zhang. Enhanced degradation of phenol by a novel biomaterial through the immobilization of bacteria on cationic straw. Water Science & Technology. vol. 84, no. 12, pp. 3791- 3798, 2021.

[12] H. D. Du Enhancement of carbofuran degradation by immobilized *Bacillus* sp. strain DT1 Environ. Eng. Res. vol.27, no. 4, pp. 1-8, 2022.

[13] M. Youssef, E. H. El-Shatoury , S. S. Ali and G. E. El-Taweel. Enhancement of phenol degradation by free and immobilized mixed culture of *Providencia stuartii* PL4 and *Pseudomonas aeruginosa* PDM isolated from activated sludge Bioremediation Journal. vol. 23, no. 2: 53-71, 2019.

[14] R. J. Varma and B. G. Gaikwad. 2010. Continuous phenol biodegradation in a simple packed bed bioreactor of calcium alginate-immobilized *Candida tropicalis* (NCIM 3556). World Journal of Microbiology and Biotechnology. vol. 26, pp. 805–809, 2010.

[15] E. Quek, Y. P. Ting and H. M. Tan. 2006. *Rhodococcus* sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products. Bioresource Technology. vol. 97, pp. 32–38, 2006.

[16] I. P. Solyanikova, E. I. Konovalova and L. A. Golovleva. Methylcatechol 1,2-dioxygenase of *Rhodococcus opacus* 6a is a new type of the catechol-cleaving enzyme. Biochemistry (Moscow). vol. 74, pp. 994–1001, 2009.

[17] A. A. Amara and S. R. Salem. Logical and experimental design for phenol degradation using immobilized *Acinetobacter* sp. culture. IIUM Engineering Journal. vol. 11, pp.89–104, 2010.

[18] A. Whiteley and M. Bailey. Bacterial community structure and physiological state within an industrial phenol bioremediation system. Applied and Environmental Microbiology. vol. 66, pp. 2400–2407, 2000.

[19] M. Nagavalli. Production of rifamycin SV using *Amycolatopsis mediterranei* (NCIM 5008). Ph.D. thesis. Department of Microbiology, Osmania University. India, 2009.

[20] J. Tampion and M. D. Tampion. Immobilized Cell: Principles and Applications. The Press Syndicate of the University of Camridge, 1987.

[21] F. O. M. Alonso, O. A. C. Antunes and E. G. Oestreicher. Enantiomerically pure *D*-phenylglycine production using immobilized *Pseudomonas aeruginosa* 10145 in calcium alginate beads. Journal of the Brazilian Chemical Society. vol. 18, pp. 566–571, 2007.

[22] K. Bandhyopadhyay, D. Das and B. R. Maiti. Solid matrix characterization of immobilized *Pseudomonas putida* MTCC 1194 used for phenol degradation. Applied Microbial Biotechnology. vol. 51, pp. 891–895, 1999.

[23] A. G. Ibrahim and L. S. Al-Ghamdi Bioremediation of Phenol by Mutated and Immobilized *Aspergillus* and *Penicillium* Species vol. 11, no. 4, pp. 1903- 1907, 2019.

[24] M. H. El-Naas, S. A. Al-Muhtaseb and S. Makhlouf. Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. Journal of Hazardous Materials. vol. 164, pp. 720–725, 2009.

[25] H. Ghorbannezhad and H. Moghimi and R. A. Taheri Enhanced biodegradation of phenol by magnetically immobilized *Trichosporon cutaneum*. Annals of Microbiology. vol. 68, pp. 485–491, 2018.

[26] B. Thu, O. Smidsrod and G. Skjak-Braek. Alginate gels - Some structure-function correlations relevant to their use as immobilization matrix for cells. In R. H. Wijffels, R. M. Buitelaar, C. Bucke and J. Tramper (eds.), Immobilized Cells: Basics and Applications, Progress in Biotechnology (Vol.:11). Elsevier Science B.V. pp.19–30, 1996.

[27] S. Manohar, C. K. Kim and T. B. Karegoudar. Enhanced degradation of naphthalene by immobilization of *Pseudomonas* sp. strain NGK1 in polyurethane foam. Applied Microbial Biotechnology. vol. 55, pp. 311–316, 2001.

[28] M. Vidali. Bioremediation. An overview. Pure and Applied Chemistry. vol. 73, pp.1163–1172, 2001.

[29] R. van der Geize and L. Dijkhuizen. Harnessing the catabolic diversity of rhodococci for environmental and biotechnological applications. Current Opinion in Microbiology. vol. 7, pp. 255–261, 2004.

[30] L. Zhao, Q. Wu and A. Ma. Biodegradation of Phenolic Contaminants: Current Status and Perspectives. 2017 International Conference on Advanced Environmental Engineering (ICAEE2017) IOP Publishing. IOP Conf. Series: Earth and Environmental Science. vol. 111, pp. 1-5, 2018.

[31] S. Chen and L. Sun. Screening of Efficient Phenol-Degrading Bacteria and Analysis of Their Degradation Characteristics. Sustainability.vol. 15, 6788, pp.1-15, 2023.

[32] S.A. Mahgoub, S.Y.A. Qattan, S.S. Salem, H.M. Abdelbasit, M. Raafat, M.F. Ashkan, D.A. Al-Quwaie, E.A. Motwali, F.S. Alqahtani and H.I. Abd El-Fattah. Characterization and Biodegradation of Phenol by *Pseudomonas aeruginosa* and *Klebsiella variicola* Strains Isolated from Sewage Sludge and Their Effect on Soybean Seeds Germination. Molecules. Vol. 28, 1203, pp. 1-19, 2023.

[33] M E J Al-Defiery and G Reddy. Lag phase and biomass determination of *Rhodococcus pyridinivorans* GM3 for degradation of phenol. IOP Conf. Series: Journal of Physics: Conf. Series.vol. 1003, 012007, pp.1-8, 2018.

[34] T. Nogina, M. Fomina, T. Dumanskaya, L. Zelena, L. Khomenko, S. Mikhalovsky, V. Podgorskyi and G. M. Gadd. A new *Rhodococcus aetherivorans* strain isolated from lubricant-contaminated soil as a prospective phenol-biodegrading agent. Applied Microbiology and Biotechnology. vol. 104, pp.3611–3625, 2020.

[35] J. Li , Y. Jia , J. Zhong , Q. Liu , H. Li , I. Agranovski. Use of calcium alginate/biochar microsphere immobilized bacteria *Bacillus* sp. for removal of phenol in water. Environmental Challenges. Vol. 9, 100599, pp.1-8, 2022.

[36] S. Asimakoula, O. Marinakos, E. Tsagogiannis and A. Koukkou. Phenol Degradation by *Pseudarthrobacter phenanthrenivorans* Sphe3. *Microorganisms*. vol. *11*, no.2, 524: 1-15, 2023.

[37] J. Liang, S. Gong, Y. Sun, J. Zhang and J. Zhang.. Enhanced degradation of phenol by a novel biomaterial through the immobilization of bacteria on cationic straw. Water Science & Technology vol 84, no.12, pp. 3791–3798, 2021.

[38] T. Nogina, M. Fomina, T. Dumanskaya, L. Zelena, L. Khomenko, S. Mikhalovsky, V. Podgorskyi and G. M. Gadd. A new *Rhodococcus aetherivorans* strain isolated from lubricant-contaminated soil as a prospective phenol-biodegrading agent. Applied Microbiology and Biotechnology vol. 104, pp.3611–3625, 2020.

[39] S. S. Mohanty and H. M. Jena. Biodegradation of Phenol By Free And Immobilized Cells of a Novel. *Pseudomonas* sp. NBM11. Brazilian Journal of Chemical Engineering. vol. 34, no. 01, pp. 75 - 84, 2017.

[40] N. C. G. eSilva , A. C. Macedo , Á. D. T. Pinheiro , M. V. P. Rocha. Phenol biodegradation by *Candida tropicalis* ATCC 750 immobilized on cashew apple bagasse. Journal of Environmental Chemical Engineering. vol. 7, no. 3, pp. 103076-103085, 2019.