Available online at http://www.journalijdr.com

ISSN: 2230-9926

International Journal of Development Research
Vol. 4, Issue, 7, pp. 1439-1442, July, 2014

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

PHENOL DEGRADATION BY IMMOBILIZED CELLS OF P*SEUDOMONAS AERUGINOSA* MTCC 4996

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ARTICLE INFO

Article History:
Received 07th April, 2014
Received in revised form
22nd May, 2014
Accepted 11th June, 2014
Published online 20th July, 2014

Key words:
Phenol degradation,
Immobilization,
Pseudomonas aeruginosa
MTCC 4996

ABSTRACT

A newly isolated *Pseudomonas aeruginosa* MTCC 4996 capable of degrading phenol *Pseudomonas aeruginosa* MTCC 4996 cells were immobilized with six different matrices. All the immobilized matrices showed faster degradation than free cells. Among the matrices, PUF immobilized cells showed maximum degradation followed by alginate and k-carrageenan, agarose, polyacrylamide and hen egg white. *P. aeuriginosa* MTCC 4996 cells immobilized with PUF were showed 100% degradation up to 1400 mg/l within 750 min. PUF immobilized non-regenerated and regenerated cells showed phenol degradation up to 150 days at constant flow rate in packed bed reactor. Regenerated cells showed 100% efficiency up to 120th days. Whereas, non-regenerated cells showed 100% efficiency of phenol degradation only up to 75 days. Then onwards, the efficiency was decreased considerably.

INTRODUCTION

Phenol is an aromatic compound consists of a benzene ring (a six-membered aromatic carbon) attached with a hydroxyl group (-OH), unlike single chain carbon molecule. It is highly stable and difficult to degrade in nature (Autenrieth *et al*., 1991). The toxicity of phenol has been widely documented and there is a great concern over the disastrous effects upon both human being and the environment. The effects on aquatic life are destructive at low concentrations and for human beings just 1 g is lethal (Bond and Straub, 1974). For human beings, the allowed exposure is 20 mg/day for people working with phenol (Bond and Straub, 1974). The presence of phenols in drinking and irrigation water represents a serious hazard for humans, animals, plants and microorganisms (Salonen *et al*., 1989; Sharma *et al*., 1997). Phenol is toxic to fish and has been lethal at concentrations of 5-25 ppm while concentrations as low as 0.1 ppm in surrounding water can taint the taste of fish (Wallace, 1991). Phenol in wastewater is highly toxic for animals and plants (Bandhyopadhyay *et al*., 1999), although effluent can be diluted and then discharged in to the waterways, this approach is not feasible as the chemical toxicity at 5-25 mg/l is hazardous.

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The need to remove these contaminations has led to the development of new technologies that emphasize the detoxification and destruction of the pollutants rather than the conventional approach of disposal. Different methods of treatment are available for reduction of phenol content in wastewater with different initial concentration. Klein and Lee (1978) have indicated the probable technologies for the treatment of wastewater containing phenol, which include chlorination, ozonation, adsorption, solvent extraction, membrane process, coagulation, flocculation and biological treatment. But physicochemical methods of the treatment of phenolic wastewater have the inherent drawbacks due to the tendency of the formation of secondary toxic materials. Moreover, the physico-chemical treatment processes have proven to be costly. Thus, biological method of treatment has turned out to be a favorable alternative for phenol degradation. Biodegradation means the biological transformation of an organic pollutant to another form (Grady, 1985). It is a cost-effective alternative to conventional disposal methods and is a new technology, which emphasizes the detoxification and destruction of pollutants by acclimatized microorganisms (Baker and Herson, 1994; Hardman *et al*., 1993). Many methods for the immobilization of bacterial cells such as entrapment, covalent binding absorption of cells and immobilization through cross-linking polymers are currently being used. However, immobilization by means of entrapment

has gained much importance. Immobilized cells are being used or investigated for many different applications such as production of pharmaceuticals and steroid transformations, biosensors, commodity and specialty chemicals, ethanol and gaseous fuels, food and beverages and waste treatment, etc., (Scott, 1987). One of the cheapest possible solutions to resolve phenol contamination problem is bioremediation using immobilized microbial cells. In the present investigation we used newly isolated strain *P. aeuriginosa* MTCC 4996 for phenol degradation. Phenol degradation compared with free cells and different immobilized cells in packed bed reactor and degradation of phenol by non-regenerated and regenerated polyurethane foam immobilized cells.

MATERIALS AND METHODS

Bacterial strain and culture media

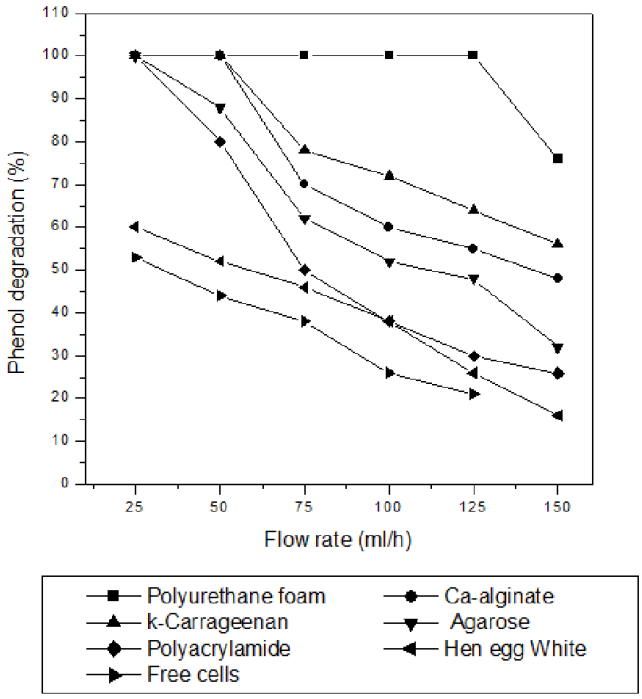
The phenol degrading *Pseudomonas aeuriginosa* MTCC 4996 used in this study was isolated by enrichment technique on phenol (Kotresha, 2005). This culture is deposited in the Institute of Microbial Technology (IMTECH), Chandigarh, India. The media used for phenol degradation studies by *P. aeuriginosa* MTCC 4996 were a mineral salts medium (MSM) contained (g/l) K_2HPO_4 6.3, KH_2PO_4, 1.82, NH_4SO_4 0.1, $MnSO_4$ 0.1, $CaCl_2$ 0.1, $MgSO_4$ 0.1, and Na_2MO_4 0.006. The minimal mineral salts medium (MMSM) contained (g/l) K_2HPO_4 0.15, MgSO4 0.20, KNO_3 1.00, $FeCl_3$ 0.05, and $CaCl_2$ 0.20. The pH of the medium was adjusted to 7.0. Culture media were sterilized at 121^{0}C for 20 min. Phenol was added after sterilization.

Immobilization of *P. aeuriginosa* MTCC 4996

P. aeuriginosa MTCC 4996 was grown in MSM medium containing phenol as a sole source of carbon and energy. The cells were harvested during the exponential growth phase by centrifugation at 5,000 rpm for 10 minutes at 4^{0}C and then immobilized in calcium alginate, polyurethane foam, k-carrageenan, polyacrylamide, agarose and hen egg white. The alginate immobilization was performed according to the method given by Chang *et al*., (2001). The polyurethane foam immobilization was carried out according to the method of Hall and Rao (1989). The k-carrageenan immobilization procedure described by Cassidy *et al.,* (1997) was used. The polyacrylamide immobilization procedure was carried out according to the methods of D'Souza and Nadkarni (1980a). Agarose immobilized procedure described by Joshi and D'Souza (1999) was used. The hen egg white immobilization was carried out using the method given by D'Souza *et al*., (1985).

Design of bioreactor for continuous treatment

A cylindrical glass column (4 × 35 cm, volume 450 ml) with inlet and outlet facility was used. The bottom of the column was packed with a circular foam pad (4 cm diameter) followed by a porous glass frit. Then the reactor was packed with the respective immobilized cell matrix to a height of 25 cm. The reactor was attached to a reservoir containing phenol in the respective medium. The medium was fed into the column continuously with the help of peristaltic pump (Machines PP10-4C) through a side arm present near the bottom of the column. During the experiments, the dynamic flow of oxygen was maintained at 1 bar (10^5 Pa) throughout the entire system, through the bottom of the column. The phenol was continuously removed from the side arm situated just above the packed bed. The schematic view of continuous flow reactor is depicted in figure 1.



The detention time of degradation was calculated using the following formula:

$$\text{Detention time} = \frac{\text{Vide volume}}{\text{Flow rate (ml/h)}} \times 100$$

Continuous degradation of phenol

The continuous degradation of phenol by free and respective immobilized cells was carried out in a continuous flow reactor. The degradation process was carried out by the continuous supply of sterile MMSM containing 200 mg/l phenol. For continues phenol degradation by polyurethane foam immobilized cells, the continuous supply of sterile MSM containing different concentration of phenol ranging from 100 mg/l to 1600 mg/l was used. The polyurethane foam immobilized cells were regenerated with the continuous supply of nutrient broth containing phenol at an interval of 30 days. The phenol degradation (600 mg/l) efficiency of non-regenerated and regenerated cells was measured over a period of 150 days.

Analytical method

The phenol concentration in the sample was determined by using 4-aminoantipyrine method (Folsom *et al*., 1990). 10 ml of reaction mixture containing 9.8 ml of distilled water, 100 µl of sample, and 25 µl of 2% 4-aminoantipyrene and 50 µl of 2 M ammonia were mixed well and added 25 µl of 8% potassium hexacyanoferrate (III). The absorbance at 500 nm was measured and compared with phenol standards.

RESULTS AND DISCUSSION

P. aeuriginosa MTCC 4996 cells immobilized with six different matrices showed a significant increase in the rate of phenol degradation as compared to free cells (Figure.1). The cells immobilized with PUF showed 100% phenol degradation up to 125 ml/h flow rate. Similarly, Alginate and k-carrageenan showed 100% degradation up to 50 ml/h flow rate and agarose and polyacrlyamide showed 100% degradation only at 25 ml/h flow rate. No 100% degradation was observed in hen egg white and with free cells. *P. aeuriginosa* MTCC 4996 cells immobilized with PUF were tested for their phenol degradability against different concentrations of phenol from 100 to 1600 mg/l. 100% degradation was observed up to 1400 mg/l within 750 min. As the phenol concentration increased, the time required was found increasing (Figure 2).

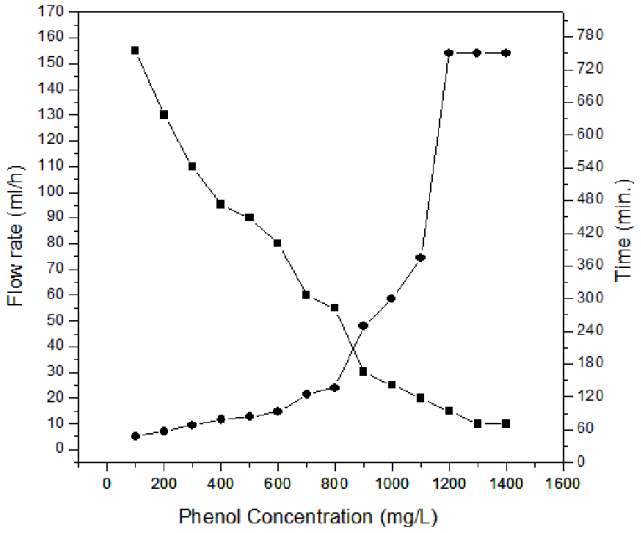


Figure 2. Continuous degradation of phenol by polyurethane foam immobilized cells of *P. aeuriginosa* MTCC 4996 in packed bed reactor

PUF immobilized non-regenerated and regenerated *P. aeuriginosa* MTCC 4996 cells were tested for their phenol degradability up to 150 days at constant flow rate and phenol concentration in packed bed reactor. Non-regenerated cells showed 100% efficiency of phenol degradation only up to 75 days. Then onwards, the efficiency was decreased considerably. On 150th day, only 28.59% degradation was recorded. Whereas, regenerated cells showed 100% efficiency up to 120th day. Then onwards, the rate of degradation gradually decreased (Figure 3). Chung *et al*. (2003) reported phenol degradation by *Pseudomonas putida* CCRC14365 and cell growth kinetics compared between the free and Ca-alginate gel-immobilized systems. Free cells could degrade phenol up to 600 mg/l, whereas, immobilized cells could tolerate a higher-level up to 1000 mg/l. Similarly, Beshay *et al*. (2002) reported *Acinetobacter* sp. strain W-17, immobilized on porous sintered glass degraded 500 mg phenol in 40 h, but free cells required 120 h. Godjevargova *et al*. (1998) immobilized *Trichosporon cutaneum* R57 cells covalently on polyamide granules. The effectiveness of the chemically immobilized cells for phenol degradation was then determined and compared with free cells. Both free and immobilized cells degraded phenol up to 1.0 g/l. The number of cycles of effective phenol degradation by immobilized cells was studied. Immobilized cells are gaining considerable importance in various fields (D'souza, 1989). One of the major uses of this technology is for environmental applications (Cassidy *et al*., 1996). Cells can be immobilized either in a viable or a nonviable form. Immobilized nonviable biomass has gained considerable importance in the bioremediation of heavy metal wastes through biosorption (Veglio and Beolchini, 1997).

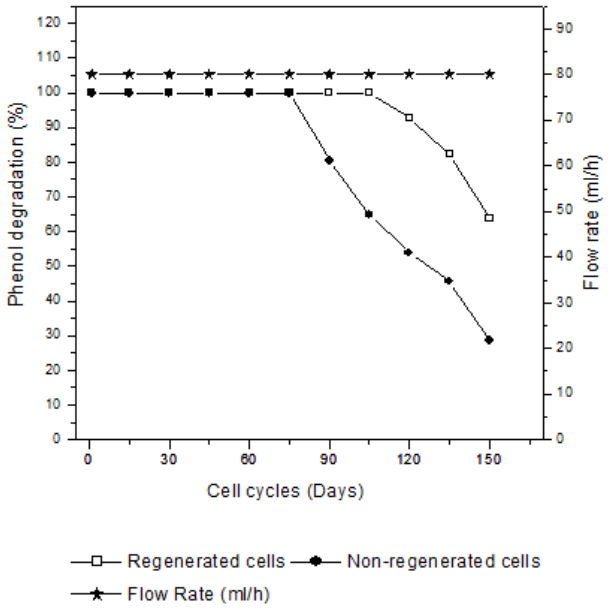


Figure 3. Continuous degradation of phenol in regenerated and non-regenerated polyurathene foam immobilized *P. aeuriginosa* MTCC 4996 cells in packed bed reactor

On the other hand immobilized viable cells can act as self-proliferating controlled catalytic biomass for complex chemical conversions in fermentation as well as in the biodegradation of organic waste. Immobilized viable cells have a number of advantages over free cells for such applications. Immobilization imparts more operational flexibility due to the fact that it prevents biomass washout in continuous flow reactors, allows the use of high cells densities than those obtainable with free cell systems, facilitates the easy separation of biomass from the treated effluents and offers the potential for improving waste water treatment and solves the problems associated with solid liquid separation in setting tank (D'souza, 1989; Cassidy *et al*., 1996). In this study, newly isolated *P. aeuriginosa* MTCC 4996 immobilized with alginate and k-carrageenan, agarose, polyacrylamide and hen egg white shows great potential of phenol degradation compared to free cells. Polyurethane foam immobilized cells showed highest phenol degradation.

Acknowledgment

The authors would like to express their gratitude to Head of the department, Dept. of Botany, Gulbarga University, Gulbarga for providing necessary facility and support to carry out the research work.

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