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Effect Temperature and Cell Carrier Loading on Phenol Degradation using Rhodococcus Erythropolis

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Abstract:
In the present study phenol degradation using *Rhodococcus erythropolis* (NCIM 5228) strain was assessed both in suspended and immobilized form. Factors affecting phenol degradation (substrate concentration, temperature and cell carrier loading) was studied. *Rhodococcus erythropolis* (NCIM 5228) was considered for degradation of phenol up to 600 ppm both in suspended and immobilized form. Phenol degradation under suspended cell system was found least dominant over immobilized cells indicated adsorbed cells on support possess high rate of degradation than suspended cells. Percentage degradation of various substrate phenol concentrations using suspended cell was found 92.4%, which was further increased up to 96% using immobilized cells. Maximum activity was observed both in suspended and immobilized cells at 30 ºC temperature and increasing the carrier loading resulted decrease in the period of degradation with efficient degradation above 99% using immobilized cell system compare to free cell system. Carrier load used for phenol degradation with *Rhodococcus erythropolis* (NCIM 5228) found preferable on the considered parameters indicated its efficiency in phenol contaminated wastewater using such combinations.

Key words: *Rhodococcus erythropolis*, degradation, immobilization, phenol degradation.

1. INTRODUCTION

The release of domestic wastes and wastes generated from extensive industrialization and urbanization due to the establishment of various industries viz. oil refineries, pharmaceutical, coal conversion and food industries etc consists of the major toxic organic pollutants such as phenol [1, 2]. Discharging phenol without its prior treatment may cause serious health and environmental problems such as eye irritation, mucous membranes, and respiratory tract if exposed directly. Phenol has its effect on flora and fauna, animal, aquatic life and humans even at very low concentration [3, 4]. Based on the recognition of its toxicity, US Environmental protection agency has considered it to be a priority organic pollutant (EPA 2014). The maximum permissible concentration decided by Central Pollution Control Board (CPCB) for effluent discharge into water bodies is 1 ppm (The Environmental Protection Rules, 1986a); similarly concentration set by World Health Organization (WHO) in drinking water is 1µg/l (WHO 2011). In order to meet the permissible limit and standards for discharge of phenol in water bodies by regulatory authorities, it is necessary to treat phenol. Various methods are available for degradation of phenol. These are physical, chemical and biological methods [6]. Physico-chemical methods include chlorination, extraction, incineration, adsorption, ozonation, biological oxidation and electrochemical methods, etc. Although, these methods are widely used for phenol removal but beside it consist of few limitations viz., formation of secondary toxic pollutant, uneconomical, high energy requirements, etc. Among these methods biological methods are considered to be more advantageous based on its regulation under ambient condition and complete mineralization of phenol, without the use or release of toxic chemicals [1, 7]. It is the process of deterioration of organic pollutants with high rate of toxicity into simplest elements using microorganisms. It is well known that a microorganism can able to degrade naturally occurring organic matter and utilizing the same as substrate or a food source, this phenomenon is known as principle of microbial infallibility (Alexander, 1965). Immobilization is considered as an efficient technique in engineering system towards cell biomass utilization [8]. Biodegradation with immobilized cells can reduce the effect caused due to substrate inhibition. Therefore, such methods are distinctive from biodegradation using suspended cells based on the presence of a material support or a carrier to overcome inhibition. Benefits associated with immobilization with respect to biodegradation are to avail the potential stability, reprocessing of cells and continuity. It is significant to consider the enhancement parameters for biodegradability on the basis of conditions relevant to it. Therefore the dynamics to be optimized for complete degradation comprises of temperature, pH, concentration, oxygen content (Paralese*t al*. 2002, Alexander 1985, and Lappin *et al.* 1985) etc. Hence, the logic behind the present study was to investigate the capability of *Rhodococcus erythropolis* (NCIM 5228) bacteria in the degradation of phenol under different conditions such as temperature, concentration, cell carrier loading both in free and immobilized form and to compare the same.

2. MATERIALS AND METHODS

2.1. Microorganism, growth and maintenance

Rhodococcus erythropolis (NCIM 5228) used in the present study was obtained in agar slant from National Collection of Industrial Microorganisms (NCIM), Pune, India. Nutrient broth

weighing 1.3 g was added to 100 ml of distilled water and autoclaved at 120°C, 1.5 Kg/cm² gauge pressure. Initial culture of *Rhodococcus erythropolis* (NCIM 5228) was added to it for suspension of culture. Meanwhile nutrient agar was prepared using agar addition of 2.1 g to above said procedure of nutrient broth and poured in a test tube and petriplates. Nutrient agar in test tubes was kept in slant position until solidification. Using sterile loop, a loopful of the suspension streaked on it and incubated at 30°C for 24 hrs. The slants prepared were covered with aluminum foil and refrigerated for further usage. The bacterial suspended culture was later grown in phenol containing liquid medium where 90 ml of nutrient broth in 250 ml conical flask was sterilized at 120 ºC and 1.5 kg/cm² gauge pressure and cooled down to room temperature. Further it was transferred in laminar flow chamber where 10 milliliter of 1000 ppm phenol was added to make the concentration up to 100 ppm in 100 ml volume of the medium. Later inoculation of the medium with freshly prepared sub cultured slant was continued on addition of two loopful organisms from it. Inoculated culture was therefore subjected to an incubation period for 24 h in an orbital shaker at 28 ºC with 120 rpm speed. This was considered as the primary culture.

2.2. Inoculum and culture medium

Nutrient broth (HIMEDIA Ltd., Mumbai, India) was used for bacterial growth. Phenol was added as the sole carbon source and was used as the inoculum for phenol degradation experiments.

2.3. Cell acclimatization and immobilization

Acclimatization and immobilization procedure was carried out according to the methodology presented by Veena et al. [9]. The cells were acclimatized up to 600 ppm of phenol concentration and immobilized on plastic support. Experiment on free and immobilized cells on plastic was carried out further to compare the efficiency of immobilized cells over free cells.

2.4. Phenol degradation studies

Biodegradation experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml of nutrient medium having phenol concentration ranging from 100 ppm to 600 ppm. After each experiment the sample was withdrawn at regular time intervals, centrifuged (10,000 rpm for 15minutes) and analyzed for residual phenol concentration. Experiment was carried out until the concentration of phenol remained constant. Effect of different conditions such as substrate concentration, temperature and carrier loading was studied on phenol degradation using suspended and immobilized cells.

2.5. Analytical procedure

The analysis on phenol concentration was carried out using spectrophotometer at wavelength of 510 nm by using 4-aminoantipyrene method as described by APHA [10], where rapid condensation of phenol on addition of 4- aminoantipyrene was carried out. Followed by it the oxidation with potassium ferricyanide under alkaline conditions resulted in the formation of red color antipyrine dye.

3. RESULTS AND DISCUSSION

3.1. Effect of influent phenol concentration on phenol degradation

The phenol degradation was studied with free cells as well with immobilized cells for influent phenol concentrations of 200, 300, 400 and 600 ppm and under the operating conditions of 30 ºC temperature, 160 rpm. When the experiments were conducted with immobilized cells same conditions were maintained with 5 g of immobilized cells added to the flask instead of free cells. The variations in effluent phenol concentration as a function of time for different influent phenol concentrations are presented (with free and immobilized cells) in Fig. 1. In the absence of any other carbon and energy source, phenol is utilized as sole source of carbon and energy for its growth. Growth in a medium corresponds to degradation of phenol but could diminish due to inhibitory effects. As observed in Fig.1 the degradation of phenol was nearly 92.4% for all influent phenol concentrations under consideration when free cells used and the percentage was increased to 96% when immobilized cells were used for the degradation of phenol. The rate of degradation is higher with immobilized form than in suspended form due to the protection of cells from harsh environment by exopolymers in the form of protein, carbohydrate and humic substance produced by microbial cells [9]. A significant effect of concentration was observed on increase of substrate phenol concentrations. A percentage decrease on phenol degradation was observed with increase in concentration from 200 to 600 ppm using suspended and immobilized cells. It was observed that percentage degradation upto 300 ppm both in suspended and immobilized cells was 93% and 97.6%, but further increase in concentration upto 600 ppm decreased the rate by 92.4% and 96% both in suspended and immobilized cell system. This resulted in phenol degradation under suspended cells appeared to be least dominant over immobilized cells indicated adsorbed cells on support possess high rate of degradation than suspended cells [11].

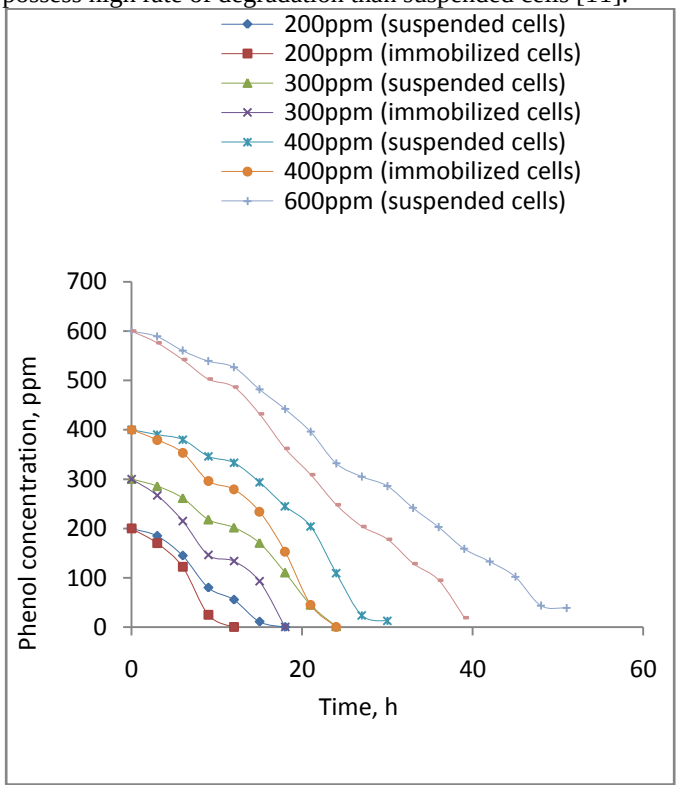

Figure.1. Phenol degradation at different influent phenol concentrations.

3.2 Effect of temperature on phenol degradation

Phenol degradation at different temperatures of 27, 30 and 33 ºC for the concentrations of 200, 400 and 600 ppm was studied by keeping other parameters constant. The plot on effect of temperature in terms of percentage degradation against various

concentrations using suspended and immobilized cells at different temperatures is presented in Fig. 2. Increased rate of degradation corresponds to increased cell growth rate [11]. Therefore the result observed was found maximum at 30 °C among the considered temperatures for phenol degradation indicated maximum metabolic activity for cell growth. The percentage degradation for various substrate concentrations was found to be upto 92.4% using suspended cell and increased upto 96% by immobilized cells at 30 ºC. Significant effect of temperature on phenol degradation was observed on increase or decrease of temperatures. The percentage degradation at 33 °C and 27 ºC temperatures was found 90% and 89% using suspended cells, which was further increased upto 94% and 93% with immobilized cell system. The result indicates percentage degradation for various phenol concentrations under different temperatures have little differences among but larger difference was obtained at 30°C in terms of their growth as observed before [12]. The complete phenol degradation at 30 ºC using immobilized cells was found after 39 h whereas it was observed 42 h and 48 h for 33 ºC and 27 ºC temperatures respectively. Phenol degradation using free cell system at 30ºC, 33ºC and 27ºC was found to be 51 h, 54 h and 57 h respectively. This indicates time consumed in the metabolic growth and degradation was comparatively less using immobilized cells than in suspended cell system.

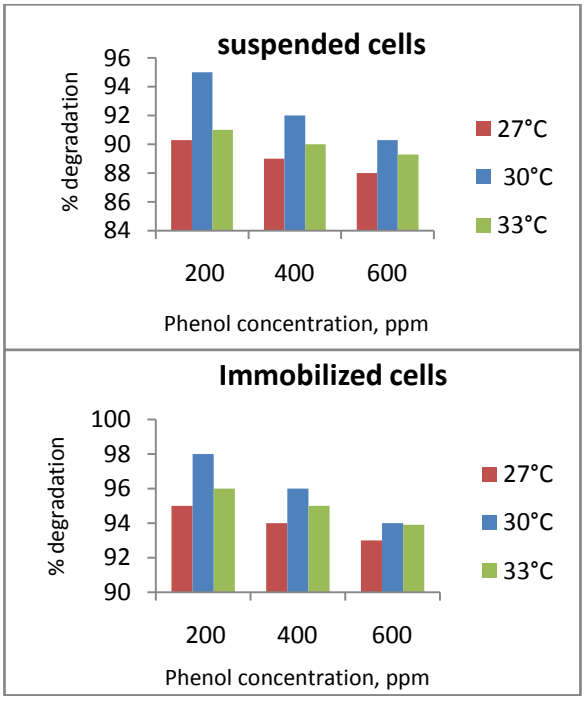

Figure.2. Effect of temperature on phenol degradation using suspended and immobilized cells with different temperature viz. 27 ºC, 30 ºC and 33 ºC

3.3 Effect of cell carrier loading on phenol degradation

Phenol degradation was studied with increased cell carrier loading of 5 g, 10 g and 20 g with other parameters constants. The plot on effect of cell carrier loading in terms of percentage degradation against phenol concentration is depicted in Fig. 3. It was observed that on increase of cell carrier loading degradation time was reduced indicating influence of cell carrier load on the rate of degradation. This signifies that on increase of carrier load surface area for bacterial growth in the form of biofilm increases and leads to increase in inoculum size. Therefore increased consumption of substrate corresponds to rapid growth with decrease in degradation time. It was found that on increase of cell carrier loading for all the phenol concentration has increased the average percentage degradation from 96% with 5 g to 97.3% with 10 g and further to 99.9% using 20 g of cell carrier loading. Also, time taken for degradation was significantly reduced from 39 h with 5 g to 27 h with 10 g and later 24 h using 20 g of carrier loading for all influent phenol concentrations. The increased efficiency on consideration of various loading is attributed to biochemical reactions on availability of substrate and oxygen leading to removal of phenol thereby increasing the percentage of degradation [13]. It was found that percentage degradation with cell carrier loading was found to be higher than free cells without loading. observed percentage degradation with free cells was only 92.4% but was enhanced to 99.9% with a carrier loading indicates effect of carrier loading on phenol degradation using immobilized system compare to suspended cell systems. However, variation in percentage degradation at different loading is attributed by the increase in mass transfer due to particle movement leading highest metabolic activity with increase of surface area followed by degradation of phenol.

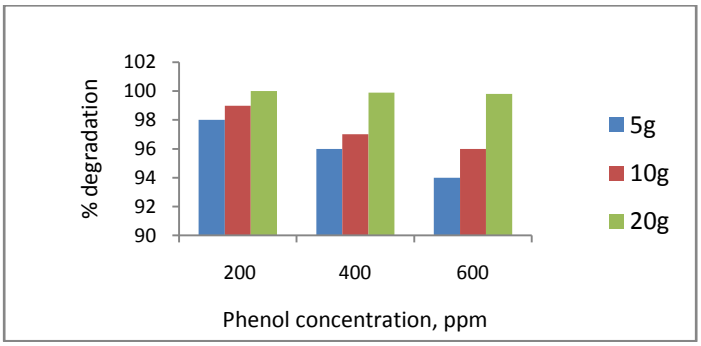

Figure. 3. Effect of cell carrier loading on phenol degradation for various concentrations with different carrier loads of 5 g, 10g and 20 g.

4. CONCLUSION

Rhodococcus erythropolis found to be capable in degradation of phenol both in suspended and immobilized form. Maximum degradation was obtained in immobilized system at 30 ºC temperature and found 99.9% on increase of cell carrier load whereas percentage degradation using free cell was only 92.4% indicates future applications of immobilized systems in wastewater treatment.

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