

Optimization of Parameters for Phenol Degradation by *Rhodococcus* UKM-P in Shake Flask Culture

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Abstract - The ability of the bacterium, *Rhodococcus* UKM-P, to degrade phenol was studied in shake flask culture. The effect of the cultivation parameters (temperature, pH, type and concentration of nitrogen source and salt concentration) on growth of the bacterium and phenol degradation was evaluated. The highest cell growth and the amount of phenol degraded (0.5 g/L) were observed in optimized cultivation conditions (30°C and initial pH 7.5) after 21 h. The culture medium supplied with 0.4 g/L ammonium sulphate gave the highest amount of phenol degraded (0.5 g/L). NaCl at a concentration of 0.1 g/L was required to enhance cell growth and phenol degradation. Results from this study showed that *Rhodococcus* UKM-P has potential to be used in bioremediation of wastewater containing phenol.

Keyword: Degradation, bioremediation, optimization, phenol degradation, *Rhodococcus* UKM-P

I. INTRODUCTION

The genus *Rhodococcus* is Gram-positive aerobic bacteria, and is closely related to the other mycolic acid containing genera: *Nocardia*, *Corynebacterium* and *Mycobacterium* [3, 8].

Recent studies on the metabolic activities of *Rhodococci* indicated that this bacterium may be exploited in various industrial applications such as pharmaceutical and environmental biotechnology. *Rhodococcus* displays a

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remarkably versatile capacity to mineralize aliphatic and aromatic hydrocarbons such as phenol, oxygenates, halogenated compounds, nitrile and various herbicides [9, 12]. Phenols released into the environment are of high concern because of their potential toxicity [5]. Various attempts have been made to degrade phenol and other aromatic compounds by free cells [4] and immobilized cells [6] of microorganisms.

Phenols are hydroxy compounds of aromatic hydrocarbons. Phenol derivatives are widely used as raw materials in many industries such as petrochemical, petroleum refineries, chemical and pharmaceutical. It is also widely used in pulp and paper mills, coking operations, coal refining, tannery and foundries. Phenolic compounds are among the most frequently found pollutants in rivers, industrial effluents, and landfill runoff waters. These compounds are toxic either by ingestion or by contact or inhalation even at low concentrations. Acute exposure of phenol causes central nervous system disorders, which leads to collapse and coma. Muscular convulsions with significant reduction in body temperature are also noted due to phenol toxicity, and this is known hypothermia. Renal damage and salivation may be induced by continuous exposure to phenol [10].

United State Environmental Protection Agency (USEPA) has set a water purification standard, in which, surface water must contains less than 1.0 µg/L phenol [7]. In this regard, industrial effluents containing phenols require proper treatment prior to discharge into the environments. Conventional methods such as solvent extraction, activated carbon adsorption, and chemical oxidation often suffer from serious drawbacks including high cost and formation of hazardous byproducts. Among various methods available, biodegradation is environmental friendly and cost effective method. Biological treatment of phenol has therefore been an increasingly important process in pollution prevention [7]. The objective of the present study was to evaluate the ability of *Rhodococcus* UKM-P to degrade phenol. The effect of various cultivation parameters such as temperature, pH, type and concentration of nitrogen source on growth of *Rhodococcus* UKM-P and phenol degradation was investigated.

II. MATERIALS AND METHODS

2.1 Microorganism and Inoculum Preparation

The bacterium, *Rhodococcus ruber* (also known as *Rhodococcus* UKM-P) was used throughout this study. This bacterium was isolated from a petroleum contaminated soil at Port Dickson, Negeri Sembilan, Malaysia and maintained at the UNISEL Culture Collection Centre, Selangor, Malaysia.

The bacterium from the stock culture was grown in nutrient broth for 24 h and the culture was used as a standard inoculum for all cultivation and degradation experiments

2.2 Cultivation and Phenol degradation experiments

The bacterium was grown in the minimal salt medium containing 500 mg/L phenol. Liquid mineral salt medium (MSM) consisted of (g/L): K_2HPO_4 , 0.4; KH_2PO_4 , 0.2; NaCl, 1; $MgSO_4$ 0.1; $MnSO_4$ 0.01; $FeSO_4 \cdot H_2O$, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $(NH_4)_2SO_4$, 0.4; and phenol, 0.5 was used in all cultivation and phenol degradation experiments. The initial pH value of the medium is adjusted to 7.5 using 30% NaOH prior to autoclaving. Phenol was sterilized separately by filtration using 0.2 μm regenerated cellulose membrane filter and added to the sterilized medium after cooled down to room temperature [2]. The medium (100 mL) in 250 mL shake flask was inoculated with 10% (v/v) inoculum to initiate the cultivation and degradation of phenol. The flask was incubated at 30°C on a rotary shaker, agitated at 160 rpm.

The variables varied for the cultivation experiments include temperature, pH, nitrogen source, ammonium sulphate concentration and NaCl concentration. During the cultivation, 10 mL of culture samples were withdrawn at different time intervals for analysis.

2.3 Analytical Procedures

The optical density of the culture was measured at 680 nm using a spectrophotometer. Cell concentration in term of dry cell weight was determined by filtration and oven dried method [13]. The known volume of culture sample was filtered through a known weight of dried membrane filter with the pore size of 0.25 μm using vacuum pump. The membrane filter with the bacterial cells were at 80°C for at least 24 h, until a constant weight was achieved.

Phenol concentration in the culture was determined by a colorimetric method based on rapid condensation with 4-aminoantipyrene (4-APP), followed by oxidation with potassium ferricyanide under alkaline conditions to give a red-colored product [1]. To perform the analysis, 1 mL of supernatant (adjusted to pH 10 using ammonium solution) was mixed with 100 μL of potassium ferric cyanide $K_3Fe(CN)_6$. The mixture was mixed with 100 μL of 4-aminoantipyrene. The absorbance of the resulting solution after incubation for 15 min (red in colour) was measured at 500 nm using a spectrophotometer.

III. RESULTS AND DISCUSSIONS

3.1 Effect of Temperature on Growth of *Rhodococcus* UKM-P and Phenol Degradation

Rhodococcus UKM-P was able to grow and degrade phenol at temperature ranging from 30°C to 40°C (Fig. 1). However, the highest growth of *Rhodococcus* UKM-P (0.394 g/L) phenol degradation was observed at 30°C, where 500 mg/L phenol was degraded after 21 h of cultivation. Although all the amount of phenol (0.5 g/L) present in the culture was degraded at 20°C, 25°C and 40°C, but the time taken was significantly longer than for cultivation at 30°C. The time taken for the degradation of 0.5 g/L phenol at 20°C, 25°C and 40°C was 120 h, 120 h and 24 h, respectively. Similar to control experiment, degradation of phenol was not observed at 50°C.

Results from this study indicated that the degradation of phenol was associated with growth of *Rhodococcus* UKM-P. High degradation rate was related to high growth rate obtained in cultivation at temperature ranging from 30°C to 40°C. Reduced growth of *Rhodococcus* UKM-P at high temperature (50°C) caused reduction in the degradation of phenol. This indicates that higher growth rate of cell will be contributed to higher rate of degradation. The highest degradation of phenol by *Rhodococcus erythropolis* was also obtained in cultivation at 30°C [11].

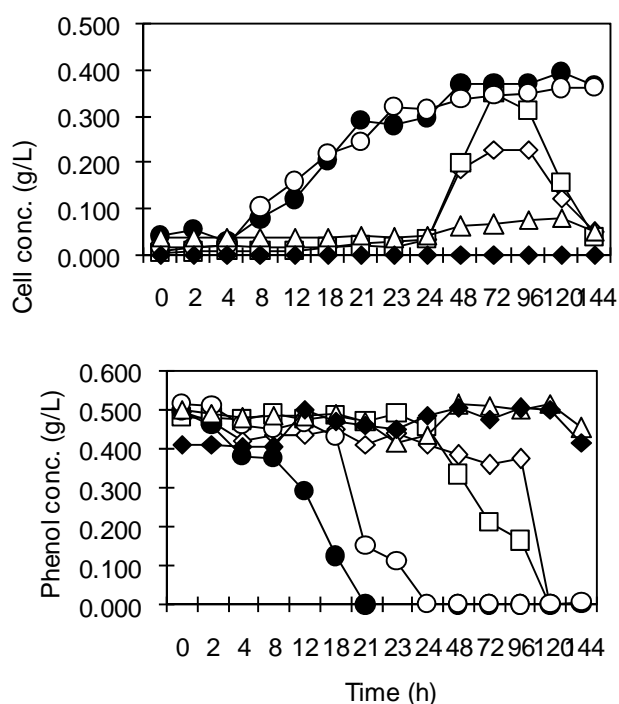


Figure 1. Effect of temperature on growth profile of *Rhodococcus* UKM-P and phenol degradation. (\diamond) 20°C; (\square) 25°C; (\bullet) 30°C; (\circ) 40°C; (Δ) 50°C; (\blacklozenge) control.

3.2 Effect of pH on Growth of *Rhodococcus* UKM-P and Phenol Degradation

Growth of *Rhodococcus* UKM-P and phenol degradation were greatly influenced by the initial culture pH (Fig. 2). The highest phenol degradation, which was associated with the highest growth of *Rhodococcus* UKM-P was obtained at neutral pH range, pH 7 to 7.5, where all the phenol present in the culture (0.5 g/L) was degraded. Growth of *Rhodococcus* UKM-P was inhibited at pH lower than 6. Although growth was not totally inhibited at pH 4.5-5, degradation of phenol was not observed. At pH higher than 8, growth of *Rhodococcus* UKM-P was slightly inhibited, which resulted to incomplete degradation of phenol present in the culture.

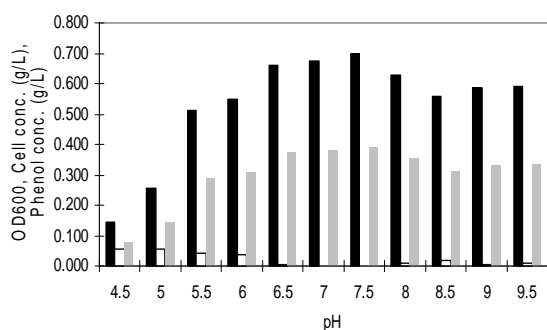


Figure 2: Effects of pH on growth *Rhodococcus* UKM-P and phenol degradation. Samples were analyzed after 21 h of cultivation. (■) OD600; (■) cell concentration; (□) phenol concentration.

3.3 Effect of Nitrogen Sources

Effect of using various carbon sources on growth of *Rhodococcus* UKM-P and phenol degradation is shown in Fig. 3. Ammonium sulphate enhanced both growth (0.463 g/L) of *Rhodococcus* UKM-P and degradation of phenol. In cultivation using ammonium sulphate, about 0.48 g/L of phenol was degraded. Although alanine stimulated growth of *Rhodococcus* UKM-P (0.492 g/L), phenol degradation was repressed, where only 0.027 g/L was degraded after 21 h cultivation. Although phenylalanine did not enhance growth of *Rhodococcus* UKM-P, about half of the phenol (0.277 g/L) present in the culture was degraded. Although substantial growth was observed in cultivation using the rest of nitrogen source, but degradation of phenol was not significance. Since NH_4SO_4 gave the highest growth and phenol degradation, it was chosen as a source of nitrogen for the subsequent experiments.

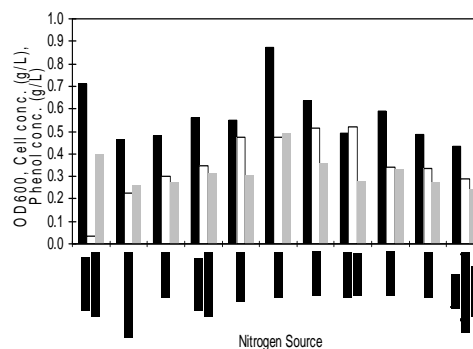


Figure 3: Effect of different types of nitrogen source on growth of *Rhodococcus* UKM-P and phenol degradation. Samples were analyzed after 21 h of cultivation. (■) OD600; (■) cell concentration; (□) phenol concentration.

3.4 Effect of Ammonium Sulphate Concentration

Various concentration of ammonium sulphate were examined for their effect on phenol degradation and cell growth, and the results are presented in Fig. 4. The highest growth of *Rhodococcus* UKM-P was obtained at two concentrations of ammonium sulphate, 0.4 g/L and 0.8 g/L, which also corresponded to the highest degradation of phenol. In cultivation using 0.4 g/L ammonium sulphate, the final cell concentration attained was 0.483 g/L and the amount of phenol degraded was 0.332 g/L.

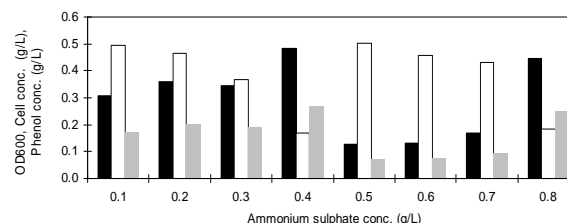


Figure 4: Effect of ammonium sulphate concentration on growth of *Rhodococcus* UKM-P and phenol degradation and. Samples were analyzed after 21 h of cultivation. (■) OD600; (■) cell concentration; (□) phenol concentration.

3.5 Effect of NaCl Concentration

Fig. 5 shows the effect of different concentrations of NaCl on growth of *Rhodococcus* UKM-P and phenol degradation. The highest degradation of phenol (0.34 g/L) was obtained when 0.1 g/L NaCl was added into the medium. Phenol degradation was repressed at NaCl concentration higher and lower than 0.1 g/L, though significant inhibition on growth was not observed.

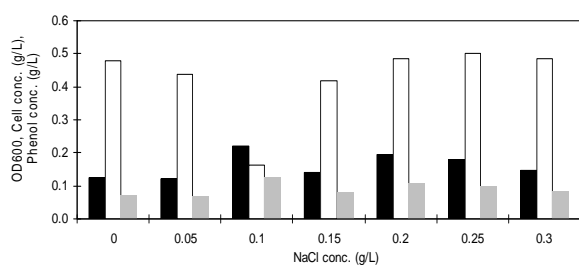


Figure 5: Effect of sodium chloride on growth of *Rhodococcus* UKM-P and phenol degradation. Samples were analyzed after 21 h of cultivation. (■) OD600; (■) cell concentration; (□) phenol concentration.

IV. CONCLUSION

Results from this study have demonstrated that *Rhodococcus* UKM-P was capable to degrade phenol and the performance was greatly influenced by the culture conditions (temperature and pH) as well as type and concentration of nitrogen sources supplied to the culture. The optimum conditions for growth and phenol degradation by *Rhodococcus* UKM-P were; 30°C, pH 7.5, 0.4 g/L ammonium sulphate and 0.1 g/L sodium chloride. In this cultivation, the final cell concentration and the amount of phenol degraded was 0.394 g/L and 0.5 g/L, respectively.

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