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Enhancement of The Growth and Degradation of Phenol by *Rhodococcus* UKMP-5M Using Various Nutrients

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Abstract: Biological method for phenol degradation was performed in this study using versatile strain of *Rhodococcus* UKMP-5M. Experiment was conducted using 250 mL shake flask. The effect of phenol concentration, additional of glucose and the effect of buffer concentration on growth and phenol degradation were investigated. This study also suggested that the strain was able to utilize phenol at concentration up to 900 mg/L, whereby growth is inhibited at lower or high phenol concentration. Additional up to 2 g/L glucose as additional carbon source was enhanced growth and phenol degradation. The concentration of buffer ranged from 50 to 150 mM also help to improve the degradation of phenol. Therefore, *Rhodococcus* UKMP-5M has potential to be used in phenol containing waste as well as in bioremediation process.

Keywords: *bioremediation, carbon source, degradation, optimization, phenol degradation, Rhodococcus UKMP-5M,*

1. Introduction

Chlorinated aromatic compounds pose one of the most serious contemporary environmental problems worldwide because they have been used in large quantities as herbicides, pesticides and solvents (Ogawa and Miyashita, 1995). In the 1980s, rapidly increasing environmental contamination raised concerns about health of ecosystems and humans and interest in biological methods of pollution cleanup (bioremediation) (Martinkova *et al*., 2009). Phenol also is a reasonably common wastewater contaminant which has been found to be either toxic or lethal to fish and most types of microorganisms at relatively low concentrations (Agarry *et al*., 2007). Due to the toxicity effects and endocrine disrupting properties of phenolic compounds, their removal from water and wastewater has gained widespread global attention (Labbe, et al., 2014).

Therefore, biodegradation of phenol at high concentration has been an interesting topics of research for many years (Bajaj *et al*., 2009) and phenol biodegradation has been chosen as a method to remediate environments contaminated by phenol (Wang *et al*., 2010; Veenagayathri and Vasudevan, 2010), which is massively discharged from uncontrolled industrial waste disposal (El Sayed *et al*., 2003; Yan *et al*., 2008).

Rhodococci may be naturally present in contaminated environment and are promising candidates for bioremediation (Bell *at al*., 1998; Martinkova *et al*., 2009). The genus *Rhodococcus* is Gram-positive aerobic bacteria and is closely related to the other mycolic acid containing genera: *Nocardia, Corynebacterium* and *Mycobacterium* (Bell *et a*l., 1998; Larkin *et al*., 1998). Recent studies on their metabolic activities have shown *Rhodococci* to be of important use in industrial,

pharmaceutical and environmental biotechnology. Furthermore, it is also proving useful in pharmaceuticals as antibiotic, anti-fungal and anti-tumor (Moore *et al*., 2005). *Rhodococcus* sp. P1 was also found to degrade up to 2.8 g/L phenol concentration (Straube *et al*., 1990).

The objective of the present study was to evaluate the ability of *Rhodococcus* UKMP-5M to degrade phenol. The effect of various phenol concentration, effect of additional of glucose and buffer concentration on growth of *Rhodococcus* UKMP-5M and phenol degradation was investigated. The information gathered from all the experiments were used to study the growth of *Rhodococcus* UKMP-5M and phenol degradation for subsequent experiments.

2. Materials and Methods

A. Microorganism and Inoculum preparation

The bacterium, *Rhodococcus rubber* (also known as *Rhodococcus* UKMP-5M 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 standard inoculums for all cultivation and degradation experiments

B. Medium

In the study of the effect of medium composition, four types of medium were used namely, medium M1 (Minimal Salt Medium). Liquid mineral salt medium (M1) consisted of (g/L): K_2HPO_4, 0.4; KH_2PO_4, 0.2; NaCl, 1; $MgSO_4$ 0.1; $MnSO_4$ 0.01; $FeSO_4H_2O$, 0.01; $Na_2MoO_4.2H_2O$, 0.01; $(NH_4)_2SO_4$, 0.4 and phenol, 0.1-1.3 was used in all cultivation and phenol degradation experiments. The initial pH value of the medium will be adjusted to 7.5 using 30% NaOH before autoclaving and phenol will be filter sterilized using 0.2 µm regenerated cellulose membrane filter (Bai *et al*., 2007). The variables varied for the cultivation experiments include phenol concentration and glucose concentration.

C. Fermentation

Batch fermentation in was carried out in 250 mL shake flask containing 100 mL medium. Medium was sterilised at 121^{o}C for 20 minutes. The medium was inoculated with 10% (v/v) inoculum that was previously grown overnight on the nutrient broth medium (NB) to initiate the cultivation and degradation of phenol. The flask was incubated at temperature 30^{o}C on a rotary shaker, agitated at 160 rpm. All fermentations were performed in triplicate and the results were presented in average value. During the cultivation, 10 mL of culture samples were withdrawn at different time intervals for analysis of cell concentration and phenol degradation.

D. Analytical Methods

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. 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 filters with the bacterial cells were at 80^{o}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-AAP), followed by oxidation with potassium ferricyanide under alkaline conditions to give a red-coloured product (APHA, 1998). 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- aminoantipyrine. The absorbance of the resulting solution after incubation for 15 min (red in colour) was measured at 500 nm using a spectrophotometer.

3. Results and Discussion

A. Effect of phenol concentration

The cell growth and biodegradation of increasing phenol concentration was shown in Figure 1. *Rhodococcus* UKMP-5M fully degraded phenol in concentrations ranging from 0.2 to 0.9 g/L. Up to 0.7 g/L, phenol was completely degraded within one day cultivation. The rest of phenol concentration had taken up to 8 days or more to degrade.

Although 0.9 mg/L phenol was only degraded after 8 days of fermentation, but the *Rhodococcus* UKMP-5M cells was significantly highest with maximum concentration of 0.27 g/L cell. Bacterium in 1.3 g/L phenol was not degraded until 10 days of fermentation. Similar to control experiment, cell growth of *rhodococcus* was inhibited at the lowest and the highest concentration of phenol. With increasing amounts of phenol lag-phases increased due to substrate inhibition by the toxic substrate phenol (Margesin *et al*., 2005).

Results from this study indicated that the degradation of phenol was non-associated with growth of *Rhodococcus* UKMP-5M. *Rhodococcus* UKMP-5M was observed can tolerate up to 0.9 g/L phenol, but the time taken was significantly longer for cultivation in 0.9 g/L phenol.

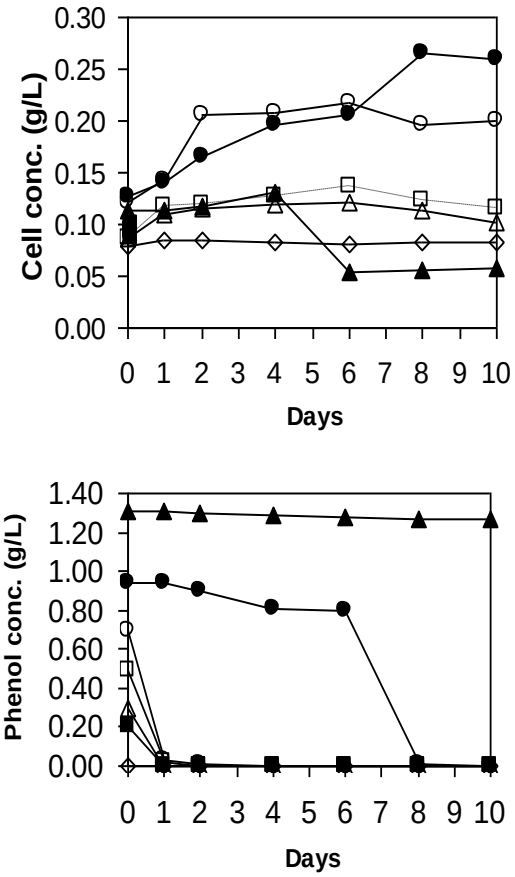

Fig. 1. Effect of phenol concentration on growth profile of *Rhodococcus* UKMP-5M and phenol degradation. Symbols: (◊) 0 g/L (control); (■) 0.2 g/L; (Δ) 0.3 g/L; (□) 0.5g/L; (○) 0.7 g/L; (●) 0.9 g/L; (▲) 1.3 g/L.

B. The effect of additional of glucose as carbon sources

In order to investigate the effect of additional carbon source on cell growth of *Rhodococcus* UKMP-5M, glucose was added at various concentration ranged from 0 to 3 g/L into Minimum Salt Medium (MSM). Experiment was take place in 250 mL shake flask. Although *Rhodococcus* consumed varies concentration of glucose up to 3 g/L, the highest cell concentration (0.570 g/L) was achieved when only 2 g/L glucose was used. 0.494 g/L phenol was degraded out of 0.5 g/L phenol added in 2 g/L glucose containing medium (Table 1). Additional of more than 2 g/L Glucose concentration was slightly inhibited the growth of *Rhodococcus* UKMP-5M (Table 1). Lob and Tar (2000) have also reported that interference in phenol uptake by glucose. This observation may be able to be used to explain this condition. Therefore, additional of glucose must be not more than 2g/L to avoid cell limitation.

Table 1: Effect of Glucose on Growth and Phenol Degradation of *Rhodococcus* UKMP-5M

Glucose (g/L)	Cell concentration (g/L)	Glucose concentration(g/L)	Glucose consumed (g/L)	Glucose consumed (%)	Phenol Degraded (g/L)
0	0.115	0.000	0.000	0.000	ND
0.1	0.275	0.004	0.096	96.20	ND
0.5	0.392	0.008	0.492	98.32	ND
1	0.416	0.008	0.992	99.25	ND
1.5	0.426	0.006	1.494	99.62	ND
2	0.570	0.091	1.909	95.46	0.494 (g/L)
2.5	0.512	0.096	2.404	96.14	ND
3	0.393	0.089	2.911	97.02	ND

ND: Not Determine

C. Effect of buffer concentration

Figure 2 shows the cell growth and phenol degradation by *Rhocococcus* UKMP-5M that was cultured in medium containing various buffer concentrations. Potassium dihydrogen phosphate and di potassium hydrogen phosphate was used as a buffer in this study. Although the highest cells, 0.427 g/L was investigated in 5 mM buffer medium, but phenol degradation was significantly degraded in medium with buffer concentration ranged from 50 to 150 mM. Growth was paralleled to phenol biodegradation where 500 mg/L phenol was completely degraded at 24 hours cultivation.

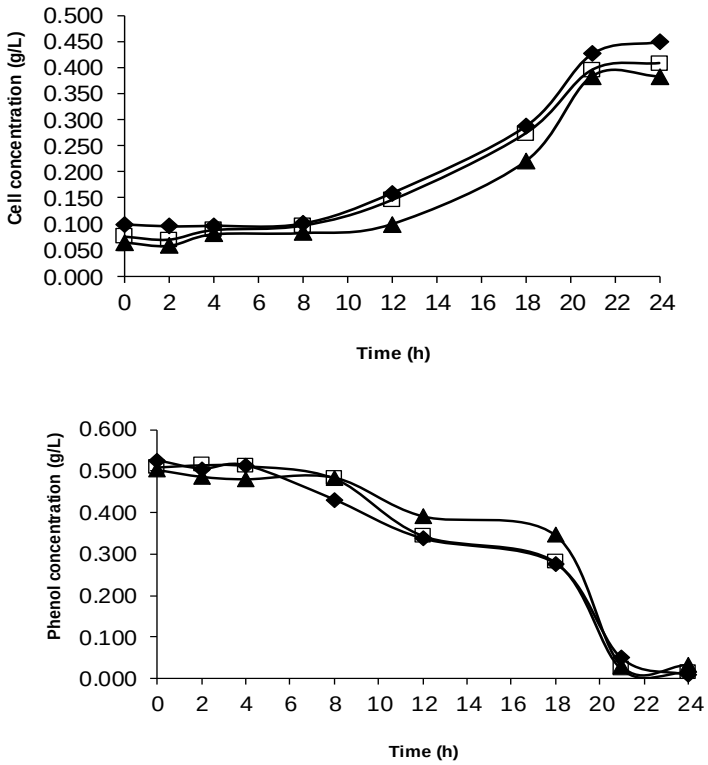

Fig.2. Effect of buffer (K_2HPO_4:KH_2PO_4) concentration on growth profile and phenol degradation of *Rhodococcus* UKMP-5M. Symbols: (♦) 5mM; (□) 50mM; (▲) 150mM.

4. Conclusion

Results from this study have demonstrated that *Rhodococcus* UKMP-5M was capable to degrade phenol and the performance was greatly influenced by the nutrients conditions (phenol concentration, glucose and buffer concentration) supplied to the culture. Even though *Rhodococcus* UKMP-5M can tolerate up to 900 mg/L phenol, only up to 700 g/L phenol was degraded in 24 hours. The other condition that affected the growth and phenol degradation were; additional of glucose up to only 2 g/L was increase the growth of *Rhodococcus* UKMP-5M as well as contribute to phenol degradation. Phenol degradation was also degraded significantly in medium with buffer condition ranged from 50-150 mM.

5. References

APHA, 1998. Standard Method for the Examination of Water and Wastewater.2 th Edition. Method 5530. pp. 540-544.

Agary, S.E, Betiku, E. and Solomon, B.O. 2007. Inhibition Kinetics of Phenol Degradation by Binary Mixed Xulture from Continuous Culture and Wash-Out Data. *Journal of Engineering and Applied Sciences* 2(6): 1020-1026.

Bai, J., Wen, J.P., Li H.M. and Jiang, Y. 2007. Kinetic modeling of growth and biodegradation of phenol and m-cresol using *Alcaligenes faecalis. Process Biochemistry* 42: 510-517.

Bajaj, M., Gallert, C. and Winter, J. 1009. Phenol degradation kinetics of an aerobic mixed culture. *Biochemical Engineering Journal* 46: 205-209.

Bell, K.S., Philip, J.C., Aw, D.W.J. and Christofi, N. 1998. The Genus *Rhodococcus*. *Journal of Applied Microbiology* 85: 195-210.

El-Sayed, W.S., Ibrahim, M.K., Abu Shady, M., El-Beih, F., Ohmura, N., Saiki, H. and Ando, A. 2003. Isolation and characterization of phenol-catabolizing Bacteria from a Coking Plant. *Bioscience Biotechnology Biochemistry* 67(9): 2026-2029.

Larkin M.J, De Mot, R., Kulakov, L.A. and Nagy, I. 1998. Applied aspects of *Rhodococcus* genetics. *Antonie Van Leeuwenhoek* 74:133–153.

Lob, K.C. and Tar, C.P.P. 2000. Effect of additional carbon sources on Biodegradation of phenol. *Bull. Environmental Contamination Toxicology* 64: 756-763.

Margesin, R., Fonteyne, P.A. and Redl, B. 2005. Low-temperature Biodegradation of High Amounts of Phenol by *Rhodococcus* spp. and Basidiomycetous Yeasts. *Research in Microbiology* 156 (2005): 68–75.

Martinkova, L., Uhnakova, B., Miroslav Patek, M., Nesvera, J. and Kren, V. 2009. Biodegradation potential of the genus *Rhodococcus. Environment International* 35: 162-177.

Marissa Choquette-Labbé, Wudneh A. Shewa, Jerald A. Lalman * and Saravanan R. Shanmugam. 2014. Photocatalytic Degradation of Phenol and Phenol Derivatives Using a Nano-TiO2 Catalyst: Integrating Quantitative and Qualitative Factors Using Response Surface Methodology. Water, 6, 1785-1806; doi:10.3390/w6061785

Ogawa. N and Miyashita, K. 1995. Recombinant of a 3-cholorobenzoate Catabolic Plasmid from *Alcaligenes eutropus* NH9 Mediated by Direct Repeat Elements. *Applied and Environmental Microbiology* 61:3788-3795.

Straube, G., Hansel, J., Niedan, C. and Straube, E. 1990. Kinetics Studies of Phenol Degradation by *Rhodococcus* sp. P1 I. Batch Cultivation. Kluwer Academic Publishers. Netherlands. *Antonie van Leeuwenhoek* 57: 29-32.

Veenagayathri, K. and Vasudevan, N. 2010. Effect of pH, nitrogen sources and salts on the degradation of phenol by the bacterial consortium under saline conditions. *International journal of Biotechnology and Biochemistry*, 6: 783-791.

Wang, L., Li, Y., Yu, P., Xie, Z., Luo, Y. and Lin, Y. 2010. Biodegradation of phenol at high concentration by a novel fungal strain *Paecilomyces variotii* JH6. *Journal of Hazardous Materials* 183:366-371.

Yan, J., Nanqi, R., Xun, C., Di, W., Liyan, Q. and Sen, L. 2008. Biodegradation of Phenol and 4-Chlorophenol by the Mutant Strain CTM 2*. *Chinese Journal of Chemical Engineering* 16(5) 796:800