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Nutrients and culture conditions requirements for the degradation of phenol by *Rhodococcus* UKMP-5M

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Abstract

The capability of *Rhodococcus* UKMP-5M, isolated from petroleum contaminated soil, in the degradation of phenol was studied using shake flask culture. The effect of nutrients and cultivation conditions on growth of this bacterium and phenol degradation was investigated. Among the different types of medium tested (M1, M2, M3 and M4), M1 was found to be the preferred medium for growth of this bacterium and phenol degradation. The optimized cultivation conditions for growth of *Rhodococcus* UKMP-5M and phenol degradation were; 30°C, initial pH 7.5 and buffer concentration ranged from 5 to 50 mM. Improvement of growth and phenol degradation was achieved in medium supplemented with 2 g l^{-1} glucose. In addition, NaCl at a concentration of 0.1 g l^{-1} was required to enhance growth and phenol degradation. The addition of 0.4 g l^{-1} $(NH_4)_2SO_4$ into the culture medium greatly enhanced phenol degradation. At optimal medium composition and cultivation condition, *Rhodococcus* UKMP-5M was able to utilize phenol at concentration up to 900 mg l^{-1}. Results of this study showed that *Rhodococcus* UKMP-5M has potential to be used in the degradation of phenol.

Key words

Cultivation parameters, *Rhodococcus* UKMP-5M, Phenol degradation

Introduction

Nowadays, contamination of the environment by hazardous and toxic chemical is considered as one of the major problems facing by the industrial nations. Phenol, a compound regarded as priority contaminants by the US Environmental Protection Agency, is normally present in wastewaters and industrial effluents as they are the waste products of many industrial activities (Santos *et al.,* 2009). The ingestion of about 1 g kg^{-1} b. wt. of phenol is considered lethal to humans and animals (Rocha *et al.,* 2007). Since phenol is very toxic, even at low levels, it poses a threat to the biosphere and aquatic life. As a result of growing awareness caused by the release of phenol to the environment, efforts are being made to minimize their adverse effects. The physicochemical methods for the removal of phenol from effluent are very costly and causing secondary pollution (Wang *et al.,* 2010). Therefore, biodegradation technique, which is environmental friendly and cost effective, has turned out to be an alternative method (Ariana *et al.,* 2004).

Intensive research on phenol biodegradation by a diverse groups of microorganisms including *Pseudomonas*, *Candida, Alcaligenes* and *Bacillus* have been reported (Bastos *et al.,* 2000; Kotresha and Vidyasagar, 2008; Benerjee

and Ghoshal, 2010). However, information on phenol degradation by *Rhodococcus* sp is still very limited. Various factors including the structure of the compound, nitrogen source, salts, temperature, salinity, pH and the availability of inorganic nutrients and oxygen greatly influenced the biodegradation of phenol in aquatic environments (Chakraborty *et al.,* 2010; Veenagayathri and Vasudevan, 2010).

The objective of the present study was to evaluate the ability of *Rhodococcus* UKMP-5M to degrade phenol. The effect of various cultivation parameters including type of medium, temperature, culture pH, buffer, type and concentration of nitrogen source, salt concentration, phenol concentration and glucose concentration on growth of *Rhodococcus* UKMP-5M and phenol degradation was investigated.

Materials and Methods

Microorganism and inoculum preparation: The bacterium *Rhodococcus* UKMP-5M was isolated from a petroleum contaminated soil at an oil refinery in Malacca, Malaysia. The stock culture of this bacterium was maintained at the Universiti Selangor (UNISEL) Culture Collection Centre, Selangor, Malaysia. The bacterium from the stock culture was grown in nutrient broth for 24 hr and the culture was used as a standard inoculum for all cultivation and degradation experiments.

Media: Four types of medium M1 [K_2HPO_4, 0.4; KH_2PO_4, 0.2; NaCl, 0.1; $MgSO_4$ 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, 0.5]; M2 [Na_2HPO_4, 10.0; KH_2PO_4, 1.0; $(NH_4)_2SO_4$, 3; FeCl, 0.018; NaCl, 0.2; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.08; yeast extract, 0.5 and phenol, 0.5]; M3 [Na_2HPO_4, 7.0; KH_2PO_4, 3.0; NaCl, 1.0; $CaCl_2$, 0.02; $MgSO_4$, 0.2 and phenol, 0.5]; M4 [Glucose, 4.0; Yeast extract, 4.0, Malt extract, 10.0 and phenol, 0.5] were initially tested. The composition of each medium is shown in Table 1. The initial pH of all media was adjusted to 7.5 using 30% NaOH before autoclaving. Phenol was sterilized by filtration through a regenerated cellulose membrane filter with pore size of 0.2 µm.

Batch cultivations of *Rhodococcus* UKMP-5M for phenol degradation were carried out in 250 ml shake flask containing 100 ml medium M1. The flask containing medium was sterilised at 121°C for 20 min. The flask was inoculated with 10% (v/v) inoculum that was previously grown overnight on nutrient broth to initiate the cultivation and degradation of phenol. The inoculated flask was incubated at 30°C on a rotary shaker, agitated at 160 rpm. Phenol concentration was determined by a colorimetric method based on rapid condensation with 4-aminoantipyrene (APHA, 2005).

The effect of different temperatures (20, 25, 30, 40 and 50°C) on growth of *Rhodococcus* UKMP-5M and phenol degradation was first studied. Subsequently, the effect of different initial pH (pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5) was studied in cultivation experiment performed at 30°C, which was the preferred temperature for growth and phenol degradation. The effect of controlled pH at different values (pH 5.8, 6.4, 7.2, 8.0) during the cultivation of *Rhodococcus* UKMP-5M was also studied. In an experiment to investigate the effect of different phosphate buffer concentrations (5, 50 and 150 mM), the initial culture pH was set at 7.5. In order to investigate the effect of different nitrogen sources ($(NH_4)_2SO_4$, phenyalanine, glycine, NH_4Cl, histidine, alanine, leucine, sodium nitrate, proline and cystein on growth of *Rhodococcus* UKMP-5M and phenol degradation, the cultivation experiments were performed at 30°C, initial pH of 7.5 and 5 mM phosphate buffer. The effect of different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.05 g l^{-1}) of the preferred nitrogen source ($(NH_4)_2SO_4$) on growth of *Rhodococcus* UKMP-5M and phenol degradation was also investigated. Subsequently, the effect of different NaCl concentrations (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3) was studied in cultivations using 0.4 g l^{-1} $(NH_4)_2SO_4$. The effect of different phenol concentrations (0, 0.2, 0.3, 0.5, 0.7, 0.9 and 1.3 g l^{-1}) and different glucose concentrations (0.1, 0.5, 1.0, 2.0, 2.5, and 3.0 g l^{-1}) on growth of *Rhodococcus* UKMP-5M and phenol degradation were also investigated in cultivations using 0.4 g l^{-1} $(NH_4)_2SO_4$ and 0.1 g l^{-1} NH_4Cl.

Cell concentration was determined in term of cell culture density and dry cell weight. The density of cell culture was measured at 680 nm using a spectrophotometer. Dry cell weight was determined by filtration and oven dried method (Ariff *et al.,* 1996).

Statistical analysis: The results were presented in average value of 3 replicate. The data were analysed using one-way ANOVA with SPSS 16.0 software.

Results and Discussion

Among the different types of medium tested, the highest phenol degradation (99.8%) was observed in M1 medium, where 500 mg l^{-1} of phenol was completely degraded after 21 hr of cultivation. In this cultivation, the highest cell concentration (0.318 g l^{-1}) was also observed after 21 hr of cultivation. Higher phenol degradation rate (0.024 g l^{-1} hr^{-1}) in M1 medium may be due to the present of trace elements. Although growth of *Rhodococcus* UKMP-5M was enhanced in cultivation using M2 (0.513 g l^{-1}) and M3 (0.482 g l^{-1}), phenol degradation was significantly reduced. The amount of phenol degraded in cultivation using M2 and M3 was only 0.386 g l^{-1} and 0.362 g l^{-1}, respectively. The presence of $CaCl_2$ in these media may repress phenol

degradation. In cultivation using M4, growth of *Rhodococcus* UKMP-5M was slightly inhibited (0.297 g l^{-1}), which resulted in incomplete degradation of phenol presence in the culture (Table 1 and Fig. 1a and b).

Phenol degradation was inhibited in cultivation with a minimal medium, though growth of *Rhodococcus* UKMP-5M was enhanced. The degradation of phenol by *Rhodococcus* UKMP-5M also required the supplement of trace elements. Degradation rate of some xenobiotic compounds can be improved with the supplement of carbon source or other nutrient components such as nitrogen, phosphate and mineral constituents (Loh and Wang, 1998). Glucose in the culture also greatly influenced phenol degradation by *Rhodococcus* UKMP-5M. Phenol degradation by *C. chrysogenum* was delayed in cultures containing 3% (w/v) glucose (Leitao *et al.,* 2007). Reduced phenol degradation at high glucose concentrations may be due to competitive inhibition. Since M1 gave the highest phenol degradation with high cell yield (1.569 g g^{-1}), this medium was chosen for the subsequent experiments.

Temperatures ranging from 30° to 40°C are suitable for *Rhodococcus* UKMP-5M to grow and to degrade phenol (Table 2). However, 30°C is the most suitable temperature for the highest growth of *Rhodococcus* UKMP-5M (0.394 g l^{-1}) and phenol degradation. In this study, 500 mg l^{-1} phenol was found to be degraded after 21 hr of cultivation. At the same temperature with lower phenol concentration (200 mg l^{-1}), *Pseudomonas putida* F1 required up to 35 hr to completely degrade all phenol presence in the culture (Hamed *et al.,* 2003). The time taken for phenol to degrade was significantly longer than cultivation in 30 °C when 0.5g l^{-1} phenol was cultured at 20°C, 25°C and 40°C. No degradation was found at 50°C, which is similar to the control experiment. Under methanogenic condition, degradations of phenol, benzoates and phthalates were restricted at 55°C (Leven and Schnurer, 2005).

In this study, degradation of phenol by *Rhodococcus* UKMP-5M showed a growth-associated process. The high degradation rate, (0.024-0.017 g l^{-1} hr^{-1}) was related to high specific growth rate (0.036–0.099 hr^{-1})

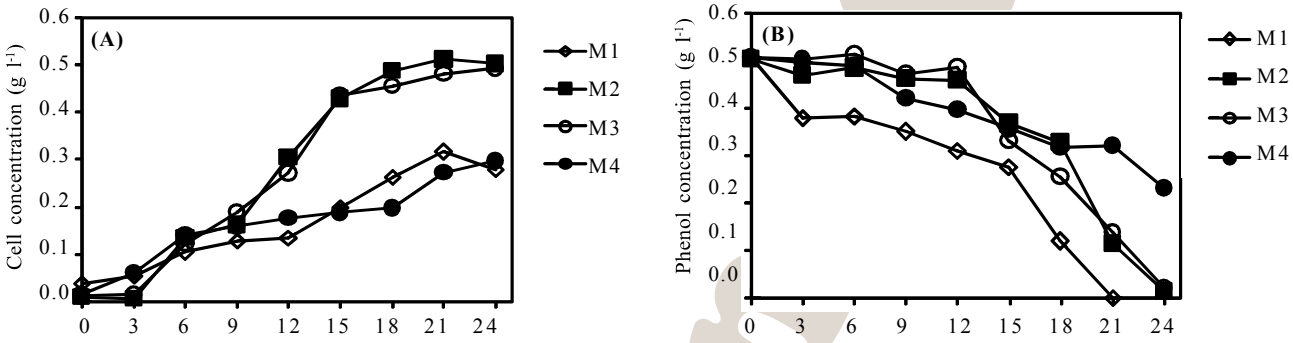

Fig. 1 : (A) Effect of medium on growth profile and (B) phenol degradation of *Rhodococcus* UKMP-5M.

Table 1 : Comparison of the kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M in different types of medium. For all experiments, initial phenol concentration and culture pH was set at 0.5 g l^{-1} and 7.5, respectively. The cultivation time for all experiments was 21 hr

Kinetics Parameters/Performance	**Medium types**			
	M1	**M2**	**M3**	**M4**
Maximum cell, X_m (g l^{-1})	0.318±0.17[c]	0.513±0.26[c]	0.482±0.28[c]	0.274±0.28[c]
Specific growth rate, μ (hr^{-1})	0.055	0.015	0.017	0.091
Specific degradation rate, P (hr^{-1})	0.124	0.140	0.081	0.000
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.559	1.303	0.981	1.433
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	0.998	0.772	0.724	0.36
Residual phenol (g l^{-1})	0.001±0.26[c]	0.114±0.36[c]	0.138±0.36[c]	0.320±0.39[c]
Phenol degraded (g l^{-1})	0.499	0.386	0.36	0.180
Phenol degradation rate (g l^{-1} hr^{-1})	0.024	0.018	0.017	0.0086
Percentage of phenol degradation (%)	99.8	77.2	72.4	36
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	1.569	0.752	0.751	0.657

Data were obtained from the time course of each fermentation run; Values are mean of three replicates ± SD; [c]Mean value in same row with different superscripts are significant different (P<0.05)

obtained in cultivation at temperature ranging from 30 to 40°C. High cell yield (0.502 and 0.663 g g^{-1}) were obtained at 30 and 40°C, respectively. Temperature of 50°C resulted in the reduction of growth and phenol degradation, indicating that higher growth rate contributed to higher rate of degradation. This is in agreement with phenol degradation by *Ewingella americana*, where reduced degradation was observed at temperature of above 37°C (Khleifat, 2006). The highest degradation of phenol by *Rhodococcus erythropolis* was also attained at 30°C (Prieto *et al.,* 2002). Phenol degradation rate declined significantly at temperature below 25°C and higher than 40°C (Wang *et al.,* 2010). In many cases, biodegradation of phenol by microorganisms was optimal at 30°C (Ahmad *et al.,* 2011).

Table 2 : Effect of temperature on kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M. For all experiments, initial phenol concentration and culture pH was set at 0.5 g l^{-1} and 7.5, respectively. The cultivation time for all experiments was 21 hr

Kinetics Parameters/Performance	Temperature (°C)				
	20	25	30	40	50
Maximum cell, X_m (g l^{-1})	0.025±0.07^c	0.019±0.08^c	0.291±0.23^b	0.246±0.22^b	0.041±0.05^c
Specific growth rate, μ (hr^{-1})	0.142	0.053	0.099	0.036	0.018
Specific degradation rate, P (hr^{-1})	0.556	0.271	0.142	0.379	0.166
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.213	0.552	0.502	0.663	0.111
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	0.178	0.058	1.000	0.694	0.054
Residual phenol (g l^{-1})	0.411±0.36^b	0.471±0.36^b	0.000±0.15^c	0.153±0.22^c	0.473±0.48^b
Phenol degraded (g l^{-1})	0.089	0.029	0.500	0.347	0.027
Phenol degradation rate (g l^{-1} hr^{-1})	0.004	0.001	0.024	0.017	0.001
Percentage of phenol degradation (%)	17.8	5.8	100.0	69.4	5.4
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	0.178	1.53	1.72	1.41	0.66

Data were obtained from the time course of each fermentation run; Values shown are mean of three replicates with ± SD; $^{b-c}$ Mean value in same row with different superscripts are significant different ($P<0.05$)

Table 3 : Effect of pH on kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M. For all experiments, initial phenol concentration was set at 0.5 g l^{-1}. The cultivation time for all experiments was 21 hr

Kinetics Parameters/ Performance	Initial culture pH without pH control during the degradation										
	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5
Maximum cell, X_m (g l^{-1})	0.080± 0.14^c	0.145 ±0.24b^c	0.287 ±0.72^a	0.308 ±0.55^{ab}	0.371 ±0.66^a	0.380 ±0.68^a	0.392 ±0.65^a	0.352 ±0.63^a	0.312 ±0.57^{ab}	0.328	0.333
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.16	0.29	0.574	0.616	0.742	0.760	0.784	0.704	0.624	0.656	0.666
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	0.890	0.888	0.912	0.924	0.990	0.998	0.908	0.978	0.964	0.990	0.982
Residual phenol (g l^{-1})	0.055 ±0.03^c	0.056 ±0.04^c	0.044 ±0.04^c	0.038 ±0.05^c	0.005 ±0.05^c	0.001 ±0.05^c	0.001 ±0.06^c	0.011 ±0.06^c	0.018 ±0.06^c	0.005 ±0.06^c	0.009 ±0.07^c
Phenol degraded (g l^{-1})	0.445	0.444	0.456	0.462	0.495	0.499	0.499	0.489	0.482	0.495	0.491
Phenol degradation rate (g l^{-1} hr^{-1})	0.021	0.021	0.022	0.022	0.024	0.024	0.024	0.023	0.023	0.024	0.023
Percentage of phenol degradation (%)	89.0	88.8	91.2	92.4	99.0	99.8	99.8	97.8	96.4	99.0	98.2
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	5.563	3.062	1.588	1.500	1.334	1.313	1.273	1.389	1.545	1.509	1.474
Controlled pH during the degradation using buffering system											
	5.8	6.4	7.2	8.0							
Maximum cell, X_m (g l^{-1})	0.071 ±0.07^c	.068 ±0.08^c	0.105 ±0.12^b	0.054 ±0.06^c							
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.082	0.043	0.152	0.065							
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	0.048	0.376	0.566	0.184							

Growth of *Rhodococcus* UKMP-5M and phenol degradation were greatly influenced by the pH of the culture (Ahmad *et al.,* 2011). The highest growth of *Rhodococcus* UKMP-5M which was related with the highest phenol degradation was attained at pH 7.5 (Table 3) and it was similar with the degradation of phenol by *E. americana* (Khleifat, 2006). At pH 7.5, all phenol presence in the culture (0.5 g l^{-1}) were degraded. At pH < 6, growth of *Rhodococcus* UKMP-5M was inhibited. Even though growth of *Rhodococcus* UKMP-5M was not completely inhibited at pH 4.5 to 5, no phenol degradation was observed. At pH > 8, growth of *Rhodococcus* UKMP-5M was inhibited followed by the decrease in phenol degradation.

The cultivation with controlled pH at 7.2 gave higher growth (0.15 g l^{-1}) and degradation of phenol (56.6%) as compared to cultivations with controlled pH at acidic (pH 5.8) and alkaline (pH 8) conditions (Table 3). However, the percentage of phenol degradation in cultivations with controlled pH was significantly lower than those obtained in cultivation without pH control. The phenol biodegradation efficiency was significantly reduced at pH lower than 7 and higher than 8. Although all phenol presence in the culture (0.5 g l^{-1}) was completely degraded in

Table 4. Effect of different types of nitrogen source on kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M. For all experiments, initial phenol concentration and culture pH was set at 0.5 g l^{-1} and 7.5, respectively. The cultivation time for all experiments was 21 hr

Kinetics Parameters/ Performance	$(NH_4)_2SO_4$	Phenyala.	Gly.	NaCl	His.	Ala.	Leu.	$NaNO_3$	Prol.	Cys.	Control
Maximum cell, X_m (g l^{-1})	0.399 ±0.71^{bc}	0.259 ±0.46^{c}	0.270 ±0.56^{c}	0.315 ±0.53^{c}	0.308 ±0.55^{c}	0.492 ±0.87^{b}	0.359 ±0.64^{bc}	0.275 ±0.49^{c}	0.332 ±0.59^{bc}	0.273 ±0.49^{c}	0.243 ±0.43^{c}
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.798	0.518	0.540	0.630	0.616	0.984	0.718	0.550	0.664	0.546	0.486
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	0.926	0.554	0.404	0.310	0.058	0.054	0	0.006	0.318	0.326	0.422
Residual phenol (g l^{-1})	0.037 ±0.04^{c}	0.223 ±0.42^{bc}	0.298 ±0.61^{b}	0.345 ±0.30^{c}	0.471 ±0.54^{b}	0.473 ±0.47^{b}	0.500 ±0.51^{b}	0.497 ±0.52^{b}	0.341 ±0.34^{bc}	0.337 ±0.34^{bc}	0.298 ±0.25^{bc}
Phenol degraded (g l^{-1})	0.463	0.277	0.202	0.155	0.029	0.027	0	0.003	0.159	0.163	0.211
Phenol degradation rate (g l^{-1} hr^{-1})	0.022	0.013	0.0096	0.0074	0.0014	0.0013	0	0.00014	0.00076	0.0078	0.010
Percentage of phenol degradation (%)	92.6	55.4	40.4	31.0	5.8	5.4	0	0.6	31.8	32.6	42.2
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	1.160	1.069	0.748	0.492	0.094	0.055	0	0.011	0.479	0.597	0.868

Data were obtained from the time course of each fermentation run; Values shown are mean of three replicates ± SD; $^{b-c}$ Mean value in same row with different superscripts are significant different (P<0.05)

Table 5 : Effect of ammonium sulphate concentration on kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M. For all experiments, initial phenol concentration and culture pH was set at 0.5 g l^{-1} and 7.5, respectively. The cultivation time for all experiments was 21 hr

Kinetics Parameters/	Ammonium sulphate concentration (g l^{-1})							
Performance	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Maximum cell, X_m (g l^{-1})	0.172 ±0.31^{b}	0.203 ±0.36^{b}	0.193 ±0.34^{b}	0.271 ±0.48^{a}	0.071 ±0.13^{c}	0.073 ±0.13^{c}	0.095 ±0.17^{c}	0.080 ±0.14^{c}
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.344	0.406	0.386	0.542	0.142	0.146	0.190	0.160
Product yield, $Y_{p/s}$ (g product g $carbon^{-1}$) (g g^{-1})	0.008	0.072	0.266	0.664	0.002	0.082	0.138	0.090
Residual phenol (g l^{-1})	0.496 ±0.48^{bc}	0.464 ±0.46^{bc}	0.367 ±0.37^{bc}	0.168 ±0.17^{c}	0.499 ±0.5^{b}	0.459 ±0.53^{c}	0.431 ±0.43^{bc}	0.455 ±0.46^{bc}
Phenol degraded (g l^{-1})	0.004	0.036	0.133	0.332	0.001	0.041	0.069	0.045
Phenol degradation rate (g $l^{-1}hr^{-1}$)	0.0002	0.002	0.006	0.016	0.00005	0.002	0.0033	0.0021
Percentage of phenol degradation (%)	0.8	7.2	26.6	66.4	0.2	8.2	13.8	9.0
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	0.023	0.177	0.689	1.225	0.014	0.562	0.726	0.563

Data were obtained from the time course of each fermentation run; Values shown are mean of three replicates with ± SD; $^{a-c}$ Mean value in same row with different superscripts are significant different (P<0.05)

cultivation where pH was controlled at 7.2, but the time taken was significantly longer than the cultivation with an initial pH 7.5 and without pH control throughout the cultivation. Phenol degradation by *Pseudomonas* sp. SA01 was optimal at pH 6.5 (Shourian *et al.,* 2009). The optimal pH for phenol degradation by the consortium of microbes was optimal at pH 7 (Veenagayathri and Vasudevan, 2010).

Growth of *Rhocococcus* UKMP-5M and phenol degradation was not significantly affected with the variation in phosphate buffer concentrations, ranging from 5 to 150 mM. Maximum cell concentration obtained in cultivation with 5, 50 and 150 mM was 0.427, 0.396 and 0.384 g l^{-1}, respectively. On the other hand, the percentage of phenol degradation obtained in cultivation with 5, 50 and 150 mM was 90.2, 95.4 and 94.2%, respectively. A slight increase in growth of *Rhodococcus* UKMP-5M was observed at very low buffer concentration (0.5 mM) with a slight reduction in percentage of phenol degradation.

The growth and phenol degradation by *Rhodococcus* UKMP-5M were enhanced using $(NH_4)_2SO_4$ and almost 96% phenol was degraded in cultivation using $(NH_4)_2SO_4$ (Table 4). Similar observation was also reported for phenol degradation by *Acinetobacter* sp. strain AQ5NOL1, where growth and phenol degrading activity were enhanced in medium containing $(NH_4)_2SO_4$ (Ahmad *et al.,* 2011).

Even though alanine only showed 5.4% of phenol degradation after 21 hr cultivation, the growth of *Rhodococcus* UKMP-5M was up to 0.492 g l^{-1}. Meanwhile, phenyalanine showed up to 54% of phenol degradation although it did not enhance the growth of *Rhodococcus* UKMP-5M. The rest of nitrogen sources showed significant growth even though phenol was not fully degraded. The percentage of phenol degradation in cultivation with (NH_4NO_3) was only 0.6%. However, high percentage of phenol degradation (75.65%) was obtained in *Pseudomonas aeruginosa* MTCC 4996 cultivation using (NH_4NO_3) as a nitrogen source (Kotresha and Vidyasagar, 2008). By showing the highest cell growth and phenol degradation, $(NH_4)_2SO_4$ was chosen as a nitrogen sources for the subsequence experiments.

Effect of various concentrations of $(NH_4)_2SO_4$ on growth of *Rhodococcus* UKMP-5M and phenol degradation is summarized in Table 5. 0.4 g l^{-1} $(NH_4)_2SO_4$ was the most suitable concentration for the highest growth of *Rhodococcus* UKMP-M. During cultivation in 0.4 g l^{-1} $(NH_4)_2SO_4$, the final cell concentration attained was 0.483 g l^{-1} with 66.4% phenol degradation. Up to 67.6% phenol was successfully degraded when 0.1 g l^{-1} NaCl was used in the culture medium. At a concentration of NaCl lower and higher than 0.1 g l^{-1}, phenol degradation was repressed even though significant inhibition on growth was not detected. The removal of phenolic compounds by microorganisms is affected by some external factor such as salts, where the activity of microorganisms is normally reduced at high salt concentration (Veenagayathri and Vasudevan, 2010).

In cultivations with initial phenol concentration ranging from 0.2 to 0.9 g l^{-1}, all phenol presence in the culture was degraded by *Rhodococcus* UKMP-5M. Results from this study indicated that growth of *Rhodococcus* UKMP-5M was greatly inhibited at very low and very high initial phenol concentrations (Table 7). Lag growth phase was also increased with increasing initial phenol concentration due to inhibition by the toxicity of phenol (Margesin *et al.,* 2005). In cultivation with phenol concentration up to 0.7 g l^{-1}, all phenol was completely degraded within 24 hr. A longer cultivation time (>192 hr) was required to degrade all phenol in cultivation with higher initial phenol concentrations (>0.8

Table 6 : Effect of sodium chloride concentration on kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M. For all experiments, initial phenol concentration and culture pH was set at 0.5 g l^{-1} and 7.5, respectively. The cultivation time for all experiments was 21 hr

Kinetics Parameters/ Performance	NaCl concentration (g l^{-1})						
	0	**0.05**	**0.1**	**0.15**	**0.2**	**0.25**	**0.3**
Maximum cell, X_m (g l^{-1})	0.069 $\pm$0.12^c	0.68 $\pm$0.12^c	0.125 $\pm$0.22^c	0.080 $\pm$0.14^c	0.109 $\pm$0.20^c	0.100 $\pm$0.32^c	0.082 $\pm$0.15^c
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	0.138	0.136	0.250	0.160	0.218	0.200	0.320
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	0.044	0.126	0.676	0.166	0.028	0.002	0.320
Residual phenol (g l^{-1})	0.478 $\pm$0.48^c	0.437 $\pm$0.44^c	0.162 $\pm$0.25^c	0.417 $\pm$0.51^c	0.486 $\pm$0.49^c	0.499 $\pm$0.50^c	0.484 $\pm$0.47^c
Phenol degraded (g l^{-1})	0.022	0.063	0.338	0.083	0.014	0.001	0.016
Phenol degradation rate (g l^{-1} hr^{-1})	0.001	0.003	0.016	0.004	0.0007	0.00005	0.00008
Percentage of phenol degradation (%)	4.4	12.6	67.6	16.6	2.8	0.2	32
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	0.319	0.093	2.704	1.038	0.128	0.01	0.195

Data were obtained from the time course of each fermentation run; Values are mean of three replicates $\pm$ SD; ^{c}Mean value in same row with different superscripts are significant different ($P<0.05$)

g l^{-1}). In comparison, *Acinetobacter* sp. and *Acinetobacter* AQ5NOL1 required more than 30 hr and up to 6 days to completely degrade phenol presence in the culture, respectively (Adav *et al.,* 2007; Ahmad *et al.,* 2011). On the other hand, *Candida tropicalis* required 50 hr to degrade 0.5 g l^{-1} phenol during the cultivation (Yan *et al.,* 2006). *Bacillus cereus* required more than 60 hr to degrade 60% of 0.5 g l^{-1} phenol presence in the culture (Benerjee and Ghoshal, 2010). A longer time (7 days) was required by *Rhodococcus* NO14-1 and NO20-3 to degrade 0.47 g l^{-1} phenol (Margesin *et al.,* 2005).

Results from this study also indicated that the degradation of phenol was not directly associated with growth of *Rhodococcus* UKMP-5M. Although the highest growth (0.27 g l^{-1}) was obtained in cultivation with high phenol concentrations (0.9 mg l^{-1}), but the time taken to degrade all phenol presence in the culture was more than 192 hr. In cultivation with very high initial phenol degradation (1.3 g l^{-1}), degradation of phenol was only started after 240 hr with a slight inhibition to growth. Growth of *Chlorella vulgaris* was greatly inhibited when phenol concentration in the culture was increased to only 0.4 g l^{-1} (Scragg, 2006).

Table 7 : Effect of initial phenol concentration on the kinetic parameters of phenol degradation by *Rhodococcus* UKMP-5M. For all experiments, the initial culture pH was set at 7.5 and not controlled during the degradation. The cultivation days for all experiments were 10 days

Kinetics Parameters/ Performance	**Phenol concentration (g l^{-1})**						
	0	**0.2**	**0.3**	**0.5**	**0.7**	**0.9**	**1.3**
Maximum cell, X_m (g l^{-1})	0.085 $\pm 0.08^{c}$	0.100 $\pm 0.09^{bc}$	0.121 $\pm 0.11^{bc}$	0.136 $\pm 0.12^{b}$	0.217 $\pm 0.18^{a}$	0.266 $\pm 0.19^{a}$	0.130 $\pm 0.09^{bc}$
Specific growth rate, μ (hr^{-1})	0.003	0.001	0.009	0.011	0.013	0.006	0.002
Specific degradation rate, P (hr^{-1})	-	0.088	0.111	0.169	0.193	0.003	0.04
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	-	0.028	0.114	1.04	0.142	13.9	1.6
Product yield, $Y_{p/s}$ (g product/g carbon) (g g^{-1})	-	0.98	0.99	0.962	0.959	0.011	0.007
Initial phenol (g l^{-1})	-	0.2	0.3	0.5	0.7	0.9	1.3
Residual phenol (g l^{-1})	-	0.004 $\pm 0.03^{c}$	0.004 $\pm 0.04^{c}$	0.019 $\pm 0.08^{c}$	0.029 $\pm 0.15^{c}$	0.890 $\pm 0.61^{b}$	1.290 $\pm 1.29^{a}$
Phenol degraded (g l^{-1})	-	0.196	0.296	0.481	0.671	0.01	0.01
Phenol degradation rate (g l^{-1} hr^{-1})	-	0.0008	0.0012	0.002	0.0028	0.00004	0.00004
Percentage of phenol degradation (%)	-	98.0	98.67	96.2	95.9	0.1	0.77
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	-	1.96	2.45	3.54	3.092	0.038	0.077

Data were obtained from the time course of each fermentation run; Values shown are mean of three replicates with ± SD; $^{a-c}$ Mean value in same row with different superscripts are significant different ($P<0.05$)

Table 8 : Effect of glucose concentration on growth of *Rhodococcus* UKMP-5M

Kinetics Parameters/ Performance	**Glucose concentration (g l^{-1})**								
	Control	**0**	**0.1**	**0.5**	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**
Maximum cell, X_m (g l^{-1})	0.115	0.291	0.275	0.392	0.416	0.426	0.638	0.512	0.393
Specific growth rate, μ (hr^{-1})	ND	0.099	ND	ND	ND	ND	0.153	ND	ND
Specific degradation rate, P (hr^{-1})	-	0.142	ND	ND	ND	ND	0.157		
Cell yield, $Y_{x/s}$ (g cell g^{-1} carbon) (g g^{-1})	-	0.058	2.75	0.784	0.416	0.284	1.154	0.205	0.131
Product yield, $Y_{p/s}$ (g product g^{-1} carbon) (g g^{-1})	ND	1.000	ND	ND	ND	ND	0.988	ND	ND
Residual glucose (g l^{-1})	-	-	0.096	0.492	0.992	1.494	1.909	2.404	2.911
Glucose consumed (g l^{-1})	-	-	0.004	0.008	0.008	0.006	0.091	0.096	0.089
Glucose consumption rate (g l^{-1} hr^{-1})	-	-	0.005	0.023	0.047	0.071	0.091	0.114	0.139
Percentage of glucose consumed (%)	-	-	96	98.4	99.2	99.6	95.45	96.16	97.03
Initial phenol (g l^{-1})	-	0.5	ND	ND	ND	ND	0.5	ND	ND
Residual phenol (g l^{-1})	-	0.000	ND	ND	ND	ND	0.06	ND	ND
Phenol degraded (g l^{-1})	-	0.5	ND	ND	ND	ND	0.494	ND	ND
Phenol degradation rate (g l^{-1} hr^{-1})	-	0.024	ND	ND	ND	ND	0.024	ND	ND
Percentage of phenol degradation (%)	-	100	ND	ND	ND	ND	98.8	ND	ND
Cell efficiency, P_m/X_m (g phenol g^{-1} cell) (g g^{-1})	-	1.72	ND	ND	ND	ND	0.774	ND	ND

ND: Not determined; Control: No additional glucose and phenol in M1 medium; 0 g l^{-1} Glucose: (0.5 g l^{-1} phenol was added to M1 medium); 2 g l^{-1} Glucose: (0.5 g l^{-1} phenol was added to M1 medium)

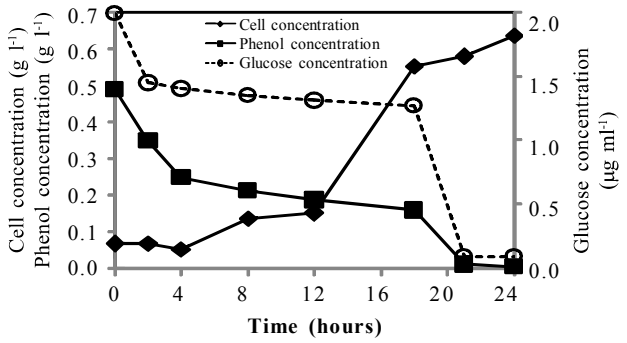

Fig. 2 : Effect of glucose on growth profile and phenol degradation of *Rhodococcus* UKMP-5M

Although *Rhodococcus* UKMP-5M was able to grow in glucose as the sole carbon source up to a concentration of 3 g l^{-1}, the highest cell concentration (0.570 g l^{-1}) was obtained at 2 g l^{-1} glucose (Table 8). Growth of *Rhodococcus* UKMP-5M was slightly inhibited at glucose concentration higher than 2 g l^{-1}, though almost all glucose presence in the culture was consumed. Phenol degradation by *C. Chrysogenum* was delayed in cultures containing high glucose concentrations due to competitive inhibition between substrate (Leitao *et al.,* 2007). A slight reduction in phenol degradation (98.8%) was observed in cultivation using 0.5 g l^{-1} phenol with the addition of 2 g l^{-1} glucose, though very high cell concentration (0.57 g l^{-1}) was achieved (Table 8). The cultures containing a supplementary carbon source grew faster with higher final cell concentration as compared to culture with phenol as the sole carbon source (Loh and Wang, 1998).

The percentage of phenol degradation in cultivation with 0.5 g l^{-1} phenol as the sole carbon source without any addition of glucose was 100%. The efficiency of cell to degrade phenol obtained in cultivation with only phenol and in cultivation in phenol with the addition of 2 g l^{-1} glucose was 1.72 g g^{-1} and 0.867 g g^{-1}, respectively. The interference of phenol uptake in the presence of glucose has been reported (Lob and Tar, 2000). A typical growth and phenol degradation profile during the cultivation of *Rhodococcus* UKMP-5M in phenol with the addition of glucose is shown in Fig. 2. All glucose added in the medium (2 g l^{-1}) was completely consumed after 21 hr of cultivation. During the cultivation, glucose and phenol were consumed concomitantly by *Rhodococcus* UKMP-5M.

Study on cell growth and phenol degradation by *Rhodococcus* UKMP-5M shows that this bacterium was able to degrade phenol and the performance was significantly influenced by the type of medium, culture conditions (temperature and pH) as well as type and concentration of nitrogen sources, salt, phenol and glucose added into the culture medium.

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