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Effects of Temperature and pH on Fluoranthene Biodegradation Kinetics by the New Strain *Rhodococcus baikonurensis* BAP-1

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An indigenous biosurfactant-producing bacterium with effective fluoranthene-degrading ability was isolated from crude oil-contaminated soil, which was identified as *Rhodococcus baikonurensis* BAP-1. Michaelis-Menten equation was used to characterize the degradation kinetics, and the present investigation examines the effect of pH and temperature on fluoranthene biodegradation kinetics. Contact time, temperature, pH, salinity, and inoculum level were used as independence variables to optimize the degradation process.2^5 full factorial, central composite design together with response surface methodology (RSM) was used to determine the optimize experiments conditions of these parameters. Under optimum condition, *Rhodococcus* sp. BAP-1 exhibited 63.56% fluoranthene. Correlation coefficient (R^2=0.976, $p<0.01$) indicated that empiric second-order polynomial model was adequate to predict. Meanwhile, lower temperature would decrease the activity of *Rhodococcus* sp. BAP-1 and cause lower biodegradation efficiency. *Rhodococcus* sp. BAP-1 would be more efficiency in biodegradation of fluoranthene under alkaline conditions. Hence, these results have significant implications be used to the bioremediationof PAHs contaminated environments.

Key words: *Rhodococcus* sp.BAP-1, fluoranthene, biodegradation, pH, temperature, optimization.

Polycyclic aromatic hydrocarbons (PAHs) are of global concern because of their toxic, mutagenic, teratogenic and carcinogenic effects. They are a typical type of persistent organic pollutants (POPs) and are also a class of petroleum pollutants (Bautista *et al.*, 2009). PAHs include a group of potential environmental pollutants and may be present at high concentrations around industrial sites associated with gas production, petroleum, coaltar preservation industries (Kumar *et al.*, 2011). Their distribution in the environment causes a significant health risk for human beings. They can enter the human body through inhalation, skin contact and ingestion. Exposure to PAHs has been linked to skin, lung, liver, intestinal and pancreatic cancers, and the toxicity of PAHs increases along with their molecular weight (Haritashand & Kaushik, 2009). Currently, more than 100 types of PAH compounds are known worldwide, and as of November 2013 the US.

Environmental Protection Agency (US EPA) had classified28 PAHs compounds as "priority pollutants". Hence, there is an increasing interest to remediate the sites contaminated with PAHs.

Compared to other treatments, microbial technology seems to be an efficient and economical choice in PAHs contaminated remediation process. The microbes can break the highly hydrophobic organic compounds into less complex metabolites through biotransformation process, then via the mineralization effect into inorganic minerals, such as H_2O, CO_2 (aerobic biodegradation) or CH_4

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(anaerobic biodegradation). Because of the hydrophobic feature, PAHs are mainly associated with organic matter in contaminated sites or existed as non-aqueous-phase liquids (NAPLs) (Woo *et al.*, 2004). So, compared to high molecular weight PAH (more than 3 aromatic rings), low molecular weight (2-3 aromatic rings) PAH are easily metabolized. Although many bacterial species, including *Sphingomonas*, *Mycobacterium*, *Brevibacillus*, *Bacillus*, *Pseudomonas*, *Rhodococcus* and *Burkholderia* have a well-known ability to degrade PAHs, but sometimes there were difficult to grow on the four rings PAHs such as pyrene and fluoranthene (Rehmann *et al.*, 1998). *Rhodococcus*species represent one of the most effective PAH-degrading bacterial genera. They are able to utilize PAHs such as phenanthrene, anthracene, benzo[a]pyrene and fluoranthene as carbon and energy sources (Song *et al.*, 2011). Adding non-ionic surfactants such as Tween 80 above the critical micelle concentration (CMC) can stimulate *Rhodococcus* to produce biosurfactants that then significantly enhance the biodegradation rate (Kolomytseva *et al.*, 2009). Additionally, some studies have investigated the stability of the biosurfactant produced by *Rhodococcus* under high-salt conditions and over a wide pH range(M. Shavandi *et al.*, 2011).

Generally, the success of bioremediation process was determined by many issues, including the species of microorganism and their degradation properties, types of the pollutants and environmental conditions for degradation (Gandolfi *et al.*, 2010). Therefore, finding the appropriate reaction conditions could enhance the rate of PAHs metabolism by microorganism and increase the removal rate of these contaminants from soils and other environments (Launen *et al.*, 1999). Classical method of optimization involves the change of "one-at-a-time-approach" which is expensive and extremely time-consuming when a number of variables are considered simultaneously (Rao *et al.*, 2007); moreover, they are essentially unable to reflect the relations among multiple parameters (Queiroga *et al.*, 2012). Statistical experimental designs using a response surface methodology (RSM), which includes factorial design and regression analysis, have been most widely applied to select optimum conditions of experiments (Puri *et al.*, 2002). Due to the variety of the advantage to their use, RSM have been widely employed to develop, improve and optimize the processes and evaluate the relative significant affecting factors (Liu *et al.*, 2010; El-Ghenymy *et al.*, 2012; Torres *et al.*, 2012).

In the present study, we investigated its potential to utilize fluoranthene as its sole carbon source. The effect of pH (5-9) and temperature (15-30) on fluoranthene biodegradation were evaluated. Also, the interactive impact of contact time, temperature, pH, salinity, and inoculum level were explored via RSM to optimize and enhance the degradation of fluoranthene by *Rhodococcus* sp. BAP-1 in MSM culture medium. Based on these statistical models, the results will be aid to optimize the biodegradation process and development of bioremediation of PAHs contaminated sites.

MATERIALS AND METHODS

Cultivation media and conditions

A *Rhodococcus* sp. BAP-1 inoculum from a nutrient agar plate was enriched in 100 ml Luria-Bertani (LB) culture medium (NaCl 5g·L^{-1}, yeast extract g·L^{-1}, tryptone 10g·L^{-1}) in a 250mL Erlenmeyer flask at 30±1°Con a shaking table (120 r·min^{-1}) for 48 h. Bacterial cells were collected by centrifugation (6000×*g*, 10 min), washed twice with mineral salts medium (MSM, pH=7.0), resuspended in sterile MSM and measured for 600 nm absorbance in a UV-visible spectrophotometer (Varian, Palo Alto, CA, USA). The final cell optical density (OD_{600}) value was adjusted to 1.5. Next, the cells were used as inoculum at 5% (v/v) in a 100 ml Erlenmeyer flask containing 50 ml of MSM. A stock solution of fluoranthene (1 g·L^{-1}) dissolved inacetone was added before inoculation as the sole carbon source. The composition of MSM was as follows (g·L^{-1}): 4.0 Na_2HPO_4; 1.5 KH_2PO_4; 1.0 NH_4Cl; 1.0 $NaNO_3$; 0.2 $MgSO_4 \cdot 7H_2O$; 0.02 $CaCl_2$; 0.03 $FeSO_4 \cdot 7H_2O$. One milliliter of a micronutrient solutionwas added, which contained (g·L^{-1}):0.005 $CuSO_4 \cdot 5H_2O$; 0.01 H_3BO_3; 0.01 $MnSO_4 \cdot 5H_2O$; 0.07 $ZnSO_4$; 0.005 $CoCl_2 \cdot H_2O$. The pH of the medium was adjusted to 7.0. Both the LB and MSM liquid culture media were sterilized by autoclaving at 121°C for 20 min.

Determination of residual fluoranthene

Remaining fluoranthene was extracted from the culture broth by extracting the whole flask

of broth (50mL) with an equal volume of n-hexane using an ultrasonic cleaning machine at 40 Hz for 30 min. The separated solvent layer was removed, and the lower aqueous phase was re-extracted in the same way. The combined extracts (2 mL) were filtered through a 0.2 µm membrane filter and transferred into an Agilent vial. Quantitative analysis of residual fluoranthene in the culture medium was performed by HPLC (U3000, DIONEX, USA) with UV detection at 254 nm. An aliquot (20 µl) was analyzed using an Agilent TC-C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase (water: acetonitrile 40:60v/v) was adjusted to 1.2 ml·min^{-1} at 25°C.

Degradation kinetics

Erlenmeyer flasks (100 mL) and MSM were each autoclaved for 20 min at 121°C. Different volumes of acetone (0.25, 0.5, 1.25, 2.5, 5, 15 mL) containing the required fluoranthene concentrations were added to autoclaved flasks to allow the acetone to evaporate. After the acetone evaporated completely, 50 mL sterile MSM was added, and different final concentrations of fluoranthene (5, 10, 25, 50, 10, 150 mg·L^{-1}) were used for the degradation kinetics studies. The flasks were inoculated with BAP-1 and incubated at 30°C at120 r·min^{-1} in a rotary shaker. Samples were aseptically collected at regular intervals.

Michaelis-Menten equation was used to characterize the degradation kinetics, in order to obtained a biodegradation activity in substrates at different concentrations and different contact times. The half-saturation constant (K_m) for degradation of fluoranthene was determined by the substrate depletion curves. From the data points, the specific substrate consumption rates were calculated at different fluoranthene concentrations and fitted to a Lineweaver-Burk plot. The maximum biodegradation rate (V_{max}) was determined by linear regression of the data points (Prenafeta-Boldú *et al.*, 2002).

Effect of pH value and temperature on fluoranthene biodegradation

Erlenmeyer flasks (100 mL) and MSM were each autoclaved for 20 min at 121°C. 1.25ml of acetone containing the required fluoranthene concentrations were added to autoclaved flasks to allow the acetone to evaporate. After the acetone evaporated completely, 50 mLsterile MSM was added, and final concentration of fluoranthene (20mg·L^{-1}) were used for the following studies. The flasks were inoculated with BAP-1 and incubated at120 r·min^{-1} in a rotary shaker. Samples were aseptically collected at regular intervals.

In order to study the effect of pH value, a series of MSM of fluoranthene concentration of 20mg·L^{-1} were carried out at 30°C at different pH values, as 5, 6, 7, 8 and 9, using inoculum level of 1% v/v fresh *Rhodococcus* sp. BAP-1 cells (corresponding to an optical density of 0.5 at 600 nm). Trial pH value was achieved by the addition of HCl (1 mol·L^{-1}) and NaOH (2 mol·L^{-1}). The original MSM pH value was 7.0. For each pH value, three independent measurements were made.

To study the effect of temperature on fluoranthene biodegradation by *Rhodococcus* sp. BAP-1, the various temperature was adjusted to 15, 20, 25and 30°C, and carried out at pH value of 7.0, using inoculum level of 1% v/v fresh *Rhodococcus* sp. BAP-1 cells (corresponding to an optical density of 0.5 at 600 nm). In different temperature conditions, three independent measurements were made.

Central composite designs and response surface analysis

A 2^5 full factorial, central composite design of RSM was employed to optimize the five most significant factors (contact time, temperature, pH, salinity and inoculum level) for enhancing the degradation of fluoranthene by *Rhodococcus* sp. BAP-1. The independent variables in terms of coded were presented in Table 3. Initial concentration of fluoranthene was 20 mg·L^{-1}. The experimental design used for study and statistical results are shown in Table 4 and Table 5, respectively. A second-order polynomial function was carried out for obtaining an empirical model fitted to the degradation of fluoranthene (Li *et al.*, 2009).

Statistical analysis and software

The statistical software package Design-Expert, Version 8.0.6 was used for the experimental designs and analyze the central composite design. The information of model fitting was estimated by the analysis of variance (ANOVA) and multiple linear regressions. The quality of fit explained by the model was given by the multiple coefficient of determined R squared (R^2) value, a good coefficient value accepted for biological sample was R^2>0.7 (Lundstedt *et al.*, 1998). The statistical significance of the model equation was determined via Fisher's

test (*F*-test) value. The general form of the second-order polynomial equation was employed to explained fluoranthene degradation (Eq.1) in five independent variables.

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii}X_i^2+\sum_{i=1}^{k-1}\sum_{j=2}^{k}\beta_{ij}X_iX_j+\varepsilon$$

Where Y is the predicted response, X_i and X_j are the independence variables, β_0 is the intercept term, β_i is the linear effect, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient and ε is a random error (Amini *et al.*, 2008).

RESULTS AND DISCUSSION

Degradation kinetics

Rhodococcus, aGram-positive bacterium, has a well-known capability to degrade PAHs. Previous studies by Mutalik *et al*. (Mutalik *et al.*, 2008) demonstrated that among *Rhodococcus* spp. this ability is often accompanied by the production of biosurfactant and trehalose lipids, which are the most important groups in the process of PAH utilization (Franzetti *et al.*, 2010). The isolated strain BAP-1 has the ability to mineralize fluoranthene when it was the sole carbon and energy source.

Degradation kinetics of contact times, present at different fluoranthene concentrations were studied (Fig. 1). Lineweaver-Burk plots were obtained for the depletion of fluoranthene at different contact times were illustrated in Fig. 2. Also, Michaelis-Menten kinetic parameters were determined (Table 1). When concentrations of substrate were higher (100 and 150 mg·L^{-1}), which caused higher degradation rates, especially at first 24h. These finding indicated that fluoranthene at all concentrations series had a drastically decrease at first 24h. Meanwhile, from Michaelis-Menten kinetic parameters which were determined in Table 1, in the first 12h, V_{max} value was 4.068 mg/L/h, which was significant higher than that in other time series. While, the K_m value for fluoranthene degradation appeared to the highest of 285.5. Additionally, the lowest K_m value for fluoranthene degradation appeared at 144h, which were 24.7.

The Michaelis-Menten model provided a good description of the degradation kinetics in batch experiments under different fluoranthene concentrations. The V_{max} obtained under each time series were consistent with the degradation pattern. At first 12h V_{max} was 4.068 mg·L^{-1}·h^{-1}, which indicated that at first 12h, biodegradation efficiency of fluoranthene increased significantly. This phenomenon can also be found from the biodegradation kinetics of fluoranthene, observed at various initial pH and temperature. Fig. 3 and Fig. 4 illustrated that in the first 12h,

Most studies have shown that neutral condition (pH=7.0) have a positive impact on PAHs biodegradation. For instance, Walter *et al.* (Walter *et al.*, 1991) indicated that pH 7.0 was determined to be optimal for microbial growth and pyrene degradation by *Rhodococcus* sp. UW1.

Effect of pH and temperature on fluoranthene biodegradation

Fig. 3 illustrated the effect of different

Table 1. Michaelis-Menten kinetic parameter for the degradation of fluoranthene by *Rhodococcus* sp. BAP-1

Time (h)	V_{max}(mg/L/h)	K_m	R^2	V_{max}/K_m
12	4.068	285.5	0.9987	0.014
24	1.387	79.18	0.9657	0.017
72	1.331	186.4	0.9988	0.007
120	0.486	97.2	0.9932	0.005
144	0.144	24.7	0.9996	0.006
168	0.501	121.4	0.9972	0.004

Table 2. 2^5 full factorial, central composite design

Coded parameters	Description	Actual values corresponding to coded value				
		-2	-1	0	1	2
X_1	Contact time/d	5	6	7	8	9
X_2	Temperature/!	21	24	27	30	33
X_3	pH	6	7	8	9	10
X_4	Salinity/%	0	1	2	3	4
X_5	Inoculum level/%	0.5	1	1.5	2	2.5

Table 3. 2^5 full factorial, central composite design for optimization of degradation of fluoranthene: experimental matrix (in coded form) and results of degradation (observed and predicted)

Exp.	Run order	Coded parameters					Degradation efficiency/%	
		X_1	X_2	X_3	X_4	X_5	Observed	Predicted
1	32	1	-1	-1	-1	-1	24.39	24.89
2	18	-1	-1	-1	-1	-1	23.70	23.86
3	17	1	1	-1	-1	-1	35.05	35.27
4	12	-1	1	-1	-1	-1	42.13	43.58
5	36	1	-1	1	-1	-1	34.19	35.54
6	50	-1	-1	1	-1	-1	36.52	37.42
7	8	1	1	1	-1	-1	25.28	25.81
8	23	-1	1	1	-1	-1	35.38	37.03
9	49	1	-1	-1	1	-1	36.58	37.44
10	47	-1	-1	-1	1	-1	29.24	31.03
11	22	1	1	-1	1	-1	45.25	45.06
12	3	-1	1	-1	1	-1	48.56	48.00
13	10	1	-1	1	1	-1	37.37	34.21
14	34	-1	-1	1	1	-1	30.41	30.72
15	9	1	1	1	1	-1	21.83	21.72
16	25	-1	1	1	1	-1	27.28	27.57
17	28	1	-1	-1	-1	1	40.61	41.20
18	42	-1	-1	-1	-1	1	29.08	30.91
19	43	1	1	-1	-1	1	42.42	43.27
20	11	-1	1	-1	-1	1	28.66	42.32
21	35	1	-1	1	-1	1	63.28	63.56
22	15	-1	-1	1	-1	1	54.07	56.18
23	44	1	1	1	-1	1	44.82	45.51
24	24	-1	1	1	-1	1	48.23	47.48
25	26	1	-1	-1	1	1	48.68	48.59
26	6	-1	-1	-1	1	1	33.37	32.92
27	45	1	1	-1	1	1	48.15	47.90
28	1	-1	1	-1	1	1	40.97	41.57
29	16	1	-1	1	1	1	56.44	57.07
30	48	-1	-1	1	1	1	40.02	44.32
31	39	1	1	1	1	1	36.46	36.26
32	41	-1	1	1	1	1	31.68	32.86
33	27	-2	0	0	0	0	41.36	42.23
34	14	2	0	0	0	0	42.90	37.79
35	7	0	-2	0	0	0	12.59	10.75
36	20	0	2	0	0	0	12.08	9.67
37	13	0	0	-2	0	0	50.17	46.81
38	2	0	0	2	0	0	49.62	48.74
39	30	0	0	0	-2	0	46.81	40.92
40	33	0	0	0	2	0	37.20	38.85
41	38	0	0	0	0	-2	37.62	36.75
42	5	0	0	0	0	2	61.72	58.35
43	37	0	0	0	0	0	28.51	29.04
44	4	0	0	0	0	0	28.71	29.04
45	19	0	0	0	0	0	28.44	29.04
46	46	0	0	0	0	0	28.29	29.04
47	29	0	0	0	0	0	28.59	29.04
48	31	0	0	0	0	0	28.32	29.04
49	21	0	0	0	0	0	28.51	29.04
50	40	0	0	0	0	0	28.72	29.04

initial pH on fluoranthene biodegradation kinetics. It's obvious that the optimum pH value for fluoranthene degradation by *Rhodococcus* sp. BAP-1 was 8.0. This indicated that the pH value of 8.0 recorded to the highest biodegradation rate, while the minimum one was at pH value of 5.0.Under such condition, besides about 10% loss in fluoranthene was attributed to abiotic factors, the removal efficiency of fluoranthene was about 53.41%. The results showed that *Rhodococcus* sp. BAP-1 would be more efficiency under alkaline conditions. Fig. 3 indicated the effect of temperature on kinetics of fluoranthene biodegradation. The optimum temperature for fluoranthene biodegradation was 25°C. Fluoranthene biodegradation rate was decreased at lower temperature (15°C). Under optimum condition, besides about 10% loss in fluoranthene was attributed to abiotic factors, the removal efficiency of fluoranthene was about 54.04%.

In addition, in the study by Boldrin et al. (B. Boldrin *et al.*, 1993), *Mycobacterium* sp. had an exponential growth in phenanthrene, fluoranthene, pyrene and fluorene when pH value was adjusted to 7.0. Fig. 2 showed that fluoranthene degradation was strongly dependent on the pH value. In the present study, with the reduction of pH value to 5.0 and 6.0, the biodegradation of fluoranthene was significantly slower, which caused lower biodegradation rates and a long period of lag phase (48h to 144h). The extent of degradation efficiency ranged from 27.7% at pH 5.0 to 53.4% at pH 8.0, which indicated that *Rhodococcus* sp. BAP-1 would be more efficiency in biodegradation of fluoranthene under alkaline conditions. It agreement with Gerbeth et al. (A. Gerbeth *et al.*, 2004) who studied the degradation of PAHs by microorganisms under alkaline conditions. Which illustrated alkaline conditions were determined to be optimum for PAHs

Table 4. 2^5 full factorial, central composite design for optimization of degradation of fluoranthene: statistical analysis of the model with highest individual coefficients upon fluoranthene degradation (R^2=0.9755; adj. R^2=0.9585; pred. R^2=0.9016; adeq precision=36.735).

Source	*SS*	*df*	*MS*	*F*	*p*
Model	5905.87	20	295.29	57.63	<0.0001
X_1	49.33	1	49.33	9.63	0.0042
X_2	2.93	1	2.93	0.57	0.4559
X_3	9.33	1	9.33	1.82	0.1876
X_4	10.75	1	10.75	2.10	0.1581
X_5	1166.18	1	1166.18	227.60	<0.0001
X_1X_2	174.66	1	174.66	34.09	<0.0001
X_1X_3	17.02	1	17.02	3.32	0.0787
X_1X_4	57.67	1	57.67	11.26	0.0022
X_1X_5	171.40	1	171.40	33.45	<0.0001
X_2X_3	808.82	1	808.82	157.86	<0.0001
X_2X_4	15.21	1	15.21	2.97	0.0956
X_2X_5	138.28	1	138.28	26.99	<0.0001
X_3X_4	385.31	1	385.31	75.20	<0.0001
X_3X_5	274.37	1	274.37	53.55	<0.0001
X_4X_5	53.25	1	53.25	10.39	0.0031
X_1^2	240.53	1	240.53	46.94	<0.0001
X_2^2	709.02	1	709.02	138.38	<0.0001
X_3^2	701.74	1	701.74	136.96	<0.0001
X_4^2	235.08	1	235.08	45.88	<0.0001
X_5^2	684.98	1	684.98	133.69	<0.0001
Residual	148.59	29	5.12		
Lack of fit	148.41	22	6.75	262.51	<0.0001
Pure error	0.18	7	0.026		
Cor total	6054.46	49			

biodegradation by some of the bacterial strain, such as *Pseudomonas* sp., *Rhodococcus* sp. and *Micrococcus* sp.

Fig. 4 showed the effect of temperature on kinetics of fluoranthene biodegradation. The optimum temperature for the rate of fluoranthene biodegradation was 25°C. Usually, lower temperature caused a negative impact on PAHs biodegradation. Studies made by Eriksson *et al.* (Eriksson *et al.*, 1999) indicated thatbiodegradation efficiency of naphthalene by indigenous microorganisms was almost 100% at 20°C while only 10% at 6°C. In the present study, when the temperature was reduced to 15°C, the biodegradation rate was considerably slower, and caused long periods of lag phase. The lower biodegradation efficiency at lower temperature related to the slower enzyme activity with the decreasing temperature (Sartoros *et al.*, 2005). Almost 54.4% of total fluoranthene were degraded within 144h at 25°C while only down to 10% of initial concentration at 15°C, which indicated that lower temperature would decrease the activity of *Rhodococcus* sp. BAP-1 and cause lower biodegradation efficiency.

Optimization of significant variables using response surface methodology

A 2^5 full factional, central composite design was applied to examine of various cultural conditions on enhance fluoranthene degradation by *Rhodococcus* sp. BAP-1, using the appropriate levels of contact time, temperature, pH, salinity and inoculum level, which was describe in Table 2. A total of 50 runs factional design with eight center points, expanded with a group of axial points were attempted. The different combination of contact time (X_1), temperature (X_2), pH (X_3), salinity (X_4) and inoculum level (X_5) was designed in Table 3. The observed and predicted degradation efficiency of the fifty experiments was also presented in Table 3. A second-order polynomial function explained the dependences of variables factors for fluoranthene degradation rates was consequently fitted to the experiment using the coded factor was given as:

$$Y = 29.04 - 1.11X_1 - 0.27X_2 + 0.48X_3 - 0.52X_4 + 0.54X_5 + 2.34X_1X_2 + 0.73X_1X_3 - 1.34X_1X_4 - 2.31X_1X_5 - 5.03X_2X_3 - 0.69X_2X_4 - 2.08X_2X_5 - 3.47X_3X_4 + 2.93X_3X_5 - 1.29X_4X_5 + 2.74X_1^2 - 4.17X_2^2 + 4.68X_3^2 + 2.71X_4^2 + 4.63X_5^2$$

A p value of less than 0.05 indicated that the model was considered to be statistically significant at >95% level (Ghevariya *et al.*, 2011). Based on the examination analyzed by Design-Expert, the results of variance were present in Table 3. The fit of the model was checked by the significant of each coefficient in the equation which was calculated by the analysis of variance (Table 5). F-value of the model was 57.63 while the low probability value (p-value) was less than 0.0001

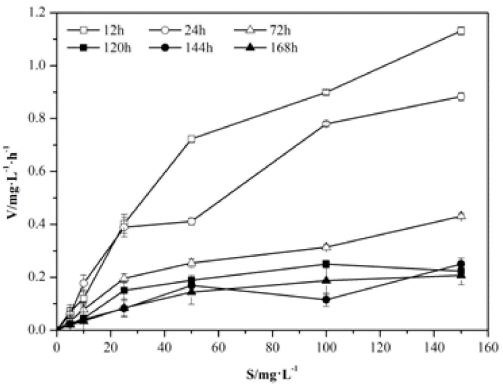

Fig. 1. Concentrations-dependent biodegradation of fluoranthene at different time series by *Rhodococcus* sp. BAP-1

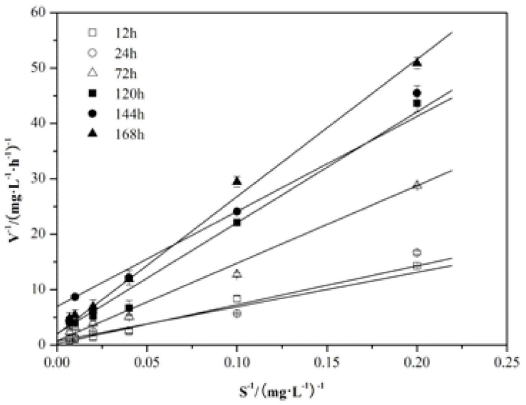

Fig. 2. Lineweaver-Burk plot from fluoranthene depletion concentrations of fluoranthene at all batch experiments had a drastically decrease. Michaelis-Menten s constant (K_m) was always used for characterization of the affinity between substrates and enzyme. Comparisons of the K_m values for degradation of fluoranthene, when contact time was 144h,curves at different time series by *Rhodococcus* sp. BAP-1.the affinity seemed much higher than others, which indicated that after 144h reactions, the biodegradation process tended to be stable

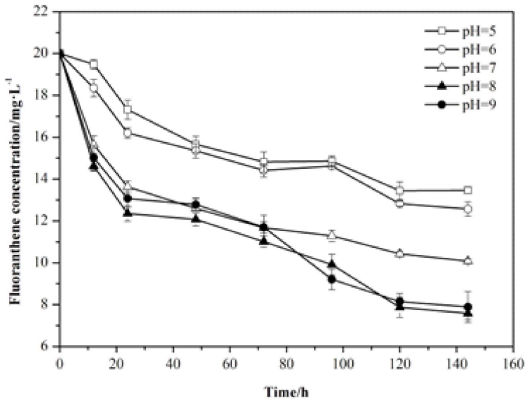

Fig. 3. Biodegradation kinetics of fluoranthene, observed at various initial pH

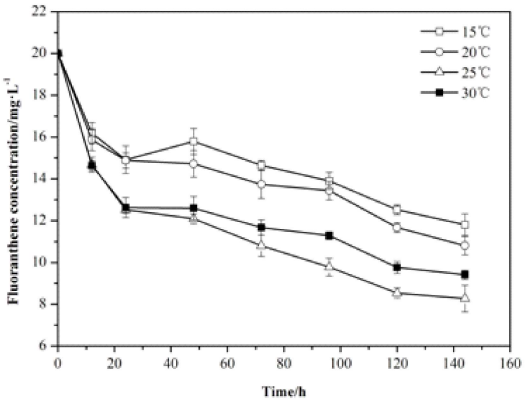

Fig. 4. Biodegradation kinetics of fluoranthene, observed at various temperature

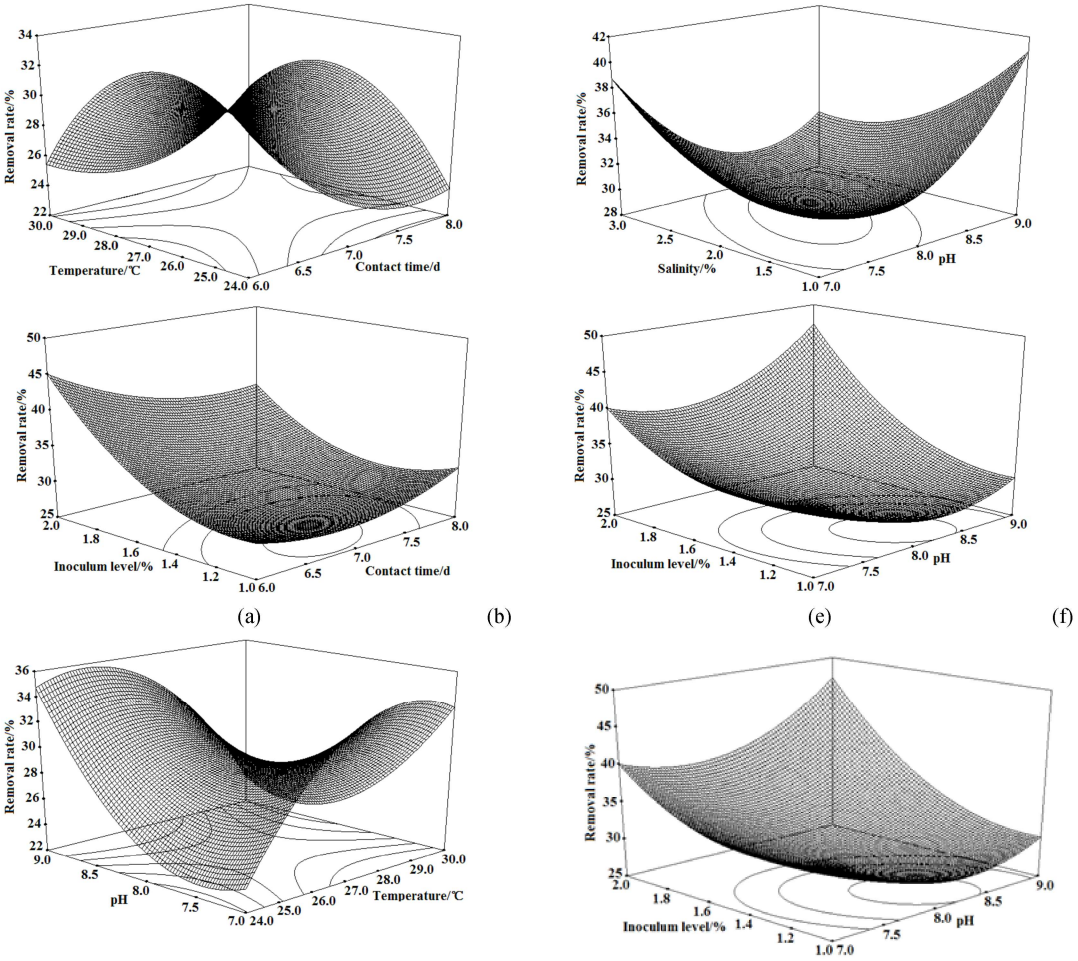

Fig. 5. Response surface curve for fluoranthene degradation by *Rhodococcus s*p. BAP-1, in terms of interaction between (a) contact time and temperature, (b) contact time and inoculum level, (c) temperature and pH, (d) temperature and inoculum level, (e) pH and salinity, (f) pH and inoculum level

implied the model was significant. And there was only a 0.01% chance that the model *F*-value this large could occur due to the noise. Determined coefficient R^2 was calculated to be 0.9755, which applied that 97.55% of the variability in the response could be explained by the model. Therefore, it could be concluded that the quadratic model postulated was adequate. Values of '*Prob>F*' less than 0.05 indicated the model terms were significant and below 0.01 indicated very significant. So, in this case, presented in Table 5, the quadratic factor of inoculum level (X_5) was significant ($p<0.01$). Among the two-factor interaction, the contact time × temperature interaction (X_1X_2), contact time × inoculum level interaction (X_1X_5), temperature × pH interaction (X_2X_3), temperature × inoculum level interaction (X_2X_5), pH × salinity interaction (X_3X_4) and pH × inoculum level interaction (X_3X_5) were significant ($p<0.01$); these results indicated that interaction effects occurred between the two variables, which applied the extent of one variable effect upon to fluoranthene degradation depended also on the actual level of another one. All factors quadratic effect were significant ($p<0.01$), but the linear effect of temperature, pH and salinity was negligible, which indicated the marginal impact of the low level of them, which increased fast when the level was higher (Queiroga *et al.*, 2012).

Three-dimensional graphical representation the response surface curve were plotted to demonstrate the combined effects between the two-factor interaction variables on fluoranthene degradation. All the interaction between the selected variables were mentioned significant ($p<0.01$) before. Meanwhile, the optimum level of each variable for maximum was determined (Fig.5). Each figure presented the effect between two factors while other factors were held at zero level. As it's shown in Fig.5 (b) (c) (d) a curve increased in removal rate was observed when the inoculum level and pH increased. However, as it's shown in Fig.5 (a) (e) (f) with temperature and contact time, salinity and pH, inoculum level and pH, the removal rate could not increase further with the increasing of these factors. Several directions would lead to the improvement and the treatment combinations would even lead to better results. This optimization strategy successfully enhanced the degradation efficiency of fluoranthene from 9.67% to 63.56%, which emphasized the practicability of this model.

Growth linked fluoranthene degradation study after optimization experiments

According to the analysis, the results predicted by the model presented that the optimum fluoranthene degradationcould be achieved when the contact time, temperature, pH, salinity and inoculum level were set at 6 d, 24°C, 9.0, 1.0% and 2%, respectively. Under the optimized condition, fluoranthene degradation percentages were 63.28% (observed) and 63.56% (predicted). Based on the results, to confirm the model adequacy for predicating the maximum fluoranthene degradation efficiency, the additional experiments were conducted in triplicate and the initial concentration of fluoranthene was set as 20 $mg \cdot L^{-1}$. The three replicate experiments yieldedaverage optimum fluoranthene degradation efficiency 64.43%, which was in good agreement with the predicted value. Hence, this model developed was considered to be reliable for the prediction of fluoranthene degradation by *Rhodococcus* sp. BAP-1. Hence, *Rhodococcus* sp. BAP-1 can be further used for its potential to remediate PAHs contaminated environments.

This work indicated that *Rhodococcus* sp. BAP-1 was capable of growing in fluoranthene-containing culture medium. According to the analysis, the optimum fluoranthene degradation could be achieved when the contact time, temperature, pH, salinity and inoculum level were set at 6 d, 24°C, 9.0, 1.0% and 2%, respectively. These levels were similar as the results we analyzed before. When contact time was 144h, the affinity seemed much higher and the process tended to be stable. However, lower temperature would decrease the activity of *Rhodococcus* sp. BAP-1 and cause lower biodegradation efficiency. Meanwhile, *Rhodococcus* sp. BAP-1 would be more efficiency in biodegradation of fluoranthene under alkaline conditions.

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