ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

The Plackett-Burman Model-Optimization of Significant Nutritional Parameters for Petroleum Bioremediation by *Pseudomonas sp.*

***Shreyasri Dutta, Padma Singh**

Department of microbiology, Kanya Gurukul Campus, Gurukul Kangri University, Haridwar-249407

Manuscript Info

Manuscript History:

Received: 15 December 2013
Final Accepted: 29 December 2013
Published Online: January 2014

Key words:
Plackett-Burman design, bioremediation, *pseudomonas sp.*

Abstract

It is worthwhile to mention that Plackett-Burman design is useful not only in evaluating the significant of some variables on the bioprocess, but also in comparing between different categories. Hence maintain a comprehensive evolution of the overall process. In this study Plackett-Burman experimental design had been applied to evaluate the significant of cultural condition affecting petroleum bioremediation by isolated *pseudomonas sp*, seven variables through eight trials were studied simultaneously, namely Temperature, pH, Peptone, KH_2PO_4, K_2HPO_4, NaH_2PO_4, Na_2HPO_4 to elucidate their effect . Based on bacterial growth by taking O.D at 600 nm, Temperature, Na_2HPO_4 were recognized as the most effective factors. The regression coefficient R^2 (0.99) ensure the adequate integrity of the model. The enhanced growth capability with appropriate cultural condition in presence of only signal carbon source as petrol of *Pseudomonas sp.* by optimizing the tools suggests a suitable method not only in evaluating the significance of some variables but also in comparing between different categories, in which was difficult to compare between their effects in conventional experiments, and hence maintain a comprehensive evaluation of the overall process.

Introduction

Due to industrialization and over use of petroleum hydrocarbon based refinery products are one of the most prevalent pollutants. The bioremediation of hydrocarbon in the natural environment is a slow process. The major factor responsible for this is the nutritional imbalance created by the oil spills and growth limiting nitrogen and phosphorus concentration (Le Petit and Barthelemy, 1968). The successful application of bioremediation depends on appropriate biodegrading microbes and environmental parameters. In OVAT method (the change of one variable at a time method) a single factor is varied while other factors are kept fixed at a specific set of condition. This method leads to unreliable results and time consuming. Therefore, the screening design is appropriate to face the large number of cultural condition under investigation. Plackett-Burman design (Plackett and Burman, 1944) comprises one type of two-level screening design. It is favored to detect the significant factors affecting the process before proceeding to the optimization stage of experimental design. (Abdel- Fattah R Yasser et al, 2002).

The aim of this work is to investigate the bioremediation of petroleum oil by isolated *pseudomonas sp.*, and applying a through optimization process. The objective was to evaluate the cultural condition. This is needed to develop a near optimal medium in order to enhance the bioremediation process by means of statistically designed experiments.

Material and Methods

Media and Chemicals

For bacterial isolation purpose mineral salt medium was used which containing following composition [in (g/L)]: KNO3 (1), $MgSo_4.7H_2O$ (1), $CaCl_2.6H_2O$ (0.1), $FeSO_4$ (0.05), trace element sol, 250ml; phosphate buffer (1M; pH-6.8), 20mL; and distilled water, 980mL. Trace element solution comprised(g)- $SnCl_2$(0.05); KI(0.05), LiCl(0.05), $MnSO_4.4H_2O$(0.08), HBO_3(0.05), $ZnSO_4.7H_2O$(0.10), $CoCl_2.6H_2O$(0.10),$NiSO_4.6H_2O$(0.10), $BaCl_2$(0.05),Ammonium molybdate (0.05) and distilled water 1000mL (All salts were dissolved in defined sequence only). (R Whittenburry et al 1970) Nutrient agar medium (NAM) containing peptone (5.0g), Beef extract (3.0g/L) sodium chloride (5.0 g) distilled water (1000ml) and pH 7.3 was used for store the bacterial pure culture at 4^0C and sub cultural purpose. Bushnell-Haas (BH) broth containing $MgSO_4$ (0.2 g/l), $CaCl_2$ (0.02 g/l), KH_2PO_4 (1 g/l), K_2HPO_4 (1 g/l), $FeCl_2$ (0.05 g/l), NH_4NO_3 (1 g/l) and pH (7), redox reagent (2% 2, 6-dichlorophenol indophenols) (Hanson et al, 1993) were used for the screening test.

Microorganism

Pseudomonas sp was isolated from petroleum contaminated soil sample from INDIAN OIL of Haldia petroleum refinery, west Bengal using the mineral salt medium (MSM) (Whittenburry *et al*., 1970). Identification was carried out on the basis of morphologcal and biochemical test according to the Bergey's Maual of Determination of Bacteriology (Holt *et al.,* 1994).

Conventional method for optimization of significant nutritional parameters

A modified S naveenkumar *et al*. (2010) conventional method was performed where *Pseudomonas sps* was taken in an inoculums and measure the optimum condition according to their growth on various temperature range (28^0C, 37^0C, 42^0C) , pH range (5,7,9), nitrogen sources (Peptone, $NaNO_3$,KNO_3,NH_4NO_3), phosphorous sources (KH_2PO_4,K_2HPO_4,NaH_2PO_4,Na_2HPO_4). Test of each nutritional parameters the previous nutritional parameter was fixed in which parameter maximum bacterial growth was observed. In all above case the carbon source was fixed as petrol.

Statistical method for optimization of significant nutritional parameters

Based on growth of *pseudomonas sps* in the prelimary experiment, seven variables (Tempeterure, pH, Peptone, KH_2PO_4, K_2HPO_4, NaH_2PO_4 and Na_2HPO_4) were selected as the various nutritional parameters for Plackett-Burman design in this study. The concentrations for the different variables were selected according to some preliminary experiments (data not shown). The above said seven variables affecting the growth were screened at two levels, maximum (+) and minimum (-) [Table1]. This said concentrations were mixed in 100 ml distilled water and adds 1ml inoculums and 0.1ml petrol. Now experiments were carried out as per the design in Table 2.They were performed in duplicates and the average growth was taken as the response (Y).Growth (i.e O.D) was read at 600 nm after 72 hours.

Table 1. Coded value and actual values of the variables.

Variables	Coded levels	
	-1	**+1**
Temperatur	37	42
pH	5	7
Peptone	.001%(w/v)	1.5% (w/v)
KH_2PO_4	.005%(w/v)	2%(w/v)
K_2HPO_4	.001%(w/v)	2%(w/v)
NaH_2PO_4	.001%(w/v)	1%(w/v)
Na_2HPO_4	.005%(w/v)	1.55%(w/v)

Table 2. Two-level Plackett-Burman design for *pseudomonas sp'*s growth

Experiments	Temperature	pH	Peptone	KH_2PO_4	K_2HPO_4	NaH_2PO_4	Na_2HPO_4	O.D
1	+	+	+	-	+	-	-	0.080
2	-	+	+	+	-	+	-	.043
3	-	-	+	+	+	-	+	.051
4	+	-	-	+	+	+	-	.030
5	-	+	-	-	+	+	+	.023
6	+	-	+	-	-	+	+	.062
7	+	+	-	+	-	-	+	.028
8	-	-	-	-	-	-	-	.026

Plackett-Burman experimental design which is based on the linear first order regression model offers a good and fast screening procedure and gives the effect of change of more than one factor in single experiment. $Y=\alpha +\Sigma\beta iXi$ Where Y is the O.D. α is the intercept, βi is the coefficient of the variable "i" estimates, Xi is the independent variable and "i" is the variable number.

The effect of each variable was calculated using the following equation.

$E=(\Sigma M+ - \Sigma M-) / N$

Where E is the effect of tested variable, M+ and M- are responses (O.D) of trials at which the parameter was at its higher and lower levels respectively and N is half the number of trials carried out, SE (Standard error) is calculated by using student's t-test.

Result and Discussion

The Plackett-Burman design method was applied to estimation the growth of *pseudomonas sp* by eight runs as shown in Table 2 were performed. Run 1 resulted in the highest O.D and run 5 shows lowest growth. Regression analyses of the results were given in Table 3.

Table 3. Regression analyses of Plackett-Burman design

Paremeters	Values
R^2	0.9912
Adjusted R^2	0.9693
Predicted R^2	0.8596
Adequate Precision	17.630

The Model F-value of 45.17 implies the model was significant. There was only a 2.18% chance that a "Model F-Value" this large could occur due to noise. In addition, R^2 (a measure of the goodness of fit of the model) was very much significant at the level of 99% meaning that the model was unable to explain only 1% of the total variations. The adjusted R^2 value of 0.96 also indicates the significance of the model to describe the experimental observations. Adequate precision, which measures the signal to noise ratio, shows value of 17.63 (A ratio greater than 4 was desirable) indicating an adequate signal. These results illustrate that the model could be used to navigate the design space.

Results of analysis of variance (ANOVA) were shown in Table 4. Two factors, i.e. temperature, Na_2HPO_4 were recognized as the most effective factors on *pseudomonas sps*'s growth respectively, based on the criterion of P-values less than 0.05.

Table 4: ANOVA Results of Plackett-Burman design using 7 factors

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2.852E-003	5	5.703E-004	45.17	0.0218
A-TEMP	4.061E-004	1	4.061E-004	32.17	0.0297
B-pH	1.201E-004	1	201E-004	9.51	0.0910
C- peptone	1.512E-005	1	1.512E-005	1.49	0.4365
D-KH_2PO_4	1.013E-005	1	1.013E-005	0.67	0.5635
E-K_2HPO4	5.513E-005	1	5.513E-005	4.37	0.1718
F-NaH_2PO4	1.901E-004	1	1.901E-004	15.06	0.0604
G-Na_2HPO4	2.080E-003	1	2.080E-003	164.76	0.0060

Table 5 shows that Na_2HPO_4 had a highest contribution where as Peptone and KH_2PO_4 had a lowest contribution for the growth of *pseudomonas sp*. One could see that the effect of various factor in Pareto chart (Fig.1), this shows that pH, peptone NaH_2PO_4 had a positive role where as other factors were not. In Figure 2 it was clear that experimental value were closer to predicted values.

Table 5: Showing different coded variables and their contribution for the growth of *pseudomonas sp*

Term	Stdized effect	Sum of Squares	% contribution
A-Temp	-0.014	4.061E-004	14.12
B-pH	7.750E-003	1.201E-004	4.18
C-Peptone	2.750E-003	1.513E-005	0.53
D-KH_2PO_4	-2.250E-003	1.012E-005	0.35
E-K_2HPO_4	-5.250E-003	5.512E-005	1.92
F-NaH_2PO_4	9.750E-003	1.901E-004	6.61
G-Na_2HPO_4	-0.032	2.080E-003	72.31

The experimental growth of *pseudomonas sp.* corroborated the validity and effectiveness of this method to be useful for petrol bioremediation. *Pseudomonas sp.*,is the most common bacterial hydrocarbon degrader reported in the literature which is widespread in nature and can degrade a wide range of petroleum degradation.

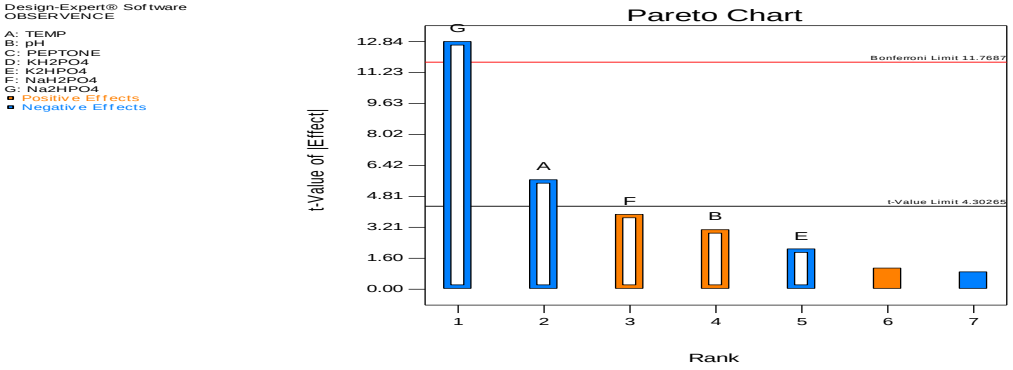

Fig 1: Showing relationship between t-value of effect and various parameters.

Similar strain was isolated by Mandri and Lin, 2007 and Salem L.B et al, 2011 and optimization was performed by S Naveen kumar et al 2010. This result implies that it can be a good degrader in presence of significant nutritional and physical parameter with in a very short period of time.

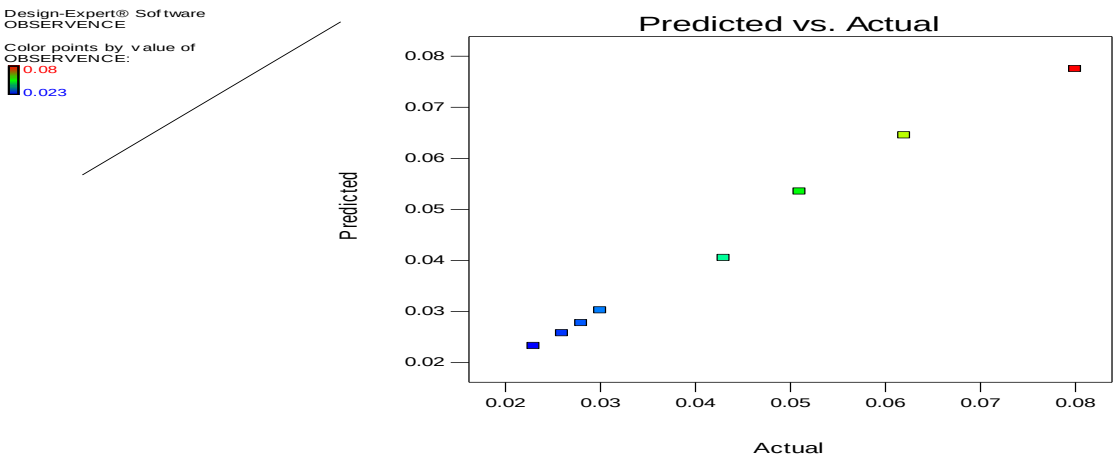

Fig 2: showing the relationship between predicted and actual value.

In the present study optimization of nutrients employing Plackett-Burman design for the bioremediation of petroleum proved to be useful. Hence the application of statistical experimental designs for optimization process is highly advantageous, relatively simple and time saving**.**

References

Addel-Fattah R Yasser, Hussein M. Hany, (2002). Numerical modeling of petroleum oil bioremediation by a local penicillium isolate as affected with culture conditions: application of Plackett-Burman design,*Arab J. Biotech, vol. 5, No(2) July: 165-172.*

Hanson KG, Desai JD, Desai AJ (1993). A rapid and simple screening technique for potential crude oil degrading microorganisms.*Biotechnology Techniques, , 7:745-748.*

Holt JG, Krieg NR, Sneath PHA, Stally JT. Williams ST (1994). Bergey's Manual of Determinative Bacteriology [M]. Ninth Edition.Baltimore, Williams and Wikins. Pp. 73, 129, 190-191.253.

Le-Petit J, Barthelemy MH (1968). The hydrocarbon in water: the problem of microorganisms using the littoral zones. *Ann. Inst. Pasteur, Paris 144:149-158.*

Mandri ,T and Lin,J.(2007). Isolation and characterization of engine oil degrade indigenous microorganisms in KwazuluNatal, SouthAfrica. *African journal of Biotechnology 6(1) : pp 23027.*

Plackett R. L. and Burman J. P. (1944). The design of optimum multifactorial experiments, *Biometrca, 33, 305-325.*

S Naveen kumar, Manoharan. N , Ganesan. S , Manivannan.S.P, Velsamy. G (2010). Isolation , screening and in vitro mutational assessment of indigenous soil bacteria for enhanced capability in petroleum degradation, *international journal of environtal sciences, vol 1 ,No 4.*

Salem L.B, Obayori O.S and G.O. Okogie, (2011). Biodegradation of bonny light crude oil by bacterial isolated from contaminated Soil .*Int. J. Agric. Biol., 13: 245–250*

Whittenburry R, Phillip K C and Wilkinson J E, (1970). Enrichment, Isolation and some properties of methane utilizing bacteria, *J Gen Microbiol, 61, 205.*