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Plackett-Burman Design of Environmental and Nutritional Parameters for Petroleum Bioremediation by *Penicillium chresogenum*

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Abstract

Bioremediation of diesel oil using microbial species is a promising method to remedy contaminated soil using *Penicillium chresogenum*. In this study, Plackett–Burman experiment is a new model for diesel oil degradation. It may save time and efforts relatively, simple in addition to making one experiment for many variables. In this study Plackett-Burman experimental design had been applied to estimate the significant of nutritional conditions affecting petroleum bioremediation by isolated *Penicillium chresogenum*. Eleven variables through 12 trials were studied in this experiment, namely ammonium sulphate, potassium carbonate, potassium dihydrogen phosphate, sodium dihydrogen phosphate, beef extract, spore suspension, sodium chloride, magnesium sulphate, calcium chloride, temperature and pH. It was found that four variables (spore suspension, (NH4)2SO4, Potassium Carbonate, and Magnesium sulphate) are affected the bioremediation process positively, while others (sodium dihydrogen phosphate, Beef extracts, Temperature, pH, Sodium chloride, NaCl and KH2PO4) have negative effects on oil bioremediation. Depending on petroleum weight loss calculations, and the above variables parameters the petroleum oil was removed by *Penicillium chresogenum*. Both, sodium chloride and temperature were found to inhibit its removal process. The experiment No (9) using *Pencillium chresogenum* was considered that the most suitable method for oil degradation with percentage removal of petroleum 97.9 %. On the hand, experiment No (10) the undesirable method for oil removal with percentage removal 4.09 %. Based on the experiment, a microbiological model is constructed to simulate microbial growth and oil decomposition. Biodegradation rate was influenced by oil concentration, temperature, water content and oxygen concentration.

Keywords: Fungi, Petroleum bioremediation, Oil removal, Marine sediment, Plackett–Burman Modeling.

Introduction

Coastal pollution by oil was found to be a very dangerous problem along the Mediterranean Sea. Hydrocarbons may come to the marine environments by anthropogenic sources such as manufacturing, refining installation, oil tanker spills; direct discharge from effluent treatment plants and accidents during transportation of the crude oil (Heul, 2009). Diesel oil is a pollutant that frequently is reported as soil contaminant (Wang et al., 2005; Ciric et al., 2009). Above a certain level diesel pollution can be toxic to plants and microorganisms (Tesar et al., 2002; Adam and Duncan, 2003; Lapinskienė et al., 2006).The increase in particulate phase hydrocarbon in winter is related to the increase in the use of domestic heating, traffic congestion, less pollutant dispersal due to meteorological conditions and the absence of photochemical degradation that occurs in summer. Bioremediation is the metabolic ability of microorganisms (*Yeast, fungi,* or *bacteria*) to transform or minimize toxic organic contaminants present in soils, sediments and water into less harmful substances. The activity of microorganisms is stimulated by many factors that affect the biodegradation of petroleum hydrocarbons such as temperature, oxygen, micro and macro nutrients, pH, Solubility, concentration of hydrocarbon. Fungi are also known to degrade hydrocarbons and are suitable for the five-ring PAHs, which are only poorly degraded by bacteria. To make a suitable bioremediation application, it can take long time and may be affecting by some factors which inhibit degradation of hydrocarbons. Anew model was proposed for using the best method for degradation of diesel oil. This method depends on using a fungal isolates from *Pencillium chresogenum*. The main method that has been used for using many factors by changing of one variable at a time method. This is an experimental method in which single factors varied, while other factors are kept constant at specific set of conditions. This method leads to unreliable results and wrong conclusions, and is inferior to the factorial design method (Krishnan et al., 1998). So, the application of this design is suitable for large number of cultural conditions under investigation. Plackett-Burman design (Plackett and Burman, 1946) comprises one type of a two-level screening design. It is favored to detect the significant factors affecting the process before proceeding to the optimization stage of experimental design. The aim of this work is to investigate the bioremediation of petroleum oil by a *Penicillium chresogenum*. and applying a thorough optimization process. The main objective was to evaluate the effects of nutritional conditions, represented as media components and environmental factors, on the bioadsorption and biodegradation of petroleum oil. This is needed to develop a near optimal medium in order to enhance the bioremediation process by means of statistically designed experiments. Abdel-Fattah *et al*. (2002).

MATERIALS AND METHODS

Media preparation

The enrichment procedure as described by Nwachukwu (2000) was used in the estimation of hydrocarbon utilizes. A minimal salt broth containing 2.0g of Na_2HPO_4, 0.17g of K_2SO_4, 4.0g of NH_4NO_3, 0.53g of KH_2PO_4, 0.10g of $MgSO_4.7H_2O$ and 5.0g of agar – agar dissolved in 1000ml of distilled water was prepared. The solution was sterilized by autoclaving. Diesel oil is added as main carbon source in concentration of 0.5% v/v.

Isolation of Pencillium chresogenum

Plates were incubated at room temperature (28^o – 30^{o}C) for 5 to 10 days. The fungus used in this study was isolated from minimal salt broth medium. It was purified and identified morphologically as *Penicillium chresogenum.*

Spore suspension preparation

Slants of minimal salt broth medium were inoculated with fungal spores and incubated till the appearance of spores. 3 ml of sterile distilled water was added to each slant and spore suspension was stored under refrigeration.

Plackett-Burman microcosm

Every variable was prepared from stock solution to obtain accurate results. Environmental factors have been evaluated. The different factors were prepared in two levels: -1 for low concentration and +1 for high concentration, depending on Plackett- Burman modeling design (Plackett and Burman, 1946). A control experiment was made for every trail in this design as the same manner of experiment without spore suspension. Table (1) illustrates the factors under investigation and their levels that used in this design. Petroleum oil concentration was kept constant in all trials at the level of 0.5%.

Table (1): List of variables under study and their coded levels.

No	Factor	Factor Name	Conc %	Low(-1)	Conc %	High(+1)
1	A	Ammonium Sulphate	0.4(**w/v**)	-1	1.2(**w/v**)	+1
2	B	Potassium Carbonate	0.03(**w/v**)	-1	0.6(**w/v**)	+1
3	C	Sodium dihydrogen phosphate	0.2(**w/v**)	-1	0.9(**w/v**)	+1
4	D	Beef extract	0.02(w/v)	-1	0.5(**w/v**)	+1
5	E	Spore suspension volume	3 ml	-1	6 ml	+1
6	F	Temperature	30ºc	-1	40ºc	+1
7	G	pH (alkaline- acidic)	4	-1	7.5	+1
8	H	Sodium chloride	0.3(**w/v**)	-1	2(**w/v**)	+1
9	J	Magnesium sulphate	1.2(**w/v**)	-1	2(**w/v**)	+1
10	K	Calcium chloride	0.1(**w/v**)	-1	0.5(**w/v**)	+1
11	L	Potassium dihydrogenphosphate	0.4(**w/v**)	-1	0.1(**w/v**)	+1

Plackett-Burma experimental design

For screening purpose, various medium components have been evaluated using Plackett–Burman (PB) statistical design (Plackett and Burman, 1946). The different factors were prepared in two levels: -1 for low level and +1 for high level. Table (1) illustrates the factors under investigation as well as levels of each factor used in the experimental design. The nitrogen compounds were prepared in equimolar bases to give 0.2 M nitrogen for higher concentration (+1) and the carbon phosphorus containing compounds were prepared to give 0.04 M phosphorus for the higher level trials (+1). Petroleum oil concentration was kept constant in all trials at the level of 0.5%.

The PB experimental design is based on the first order model **[2]:**

$$Y = a_0 + \sum_{i=1}^{n} a_i x_i \quad (1)$$

Where Y is the response (productivity or specific activity), a_0 is the model intercept and a_i is the variable estimates. This model describes no interaction among factors and issued to evaluate the important factors that influence petroleum oil bioremediation and fungal growth. Eleven variables were screened in twelve experiments; each variable being either medium constituent or environmental variable. Variables with high confidence levels are considered significant on their effect on petroleum bioremediation.

Screening of significant medium variables by PB design:

The PB design was applied to obtain the estimates of the different culture determinants for petroleum removal by *Penicillium chresogenum.* A polynomial model for petroleum removal% was developed by using the estimated coefficients (coded units) and given in Eq. (2)**.**

$$\begin{aligned} Y = {} & 56.66+0.61A+3.96B-9.43C \\ & -1.66D+25.81E-5.09F-6.40G \\ & -3.03H+3.22J-2.93K-2.5L \quad (2) \end{aligned}$$

Results and Discussion

Twelve experiments were done according to PB design and the response, petroleum removal was obtained as given in Table 2.The responses were ranging from 4.09% that was obtained in the combination number 10 to 97.9% that was obtained in the combination number 9. Additionally, it can be said that the variability created in the petroleum bioremediation results in the different trials reflects the importance of studying the effect of different variables (either nutritional or environmental) on this microbiological process. Statistical analysis of these data revealed that the value of determination coefficient R^2, that measures the goodness of model fitting, is> 0.99. This indicates that less than 1% of the total variations is not explained by the model, which ensures the good adjustment of the model (in equation 2) to experimentalize the results.

The factors tested in this design contributed differently on petroleum bioremediation by the *Penicillium chresogenum*, which means that some of them affected positively and others negatively. **Fig. (1)** shows that spore suspension, $(NH_4)_2SO_4$, Potassium Carbonate, and Magnesium sulphate promoted petroleum removal by *Penicillium chresogenum*. While, sodium dihydrogen phosphate, Beef extracts, Temperature, pH, Sodium chloride, NaCl and KH_2PO_4 inhibited the oil bioremediation process. **Fig. (2)** shows the ranking of factor estimates in a Pareto chart. The Pareto chart displays the magnitude of each factor estimate (independent on its contribution, either positive or negative) and is a convenient way to view the results of a PB design (**Strobel and Sullivan, 1999**). The highest positive significant variable is spore suspension, while pH is the highest negative significant variable. **Shreyasri Dutta and Padma Singh (2014)** 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.

Accuracy of the model

To examine the model validation, a comparison was held between estimated and predicted results as shown in **Fig. (3).** The linearity of correlation is an evidence of the excellent agreement between experimental and predicted data. The created model (as in equation **2**) could be used to predict the response (petroleum removal percentage) when using different culture conditions. It was clear that experimental value were closer to predicted values.

Table (2): PB experimental design for evaluating the effect of different nutritional and environmental categories on oil bioremediation.

Run Order	**Ammonium Sulphate**	**Potassium Carbonate**	**Sodiumdihydrogen phosphate**	**Beef extract**	**Spore suspension volume**	**Temperature**	**pH**	**Sodium chloride**	**Magnesium sulphate**	**Calcium chloride**	**Potassium dihydrogen phosphate**	**Oil removal**
1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	95.5
2	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	86.19
3	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	22.9
4	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	32.15
5	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	48.43
6	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	77.22
7	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	23.4
8	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	71.2
9	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	97.9
10	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	4.09
11	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	66.8
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	54.1

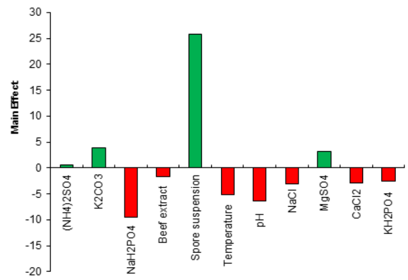

Fig (1): Effects of different culture conditions on petroleum removal %.

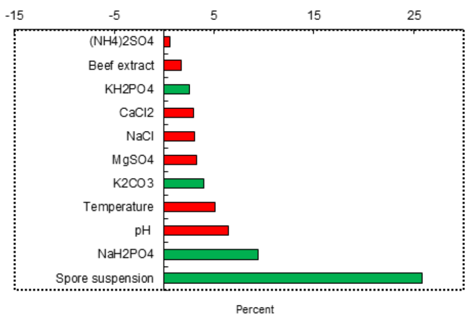

Fig (2): Pareto plot for PB parameters estimates.

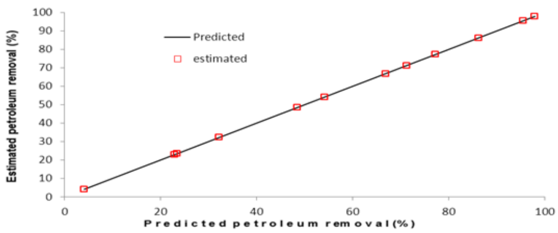

Fig (3): Relation between predicted removal and estimated removal of petroleum.

Conclusion

Plackett- Burman design is considered a suitable model for choosing the best experiment for hydrocarbon degradation using *Pencillium chresogenum*. It also evaluates the significance of some variables on the bioprocess, but also in comparing between different categories, in which is difficult to compare between their effects in conventional experiments, and hence maintain a comprehensive evaluation of the overall process. It is found that four variables are affected the bioremediation process positively, while others have negative effects on oil bioremediation.

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الملخص العربي

عنوان البحث: نموذج بلاكت بيرمان لإزالة التلوث البترولي بواسطة عزله فطرة البنسليوم

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نموذج بلاكت بيرمان هو نموذج إحصائي تم استخدامه لتحديد أمثل الطرق للتحلل البيولوجي لزيت البترول بواسطة عزلة من فطرة البنسليوم. والذي يدرس العلاقة بين مجموعة من المتغيرات في تجربة واحدة. ولذلك تم إجراء ١٢ تجربة في نفس الوقت لتحديد انسب الظروف لإزالة التلوث البترولي في الوسط قبل وبعد إجراء التجربة. وقد وجد أن كربونات البوتاسيوم وكبريتات الأمونيوم وكبريتات الماغنسيوم لهم تأثير فعال في ازالة الهيدروكربون وعلى النقيض وجد أن باقي المتغيرات تثبط من ازالة الهيدروكربون كما وجد أن التجربة رقم ٩ انسب التجارب الممكن إجراؤها لإزالة الهيدروكربون بنسبة ٩٧% وعلى النقيض وجد أن التجربة رقم ١٠ أقل الظروف الممكن إتباعها في إزالة الهيدروكربون بنسبة ٤% .