Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Online International Journal Available at http://www.cibtech.org/jls.htm
2012 Vol. 2 (4) October-December, pp.54-60/ Prajapati et al.

Research Article

OPTIMIZATION OF MEDIUM COMPONENTS FOR POTASSIUM SOLUBILIZING FUNGUS - *ASPERGILLUS TERREUS (KSF 1)* BY RESPONSE SURFACE METHODOLOGY

[*]Kalavati Prajapati[1], M.C. Sharma[1] and H.A. Modi[2]
[1]*Department of Biotechnology, Kadi Sarva Vishwavidyalaya, Gandhinagar*
[2]*Life Science Department, School of Sciences, Gujarat University, Ahmadabad*
[*]*Author for Correspondence*

ABSTRACT
Response surface methodology was employed to optimize the composition of medium for potassium solubilization by *Aspergillus terreus (KSF-1)*, which is less well known as potassium solubilizing plant-associated bacteria. Feldspar (rock potassium), yeast extract and $FeCl_3$ were found to have significant effects on potassium solubilization by the Plackett-Burman design. The response surface method was used to access the optimal region of the medium composition. The analysis revealed that the optimum values of the tested variables were Yeast extract 0.6 g%, Feldspar 2.5 g% and $FeCl_3$ 0.0006 g% Potassium solubilization of 4.0 mg%, which was in agreement with the prediction.

Key Words: *Potassium Solubiliziation, Aspergillus Terreus, Feldspar, Optimization and Response Surface*

INTRODUCTION
Potassium (K) is one of the major plant nutrients limiting plant growth. Most agricultural soils contain large reserves of Potassium, a considerable part of which has accumulated as a consequence of regular applications of K fertilizers (Richardson *et al.,* 1994). However, a large portion of soluble inorganic Phosphorus and potassium applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Dey, 1988). Thus, the release of insoluble and fixed forms of K is an important aspect of increasing soil K availability. On the other hand, Potassium solubilizing microorganisms are able to total solubilize rock K mineral powder, such as micas, illite and available K, through production and excretion of organic acids (Friedrich, 1991 and Ullman, 1996). Silicate solubilizing bacteria were found to resolve potassium, silicon and aluminum from insoluble minerals (Aleksandrov *et al*., 1967; Barker *et al.*, 1998 and Sheng *et al*., 2003). Silicate solubilizing bacteria exert beneficial effects upon plant growth. Their uses as biofertilizers or control agents for agriculture improvement and environmental protection have been a focus of recent research (Glick, 1995). The occurrence of bacterially promoted potassium release suggests that a similar effect should also come about in fungus- mineral interactions (Blum *et al.,* 2002; Weiner and Dove, 2003). A strain of thermophilic fungus *Aspergillus fumigatus* was cultured with K-bearing minerals to determine if microbe–mineral interactions enhance the release of mineral potassium (Lian *et al.*, 2008). Accelerated potassium release from aluminosilicate minerals by fungi was observed as well (Wallander and Tonie, 1999; Yuan *et al*., 2000; Glowa *et al*., 2003; Yuan *et al.*, 2004). Various kinds of bacteria have been isolated and characterized for their ability to solubilize unavailable reduced Potassium to available forms. This work was to apply the Plackett-Burman design, followed by response surface methodology to optimize the culture medium composition for Potassium solubilization by *Aspergillus terreus* (KSF-1).

MATERIALS AND METHODS
Microorganism and Medium
Fungal strain selected in this study was the higher potassium solubilizer from the all isolated potassium solubilizers from ceramic industry soil of Gujarat, India. The medium used for the isolation and screening

of the potassium solubilizers was Aleksandrov agar medium constituted 1% glucose, 0.5% Yeast extract, 0.05% $MgSo_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPo_4$ and 0.5% Feldspar, 3 % agar and pH-4.5 (P. Sugumaran and B. Janartham; 2007) The selected fungal strain was identified as *Aspergillus terreus* (KSF-1) using standard cultural, morphological and biochemical methodology, but its identity was re-evaluated by 18 S rRNA gene sequence analysis.

Analytical Method

Solubilization of K *Aspergillus terreus* (KSF-1) were estimated using Feldspar as an insoluble potassium source. The composition of the medium was 1% Glucose, 0.05% yeast extract, 0.5% feldspar, 100 ml distilled water, pH-6.0. 1x 10^6 spores were transferred to 250 ml Erlenmeyer flask containing 100 ml of the above medium and incubated on shaking condition at 30^0 C on 120 rpm for 7 days by using two insoluble source of potassium feldspar. After each 24 hours the potassium released were determined by using Sodium cobaltinitrite and Folin-Ciocalteu Phenol reagent (Abul Fadl, 1948). The pH was determined with pH meter.

Experimental Designs

Plackett-Burman Design

Plackett-Burman design, a rapid screening multifactor to find the most significant independent factors (Plackett and Burman, 1946; Wang *et al*., 2007; Xiao *et al*., 2007), was used in the present study to screen the important variables that significantly influenced potassium solubilization. In this study, a 12-run Plackett-Burman design (Table 1) with a first-order polynomial equation was applied to evaluate seven factors (including four dummy variables). Each variable was examined at two levels: -1 for the low level and +1 for the high level. The fitted first-order model is

$Y= \beta 0 + \Sigma \beta i\, xi$ ------------- (1)

Y is the predicted response, $\beta 0$ and βi are constant coefficients, and *xi* is the coded independent factors.

Table 1: The Placket-Burman design variables (in coded levels) with soluble potassium as response

Run	Glucose X_1	Yeast extract X_2	Feldspar X_3	$MgSo_4$ X_4	$FeCl_3$ X_5	$CaCo_3$ X_6	$CaPo_4$ X_7	K Solubilization (mg %)
1	-1	+1	-1	+1	+1	-1	+1	11.56
2	+1	+1	-1	+1	+1	+1	-1	15.81
3	+1	-1	+1	+1	+1	-1	-1	22.5
4	+1	-1	-1	-1	+1	-1	+1	15.67
5	-1	-1	-1	+1	-1	+1	+1	10.89
6	+1	+1	-1	-1	-1	+1	-1	18.14
7	-1	-1	-1	-1	-1	-1	-1	13.29
8	-1	+1	+1	-1	+1	+1	+1	19.36
9	-1	+1	+1	+1	-1	5	-1	15.14
10	-1	-1	+1	-1	+1	+1	-1	23.66
11	+1	-1	+1	+1	-1	+1	+1	14.05
12	+1	+1	+1	-1	-1	-1	+1	22.34

Response surface methodology

A central composite design (CCD) of RSM was employed to optimize the three most significant factors (Feldspar, Yeast extract, $FeCl_3$) for enhancing K solubilization by *Aspergillus terreus* screened by Plackett-Burman design. The three independent factors were investigated at five different coded levels (-1.682, -1, 0, +1, +1.682) and the experimental design used for study is shown in

Research Article

Table 2: The variables were coded as in Equation 2:
$xi = (Xi - m)\ li$, $i = 1,2,\ldots, k$,------------ (2)
Where *Xi* is the real value of the independent variable; *xi* is the coded value; *mi* is the value of *Xi* at the center point and *li* is the step change value. The potassium solubilization was fitted using a second-order polynomial equation and a multiple regression of the data was carried out for obtaining an empirical model related to the most significant factors. The general form of the second-order polynomial equation is:
$Y = \beta 0 + \Sigma \beta i\ xi + \Sigma \beta ii xi^2 + \Sigma \beta ij\ xi\ xj$------------ (3)
Where *Y* is the predicted response, *xi* and *xj* are independent factors.

Table 2: Experimental design and results of the central composite design

Run	**Feldspar (gm**		**Yeast extract (mg %)**		**$FeCl_3$ (ug %)**		**K solubilization (mg %)**
	Coded	**Actual**	**Coded**	**Actual**	**Coded**	**Actual**	
1	0	2.25	0	275	0	600	15.2
2	0	2.25	0	275	0	600	20.35
3	-1	0.5	-1	50	+1	1000	19.53
4	-1	0.5	+1	500	-1	200	22.47
5	0	2.25	0	275	-1.682	-72.72	18.65
6	0	2.25	0	275	0	600	19.85
7	0	2.25	0	275	0	600	27.9
8	-1	0.5	+1	500	+1	1000	18
9	+1	4	-1	50	-1	200	14.3
10	0	2.25	0	275	0	600	18.55
11	0	2.25	-1.682	-103.4	0	600	24.6
12	+1	4	-1	50	+1	1000	25.9
13	-1.682	-0.69	0	275	0	600	26.8
14	+1.682	5.19	0	275	0	600	19.5
15	0	2.25	+1.682	653.4	0	600	15.65
16	+1	4	+1	500	-1	200	14.57
17	0	2.25	0	275	0	600	20.4
18	0	2.25	0	275	+1.682	1272.72	20.73
19	-1	0.5	-1	50	-1	200	19.85
20	+1	4	+1	500	+1	1000	12.25

Statistical Analysis
Statistical analysis of the model was performed to evaluate the analysis of variance (ANOVA). The quality of the polynomial model equation was judged statistically by the coefficient of determination R2, and its statistical significance was determined by an *F*-test.

RESULTS AND DISCUSSION

Plackett-Burman Design
The Plackett-Burman design is a rapid method for screening significant factors. Twelve runs were carried out to analyze the effect of 7 variables on K solubilization and the results are demonstrated in Table 3. Analysis of the regression coefficients, *t*-values and *P*-values of 7 factors showed that X_2, X3 and X_5 had positive effects on potassium solubilization; The Model F-value of 16.93 implies the model is significant.

There is only a 0.18% chance that a "Model F-Value" this large could occur due to noise.whereas X1, X4, X6 and X7 had negative effects. The variable with $P<0.05$ is considered as significant parameter. It was clear that variables X2, X3 and X5 were the significant factors, while X1, X4, X6, and X7, with $P>0.05$, were considered insignificant and were not included in the CCD experiments.

Table 3: ANOVA for selected factorial model for Plackett-Burman design

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	162.34	6	27.06	3.11	0.1168
X2-Yeast Extract	0.44	1	0.44	0.050	0.8315
X3-Feldspar	83.69	1	83.69	9.62	0.0268
X5-Fecl3	18.03	1	18.03	2.07	0.2094
Residual	106.92	6	17.82		
Cor Total	1615.3	11			

Optimization by Response Surface Methodology

CCD was employed to study the interactions between the significant factors and also to determine their optimal levels. Data were analysed by using analysis of variance for responce surface quadratic model. The design matrix of tested variables and the experimental results are represented in Table 4. The Model F-value of 15.41 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms.

Table4: Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of K solubilization

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	336.76	9	37.42	62.47	< 0.0001
X3-Feldspar	322.55	1	322.55	538.46	< 0.0001
X2-yeast extract	0.94	1	0.94	1.57	0.2391
X5-FeCl3	7.38	1	7.38	12.33	0.0056
X2X3	0.14	1	0.14	0.23	0.6418
X3X5	4.22	1	4.22	7.04	0.0241
X2X5	0.48	1	0.48	0.79	0.3940
$X3^2$	0.62	1	0.62	1.03	0.3331
$X2^2$	0.31	1	0.31	0.51	0.4897
$X5^2$	0.027	1	0.027	0.046	0.8347
Residual	5.99	10	0.60		
Lack of Fit	3.70	5	0.74	1.61	0.3059
Pure Error	2.29	5	0.46		
Cor Total	342.75	19			

Table 5: Various Analysed values for response surface model

Std. Dev.	0.77	R-Squared	0.9825
Mean	19.75	Adj R-Squared	0.9668
C.V. %	3.92	Pred R-Squared	0.9083
PRESS	31.42	Adeq Precision	29.869

A P-value of less than 0.05 indicates that the model terms are significant. In this case, Feldspar, yeast extract, $FeCl_3$ had a significant effect on potassium solubilization, as well as the interaction terms. The "Lack of Fit F-value" of 1.61 implies the Lack of Fit is not significant relative to the pure error. There is a 30.59 % chance that a "Lack of Fit F-value" this large could occur due to noise.
The "Pred R-Squared" of 0.6591 is not as close to the "Adj R-Squared" of 0.8722 as one might normally expect was also satisfactory to confirm the significance of the model.
Final Equation for K solubilization in Terms of Coded Factors is,

$$Y = +19.74 + 4.86 \times X_3 + 0.26 \times X_2 + 0.74 \times X_5 + 0.13 \times X_2 X_3 + 0.73 \times X_3 \times X_5 + 0.24 \times X_2 \times X_5 + 0.21 \times X_3^2 - 0.15 \times X_2^2 - 0.044 \times X_5^2$$

The response surface curves are plotted to explain the interaction of the variables and to determine the optimum level of each variable for maximum response. The response surface curves are shown in Figures 1 to 3. Each figure demonstrated the effect of two factors while the other factors were fixed at zero level. The model predicted the optimal values (coded) of the three most significant variables were X2 =0.6 g%, X3 = 2.5 g% and X5 = 0.0006 g% correspondingly, the values of Yeast extract, Feldspar and $FeCl_3$ were, and, respectively. The maximum predicted phosphate solubilization was 4.0 mg%.

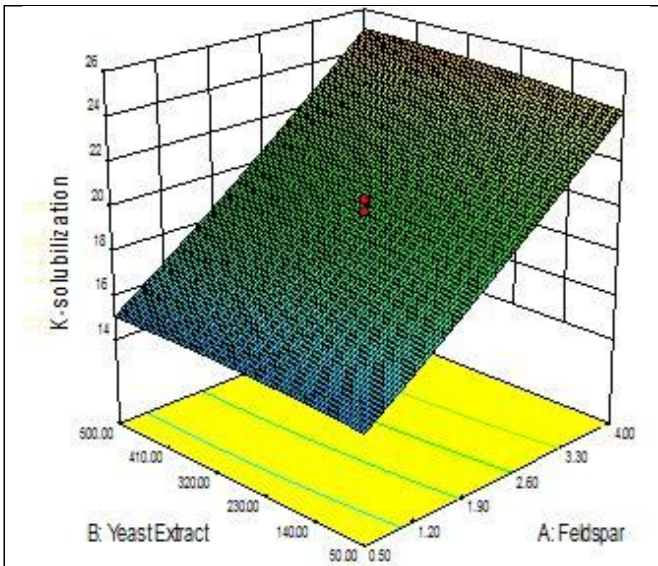

Figure 1: Response surface curve for Potassium solubilization by *Aspergillus terreus* showing the interaction between yeast extract and Feldspar

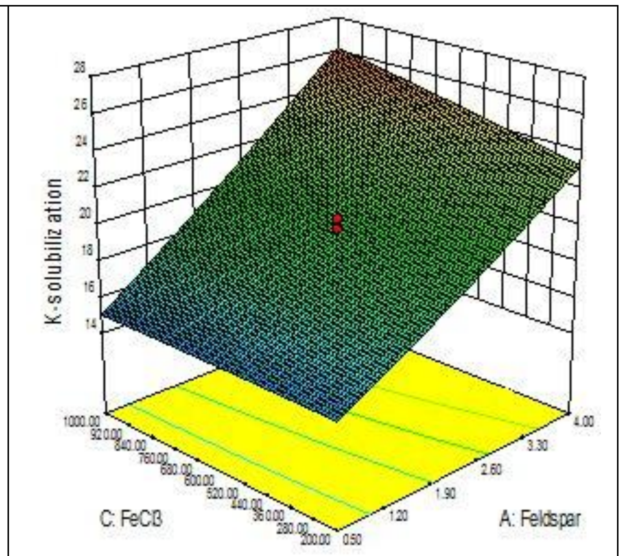

Figure 2: Response surface curve for Potassium solubilization by *Aspergillus terreus* showing the interaction between $FeCl_3$ and Feldspar

Research Article

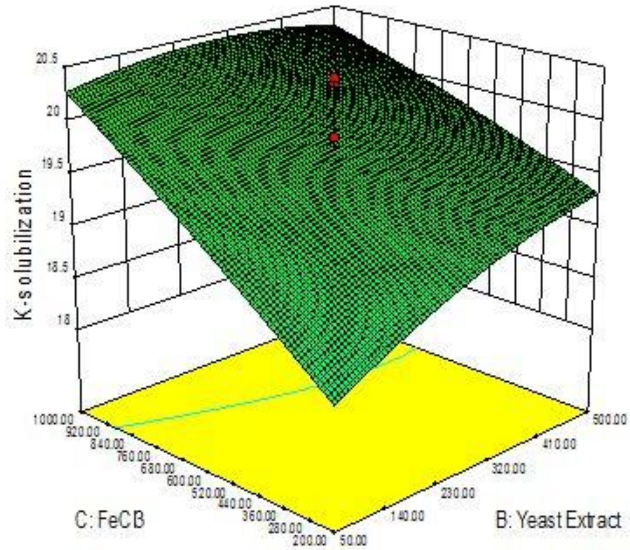

Figure 3: Response surface curve for Potassium solubilization by *Aspergillus terreus* showing the interaction between yeast extract and $FeCl_3$

Conclusion

The high adequacy of the model was proven by the good fit between the experimental and predicted values. Response surface methodology was a better choice to optimize the biological process than that of a "one-condition-at-a-time" because response surface methodology has factor interaction analysis and fewer treatments than a full factorial design (Barrington and Kim, 2008). Response surface method had been proved to be effective on optimizing potassium solubilization by *Aspergillus terreus* was 1% glucose, 0.6 % Yeast extract, 0.05% $MgSo_4.7H_2O$, 0.0006% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPo_4$ and 2.25 % Feldspar, 3 % agar and pH-4.5. This resulted in an overall 1.8-fold increase compared with that using the original medium.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Biotechnology and Management of Kadi Sarva Vishwavidyalaya (KSV), Gandhinagar, for providing the facilities for this research.

REFERENCES

Abul Fadl MAM (1948). Colorimetric Determination of Potassium by Folin-Ciocalteu phenol reagent. Postgraduate Medical School London 282-285.

Aleksandrov VG, Blagodyr RN and Iiiev IP (1967). Liberation of phosphoric acid from apatite by silicate bacteria. *Mikrobiyol Zh (Kiev)* **29** 111-114.

Barrintogn S and Kim JW (2008). Response surface optimization of medium components for citric acid production by *Aspergillus Niger* NRRL 567 grown in peat moss. *Bioresource Techonology* **99** 368-377.

Bin Lian, Bin Wang, Mu Pan, Congqiang Liu and Henry Teng H (2008). Microbial release of potassium from K-bearing minerals by thermophilic fungus Aspergillus *fumigatus. Geochimica et Cosmochimica Acta* **72** 87-98.

Blum JD, Klaue A, Nezat CA, Driscoll CT, Johnson CE, Siccama TG, Eagar C, Fahey TJ and Likens GE (2002). Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* 417 729-731.

Dey K (1988). Phosphate solubilizing organisms in improving fertility status. *In Biofertilizers*: Potentialities and Problems 237-248.

Friedrich S, Platonova NP, Karavaiko GI, Stichel E and Glombitza F (1991). Chemical and microbiological solubilization of silicates. *Acta Biotechnology* **11** 187-196.

Glick BR (1995). The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology* 41 109-117.

Glowa KR, Arocena JM and Massicotte HB (2003). Extraction of potassium and/or magnesium from selected soil minerals by Piloderma. *Geomicrobiology Journal* **20** 99-112.

Plackett RL and Burman JP (1946). The design of optimum multifactorial experiments. *Biometrika* **33** 305-325.

Sugumaran P and Janartham B (2007). Solubilization of potassium minerals by bacteria and their effect on plant growth. *World Journal of Agricultural Sciences* **3**(3) 350-355.

Richardson A, Pankhurst C, Doube B, Gupta V and Grace P (1994). Soil microorganisms and phosphorus availability. *Soil biota: management in sustainable farming systems* 50-62.

Sheng XF, Huang WY and Yin YX (2003). Effects of application of silicate bacteria fertilizer and its potassium release. *Journal of Nanjing Agricultural University* **23** 43-46.

Ullman WJ, Kirchman DL and Welch SA (1996). Laboratory evidence for microbially mediated silicate mineral dissolution in nature. *Chemical Geology* **132** 11-17.

Wallander H and Tonie W (1999). Biotite and microcline as potassium sources in ectomycorrhizal and non-mycorrhizal Pinus sylvestris seedlings. *Mycorrhiza* **9** 25-32.

Wang X, Yin M, Xiao Z, Ma C, Lin Z, Wang PG and Xu P (2007). Production of uridine 5'-monophosphate by Corynebacterium ammoniagenes ATCC 6872 using a statistically improved biocatalytic process. *Applied Microbiology and Biotechnology* **76** 321-328.

Weiner S and Dove PM (2003). An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry* **54** 1-29.

Xiao Z Liu P Qin J Xu P 2007: Statistical optimization of medium components for enhanced acetoin production from molasses and soybean meal hydrolysate. *Applied Microbiology and Biotechnology* **74** 61-68.

Yuan L, Fang DH, Wang ZH, Shun H and Huang JG (2000). Bio-mobilization of potassium from clay minerals: I. By ectomycorrhizas. *Pedosphere* **10** 339-346.

Yuan L, Huang JG, Li XL and Christie P (2004). Biological mobilization of potassium from clay minerals by ectomycorrhizal fungi and eucalypt seedling roots. *Plant Soil* **262** 351-361.