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# Development of Medium for Griseofulvin Production: Part II. Optimization of Medium Constituents Using Central Composite Design

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**Abstract** Central composite experimental design was employed to determine the optimal concentration of medium constituents for griseofulvin production by *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004. The optimal concentration of sucrose, $K_2HPO_4$, $NaNO_3$, and $FeSO_4{\cdot}7H_2O$ were found to be 48.08 g/l, 1.228 g/l , 2.7 g/l, and 0.011 g/l, respectively, for *Penicillium griseofulvum* MTCC 1898, and for *Penicillium griseofulvum* MTCC 2004, 23.52 g/l, 43.67 g/l, and 0.0434 g/l of glucose, lactose, and $MnSO_4{\cdot}H_2O$, respectively. The yield of griseofulvin under optimal composition of medium constituents increased by 1.26 and 1.38 times than prior to optimization, for *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively.

**Key words:** *Penicillium griseofulvum*, griseofulvin, optimization, central composite experimental design

The development of a medium for maximum production of any metabolite is a key step in industrial scale fermentation. The screening and optimization of medium constituents, which significantly influence the production, have been carried out for the development of medium. The medium constituents sucrose, $K_2HPO_4$, $NaNO_3$, and $FeSO_4{\cdot}7H_2O$ for *Penicillium griseofulvum* MTCC 1898 and lactose, glucose, and $MnSO_4{\cdot}H_2O$ for *Penicillium griseofulvum* MTCC 2004, respectively, were screened using Plackett-Burman experimental design for griseofulvin production [11]. It is necessary to optimize the screened medium constituents to enhance griseofulvin production. Response surface methodology has been adopted to determine the optimal concentrations of carbon, nitrogen, and phosphorous for citric acid production by *Aspergillius foetidus* [3]. The combined effect of sorbitol and yeast extract on tartaric production by *Gluconobactor suboxydans* was determined using response surface methodology [6]. The production of tetracycline with κ-carrageenan-immobilized *Streptomyces aureofaciens* was enhanced eight times in comparison with free-cell mycelial cultures [10]. The production of chitinase has been enhanced under optimal levels of chitin, ammonium sulfate, peptone, and urea [5]. Central composite design was applied to find the optimal combinations of medium constituents for improved production of propionic acid [1]. The method of studying one variable at a time, while keeping all others at a predetermined constant level, is very inefficient in many cases [9]. One variable at each experiment is also a time-consuming technique and fails to explain the interactions between and among the variables [7]. The objective of this study was to optimize the screened medium constitutions to improve griseofulvin production by *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004. In this communication, a novel method of response surface technique has been applied to study the effect of medium constituents on griseofulvin production. Central composite design has been applied to evaluate the optimal combinations of medium constituents.

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## MATERIALS AND METHODS

### Organisms

*Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004 were obtained from the Institute of Microbial Technology, Chandigarh, India.

### Culture Maintenance

*Penicillium griseofulvum* MTCC 1898 was maintained on agar slants containing (g/l) sucrose, 30.0; yeast extract, 5; $K_2HPO_4$, 1.0; $NaNO_3$, 3; KCl, 0.5; $MgSO_4{\cdot}7H_2O$, 0.5; $FeSO_4{\cdot}7H_2O$, 0.01. *Penicillium griseofulvum* MTCC 2004 was maintained on agar slants containing (g/l) sucrose, 30.0; $K_2HPO_4$, 1.0; $NaNO_3$, 2; KCl, 0.5; $MgSO_4{\cdot}7H_2O$, 0.5; $FeSO_4{\cdot}7H_2O$, 0.01; $ZnSO_4{\cdot}7H_2O$, 0.01, and $CuSO_4{\cdot}5H_2O$, 0.005.

Both organisms were subcultured every month and stored at 4±1°C.

### Seed Culture Medium

The composition of the medium used for development of seed culture was the same as that described for culture maintenance, with 10 g/l glucose being the additional constituent added for both the strains. Five hundred ml Erlenmeyer flasks containing 100 ml of inoculum medium was autoclaved at 121°C for 20 min. The initial pH of the media was adjusted to 6.5 before sterilization. The seed culture medium was inoculated with 1 ml spore suspension (in sterile distilled water) containing $1.8\times10^7$ spores. The age of organisms in slant growth, seed age, and inoculum levels were maintained as reported by Venkata Dasu and Panda [12].

### Cultivation Medium and Culture Conditions

The constituents of the cultivation medium are (in g/l) yeast extract, 5; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; and various levels of sucrose, $K_2HPO_4$, and $FeSO_4 \cdot 7H_2O$ (according to the experimental plan given in Table 3) were used for *Penicillium griseofulvum* MTCC 1898. For *Penicillium griseofulvum* MTCC 2004, the medium was composed of (g/l) corn steep liquor, 1.0; $KH_2PO_4$, 0.5; $NaNO_3$, 3; KCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.02; $ZnSO_4 \cdot 7H_2O$, 0.044; $CaCO_3 \cdot 2H_2O$, 1.0; and various levels of glucose, lactose, and $MnSO_4 \cdot H_2O$ were used for griseofulvin production (experimental plan given in Table 4). The initial pH of the medium was adjusted to 6.5 before sterilization. One hundred mlof sterile medium in 500-ml Erlenmeyer flasks were inoculated with 12% (v/v) and 9% (v/v) seed culture of *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively. The cultures were incubated on a rotatory shaker at 160 rpm at 30°C. Samples were withdrawn at regular interval of 24 h and assayed for griseofulvin [4]. All experiments were performed in duplicate.

### Experimental Design

Central composite design was used to optimize the medium constituents [2]. The medium constituents were considered as independent variables. According to the central composite design, the total number of treatment combinations was $2^K+2K+n_0$, where K is the number of independent variables and $n_0$ is the number of repetition of the experiments at the center point.

The variables $X_i$ were coded as $x_i$ according to the following equation

$$x_i=(X_i-X_0)/\Delta X_i \quad i=1, 2, 3, -, -, K \qquad (1)$$

where,

$x_i$=dimensionless coded value of the variable $X_i$
$X_0$=the variable of $X_i$ at center point
$\Delta X_i$=step change

The behavior of the system was explained by the following second-order polynomial equation

$$Y=\beta_0+\Sigma\beta_i x_i+\Sigma\beta_{ii} x_i^2+\Sigma\beta_{ij} x_i x_j \qquad (2)$$

where

Y=predicted response
$\beta_0$=offset term
$\beta_i$=linear term
$\beta_{ii}$=squared term
$\beta_{ij}$=interaction term

The second-order polynomial equations were used to estimate the response of the dependent variable (griseofulvin production). These regression equations were optimized by the iteration method to obtain the optimum values [8]. Analysis of variance (ANOVA) was performed on the data. Design expert (Stat-Ease, Inc., Minneapolis, MN, U.S.A.) was used for this study.

### Optimization of the Medium Constituents for Griseofulvin Production by *Penicillium griseofulvum* MTCC 1898

The medium constituents sucrose ($x_1$), $K_2HPO_4$ ($x_2$), $NaNO_3$ ($x_3$), and $FeSO_4 \cdot 7H_2O$ ($x_4$) were considered as independent variables. A $2^4$-factorial experimental design, with eight axial points ($\alpha$=2.0) and six replicates at the center point with a total of 30 experiments were employed for this study. The coded values of the variables are given in Table 1.

### Optimization of the Medium Constituents for Griseofulvin Production by *Penicillium griseofulvum* MTCC 2004

The medium constituents glucose ($x_1$), lactose ($x_2$), and $MnSO_4 \cdot H_2O$ ($x_3$) were considered as independent variables. A $2^3$-factorial experimental design with six axial points, 12 factorial portion points, and six replicates at the center point with a total of 20 experiments were employed for optimization of the medium constituents. The coded values of the variables are given in Table 2. The griseofulvin production was taken as the dependent variable. All experiments were carried out in duplicate.

**Table 1.** Variables and their levels employed in the central composite experimental design for optimization of sucrose, $K_2HPO_4$, $NaNO_3$, and $FeSO_4 \cdot 7H_2O$ for optimal griseofulvin production by *Penicillium griseofulvum* MTCC 1898.

| Variables | Coded levels | | | | |
|---|---|---|---|---|---|
| | −2 | −1 | 0 | +1 | +2 |
| | Actual values (g/l) | | | | |
| Sucrose ($X_1$), g/l | 2.5 | 20.0 | 37.5 | 55.0 | 72.5 |
| $K_2HPO_4$ ($X_2$), g/l | 0.25 | 0.75 | 1.25 | 1.75 | 2.25 |
| $NaNO_3$ ($X_3$), g/l | 0.25 | 2.0 | 3.75 | 5.5 | 7.25 |
| $FeSO_4 \cdot 7H_2O$ ($X_4$), g/l | 0.0025 | 0.0075 | 0.0125 | 0.0175 | 0.0225 |

$x_i$=coded value of the variable $X_i$.

**Table 2.** Variables and their levels employed in the central composite experimental design for optimization of glucose, lactose, and $MnSO_4 \cdot H_2O$ for griseofulvin production by *Penicillium griseofulvum* MTCC 2004.

| Variables | Coded levels | | | | |
|---|---|---|---|---|---|
| | −1.682 | −1 | 0 | +1 | +1.682 |
| | Actual values (g/l) | | | | |
| Glucose ($X_1$), g/l | 4.372 | 13.75 | 27.5 | 41.25 | 50.627 |
| Lactose ($X_2$), g/l | 24.77 | 35.0 | 50.0 | 65.0 | 75.23 |
| $MnSO_4 \cdot H_2O$ ($X_3$), g/l | 0.00795 | 0.025 | 0.05 | 0.075 | 0.09205 |

$x_i$=coded value of the variable $X_i$.

## RESULTS AND DISCUSSION

The experiments were performed according to the experimental plan given in Tables 3 and 4 for *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively. By applying multiple regression analysis on the experimental data, the following second-order polynomial equations have been found to explain the giseofulvin production (predicted values).

$$Y=0.821517+0.1425\,x_1+0.007208\,x_2-0.026958\,x_3-0.022983\,x_4-0.11399\,x_1^2-0.06009x_2^2-0.02454\,x_3^2-0.031477\,x_4^2-0.006438\,x_1x_2+0.000562\,x_1x_3+0.015712\,x_1x_4+0.01035\,x_2\,x_3+0.008775\,x_2\,x_4+0.007775\,x_3\,x_4 \quad (3)$$

and

$$Y=0.527862-0.015065\,x_1-0.03698\,x_2-0.010062\,x_3-0.045893\,x_1^2-0.054888\,x_2^2-0.048226\,x_3^2-0.013775\,x_1x_2-0.0221\,x_1x_3-0.021\,x_2x_3 \quad (4)$$

The predicted values (using above equations) were compared with the experimental values for griseofulvin production by *Penicillium griseofulvum* MTCC 1898 and *Penicillium*

**Table 3.** Central composite design matrix and corresponding griseofulvin production by *Penicillium griseofulvum* MTCC 1898. Maximum griseofulvin production was achieved on the 8th day of fermentation.

| Run # | Sucrose $x_1$ (≡$X_1$, g/l) | $K_2HPO_4$ $x_2$ (≡$X_2$, g/l) | $NaNO_3$ $x_3$ (≡$X_3$, g/l) | $FeSO_4 \cdot 7H_2O$ $x_3$ (≡$X_3$, g/l) | Griseofulvin (g/l) | |
|---|---|---|---|---|---|---|
| | | | | | Experimental | Predicted |
| 1 | +1 (≡ 55.0) | −1 (≡ 0.75) | +1 (≡ 5.5) | +1 (≡ 0.0175) | 0.74 | 0.6986 |
| 2 | +1 (≡ 55.0) | +1 (≡ 1.75) | +1 (≡ 5.5) | −1 (≡ 0.0075) | 0.76 | 0.7199 |
| 3 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.82 | 0.8320 |
| 4 | −1 (≡ 20.0) | −1 (≡ 0.75) | +1 (≡ 5.5) | −1 (≡ 0.0075) | 0.40 | 0.4476 |
| 5 | +1 (≡ 55.0) | −1 (≡ 0.75) | −1 (≡ 2.0) | −1 (≡ 0.0075) | 0.88 | 0.8042 |
| 6 | −1 (≡ 20.0) | +1 (≡ 1.75) | +1 (≡ 5.5) | +1 (≡ 0.0175) | 0.36 | 0.4338 |
| 7 | −1 (≡ 20.0) | +1 (≡ 1.75) | −1 (≡ 2.0) | −1 (≡ 0.0075) | 0.49 | 0.5279 |
| 8 | +1 (≡ 55.0) | +1 (≡ 1.75) | −1 (≡ 2.0) | +1 (≡ 0.0175) | 0.80 | 0.7549 |
| 9 | −1 (≡ 20.0) | −1 (≡ 0.75) | −1 (≡ 2.0) | +1 (≡ 0.0175) | 0.39 | 0.4284 |
| 10 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.82 | 0.8320 |
| 11 | −1 (≡ 20.0) | −1 (≡ 0.75) | −1 (≡ 2.0) | −1 (≡ 0.0075) | 0.46 | 0.5086 |
| 12 | −1 (≡ 20.0) | +1 (≡ 1.75) | +1 (≡ 5.5) | −1 (≡ 0.0075) | 0.38 | 0.4478 |
| 13 | +1 (≡ 55.0) | +1 (≡ 1.75) | −1 (≡ 2.0) | −1 (≡ 0.0075) | 0.77 | 0.7372 |
| 14 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.82 | 0.8017 |
| 15 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.82 | 0.8017 |
| 16 | +1 (≡ 55.0) | −1 (≡ 0.75) | −1 (≡ 2.0) | +1 (≡ 0.0175) | 0.78 | 0.7263 |
| 17 | +1 (≡ 55.0) | −1 (≡ 0.75) | +1 (≡ 5.5) | −1 (≡ 0.0075) | 0.76 | 0.6849 |
| 18 | +1 (≡ 55.0) | +1 (≡ 1.75) | +1 (≡ 5.5) | +1 (≡ 0.0175) | 0.74 | 0.7081 |
| 19 | −1 (≡ 20.0) | −1 (≡ 0.75) | +1 (≡ 5.5) | +1 (≡ 0.0175) | 0.29 | 0.3379 |
| 20 | −1 (≡ 20.0) | +1 (≡ 1.75) | −1 (≡ 2.0) | +1 (≡ 0.0175) | 0.34 | 0.4223 |
| 21 | 0 (≡ 37.5) | 0 (≡ 1.25) | +2 (≡ 7.25) | 0 (≡ 0.0125) | 0.69 | 0.6787 |
| 22 | +2 (≡ 72.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.45 | 0.6598 |
| 23 | 0 (≡ 37.5) | +2 (≡ 2.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.65 | 0.6048 |
| 24 | 0 (≡ 37.5) | 0 (≡ 1.25) | −2 (≡ 0.25) | 0 (≡ 0.0125) | 0.78 | 0.7865 |
| 25 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.82 | 0.8307 |
| 26 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.82 | 0.8307 |
| 27 | 0 (≡ 37.5) | −2 (≡ 0.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.53 | 0.5760 |
| 28 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | +2 (≡ 0.0225) | 0.68 | 0.6589 |
| 29 | 0 (≡ 37.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | −2 (≡ 0.0025) | 0.73 | 0.7508 |
| 30 | −2 (≡ 2.5) | 0 (≡ 1.25) | 0 (≡ 3.75) | 0 (≡ 0.0125) | 0.30 | 0.0898 |

$R^2$=0.8677, R=0.9315.

Values in the parenthesis are the decoded figures.

**Table 4.** Central composite design matrix and corresponding griseofulvin production by *Penicillium griseofulvum* MTCC 2004. Maximum griseofulvin production was achieved on the 7th day of fermentation.

| Run # | Glucose $x_1$ (≡$X_1$, g/l) | Lactose $x_2$ (≡$X_2$, g/l) | $MnSO_4 \cdot H_2O$ $x_3$ (≡$X_3$, g/l) | Griseofulvin (g/l) | |
|---|---|---|---|---|---|
| | | | | Experimental | Predicted |
| 1 | +1 (≡ 41.25) | +1 (≡ 65.5) | +1 (≡ 0.075) | 0.32 | 0.3063 |
| 2 | 0 (≡ 27.5) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.55 | 0.5743 |
| 3 | 0 (≡ 27.5) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.54 | 0.5743 |
| 4 | 0 (≡ 27.5) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.55 | 0.5743 |
| 5 | -1 (≡ 13.75) | +1 (≡ 65.5) | -1 (≡ 0.025) | 0.43 | 0.4261 |
| 6 | 0 (≡ 27.5) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.54 | 0.5743 |
| 7 | +1 (≡ 41.25) | +1 (≡ 65.5) | -1 (≡ 0.025) | 0.43 | 0.4126 |
| 8 | -1 (≡ 13.75) | -1 (≡ 35.0) | -1 (≡ 0.025) | 0.45 | 0.4305 |
| 9 | +1 (≡ 41.25) | -1 (≡ 35.0) | +1 (≡ 0.075) | 0.47 | 0.4498 |
| 10 | +1 (≡ 41.25) | -1 (≡ 35.0) | -1 (≡ 0.025) | 0.48 | 0.4721 |
| 11 | -1 (≡ 13.75) | +1 (≡ 65.5) | +1 (≡ 0.075) | 0.43 | 0.4082 |
| 12 | -1 (≡ 13.75) | -1 (≡ 35.0) | +1 (≡ 0.075) | 0.51 | 0.4966 |
| 13 | +1.682 (≡ 50.627) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.31 | 0.3263 |
| 14 | -1.682 (≡ 4.372) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.35 | 0.3769 |
| 15 | 0 (≡ 27.5) | +1.682 (≡ 75.23) | 0 (≡ 0.05) | 0.25 | 0.2639 |
| 16 | 0 (≡ 27.5) | 0 (≡ 50.0) | -1.682 (≡ 0.00795) | 0.35 | 0.3619 |
| 17 | 0 (≡ 27.5) | 0 (≡ 50.0) | +1.682 (≡ 0.09205) | 0.30 | 0.3281 |
| 18 | 0 (≡ 27.5) | -1.682 (≡ 24.77) | 0 (≡ 0.05) | 0.36 | 0.3884 |
| 19 | 0 (≡ 27.5) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.54 | 0.4815 |
| 20 | 0 (≡ 27.5) | 0 (≡ 50.0) | 0 (≡ 0.05) | 0.54 | 0.4815 |

$R^2$=0.9153, R=0.9567.
Values in the parenthesis are the decoded figures.

*griseofulvum* MTCC 2004, given in Tables 3 and Table 4, respectively. The regression equations (3) and (4) were optimized to determine the optimal concentration of each medium constituent [12]. The optimal levels of sucrose ($x_1$), $K_2HPO_4$ ($x_2$), $NaNO_3$ ($x_3$), and $FeSO_4 \cdot 7H_2O$ ($x_4$) were found to be 48.08 g/l, 1.228 g/l, 2.7 g/l, and 0.011 g/l, respectively, for *Penicillium griseofulvum* MTCC 1898. Similarly, for *Penicillium griseofulvum* MTCC 2004, the optimal levels of glucose ($x_1$), lactose ($x_2$), and $MnSO_4 \cdot H_2O$ ($x_3$) were 23.52 g/l, 43.67 g/l, and 0.0434 g/l, respectively.

The griseofulvin production by *Penicillium griseofulvum* MTCC 1898 for run numbers 22 and 30 (extreme levels of sucrose) was less compared to the central design point (for example, run number 3) (Table 3). It appears that a concentration above 55 g/l reduces griseofulvin production (cf. run number 22 compared with the run numbers 1, 2, 5, 8, 13, 16, and 18 in Table 3). These results suggested that the optimum concentration of sucrose lies between 37.5 g/l and 55.0 g/l. For run numbers 23 and 27 (extreme levels of $K_2HPO_4$), the griseofulvin was again less compared to the central design point (run number 3). This suggests that the optimum concentration of $K_2HPO_4$ lies between 0.25 g/l and 1.75 g/l. The production of griseofulvin for run numbers 21 and 24 (at extreme design points of $NaNO_3$) was less compared to the center point. Similarly, the griseofulvin production at extreme design points of $FeSO_4 \cdot 7H_2O$ (run numbers 28 and 29) was less compared to the center point (for example, run number 26). This appears to suggests that the optimal concentrations of $NaNO_3$ and $FeSO_4 \cdot 7H_2O$ lies between 0.25 g/l to 7.25 g/l, and 0.0025 g/l to 0.0225 g/l, respectively.

The production of griseofulvin by *Penicillium griseofulvum* MTCC 2004 for run numbers 13 and 14 (at extreme design points of glucose) was less compared to the central design point (for example, run number 19) (Table 4). This appears

**Table 5.** ANOVA Table: For optimization of sucrose, $K_2HPO_4$, $NaNO_3$, and $FeSO_4 \cdot 7H_2O$ by *Penicillium griseofulvum* MTCC 1898.

| Source | Sum of squares | Degrees of freedom | Mean square | F Value | Prob. > F |
|---|---|---|---|---|---|
| Model | 0.940143 | 14 | 0.067153 | | |
| Error | 0.144268 | 13 | 0.011098 | 6.051 | 0.0012 |
| Cor. total | 1.084411 | 27 | | | |

**Table 6.** ANOVA Table: For optimization of glucose, lactose, and $MnSO_4 \cdot H_2O$ by *Penicillium griseofulvum* MTCC 2004.

| Source | Sum of squares | Degrees of freedom | Mean square | F Value | Prob. > F |
|---|---|---|---|---|---|
| Model | 0.121639 | 9 | 0.013515 | | |
| Error | 0.015606 | 9 | 0.001734 | 7.795 | 0.0027 |
| Cor. total | 0.137245 | 18 | | | |

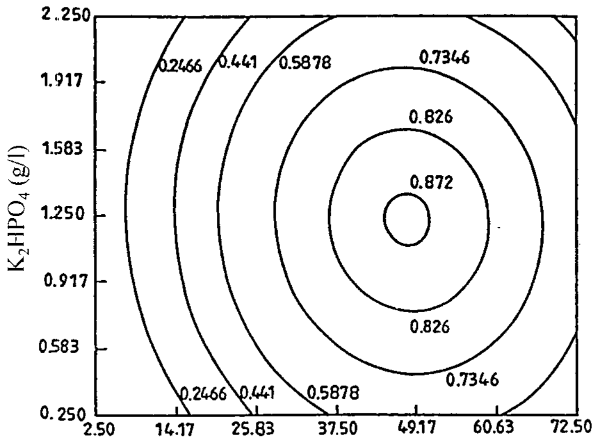


**Fig. 1.** Isoresponse contour plot showing the effect of sucrose and $K_2HPO_4$ concentrations on griseofulvin yield (at constant concentrations of $NaNO_3$ and $FeSO_4 \cdot 7H_2O$).

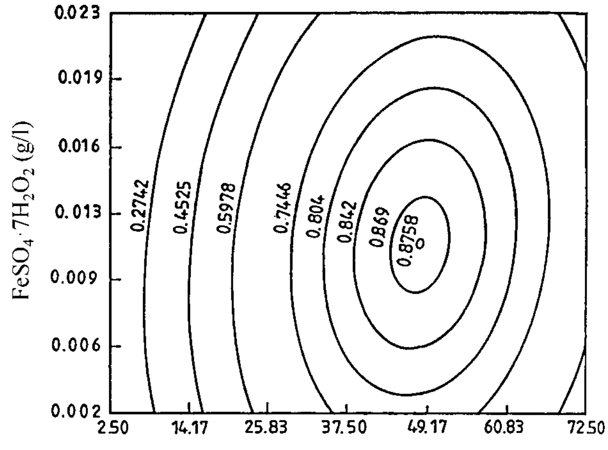


**Fig. 3.** Isoresponse contour plot showing the effect of sucrose and $FeSO_4 \cdot 7H_2O$ concentrations on griseofulvin yield (at constant concentrations of $K_2HPO_4$ and $NaNO_3$).

to suggests that the optimal glucose concentration lies between 4.37 g/l and 41.25 g/l (cf. run number 19 compared with run numbers 7, 9, 10, and 13). The griseofulvin production for extreme concentration of lactose (run numbers 15 and 18) was less compared to the center point (for example, run number 19). It appears that lactose at a concentration above 65 g/l reduces the griseofulvin production (cf. run number 19 compared with the run numbers 1, 5, 7 11, and 15). This suggests that the optimum concentration of lactose lies between 24.77 g/l to 65.0 g/l. Similarly, the griseofulvin production for run numbers 16 and 17 (at extreme design point of $NaNO_3$) was less compared to the center point. It appears that $MnSO_4 \cdot H_2O$ at a concentration of 0.075 g/l decreases the production (cf. run number 6 compared with the run numbers 9, 11, 12, and 17 of Table 4). This appears to suggests that the optimal concentration of $MnSO_4 \cdot H_2O$ lies between 0.05 g/l and 0.075 g/l.

The value of R (coefficient of correlation) for production of griseofulvin by *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004 are 0.9315 and 0.9567, respectively. This indicates a good agreement between experimental and predicted values of griseofulvin production (Tables 3 and 4). The values of $R^2$ (coefficient of determination) for production of griseofulvin by *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004 are 0.8677 and 0.9153, respectively. The observed sample variation of 86.77% and 91.53% for griseofulvin production by *Penicillium griseofulvum* MTCC

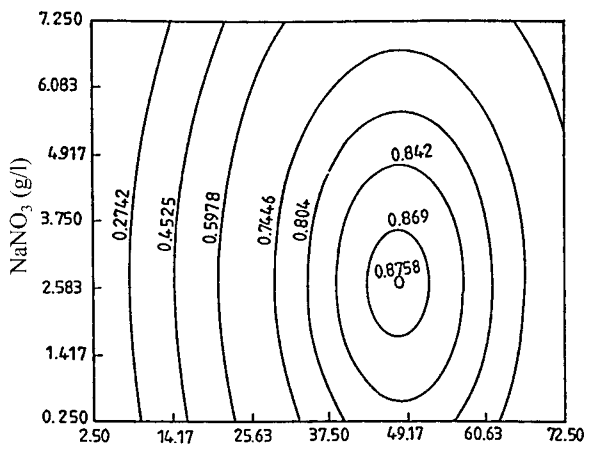


**Fig. 2.** Isoresponse contour plot showing the effect of sucrose and $NaNO_3$ concentrations on griseofulvin yield (at constant concentrations of $K_2HPO_4$ and $FeSO_4 \cdot 7H_2O$).

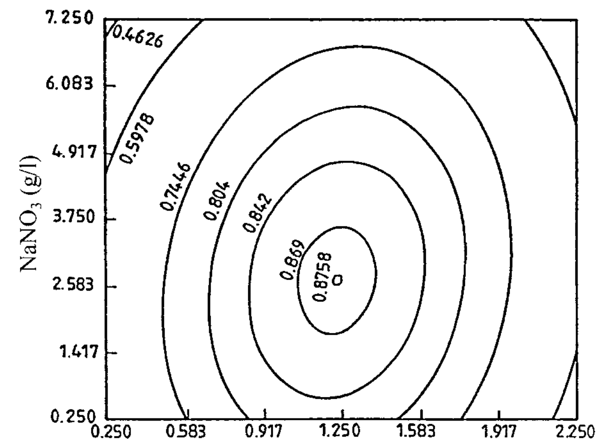


**Fig. 4.** Isoresponse contour plot showing the effect of $K_2HPO_4$ and $NaNO_3$ concentrations on griseofulvin yield (at constant concentrations of sucrose and $FeSO_4 \cdot 7H_2O$).

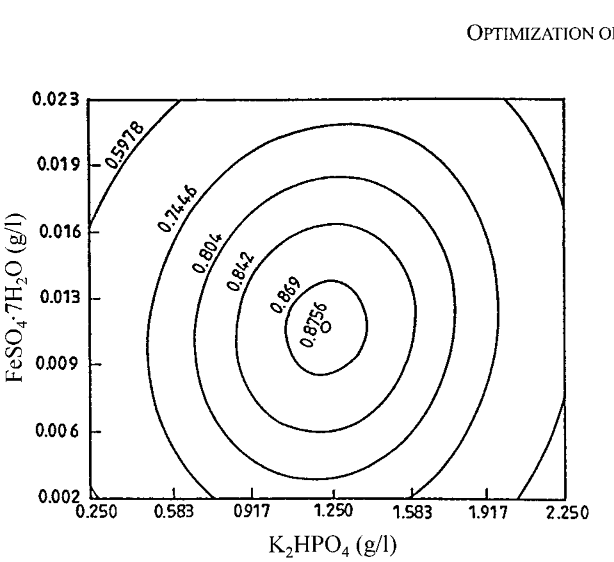


**Fig. 5.** Isoresponse contour plot showing the effect of $K_2HPO_4$ and $FeSO_4{\cdot}7H_2O$ concentrations on griseofulvin yield (at constant concentrations of sucrose and $NaNO_3$).

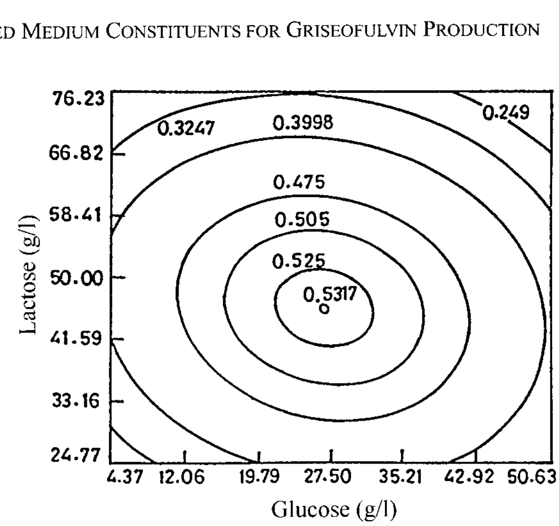


**Fig. 7.** Isoresponse contour plot showing the effect of glucose and lactose concentrations on griseofulvin yield (at constant concentration of $MnSO_4{\cdot}H_2O$).

1898 and *Penicillium griseofulvum* MTCC 2004 can be explained by independent variables. Statistical testing of the model was done by the Fisher's statistical test for analysis of variance (ANOVA). Tables 5 and 6 represent the corresponding ANOVA. The $F_{statistic}$ values of 6.051 and 7.795 for *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively, are greater than tabulated $F_{14,13}$ and $F_{9,9}$ within a rejection region having an α-level that is 0.0012 and 0.0027, respectively. If the values of $F_{statistic}$ exceed the tabulated $F_{14,13}$ and $F_{9,9}$, the lack of fit can be detected at the α-level of significance. It indicates that the fitted model exhibits lack of fit at the confidence level.

The contour plots to estimate production surface over independent variables $x_1$, $x_2$, $x_3$, and $x_4$ for *Penicillium griseofulvum* MTCC 1898, and $x_1$, $x_2$, and $x_3$ for *Penicillium griseofulvum* MTCC 2004 are shown in Figs. 1-6 and Figs. 7-9, respectively.

The contour plots given in Figs. 1-6 show the relative effects of any two variables, when the concentration of remaining two variables are maintained at a constant levels individually. Similarly, the contour plots given in Figs. 7-9 show the relative effects of any two variables, when the concentration of the third variable is kept constant. The trends of mutual interactions between the variables are elliptical. A similar type of trend was observed for optimization of medium constituents for citric acid production [2]. The stationary point or center point is the point at which the slope of the response surface is zero in all directions. The coordinates of the central point within the highest contour levels in each of these figures will correspond to the

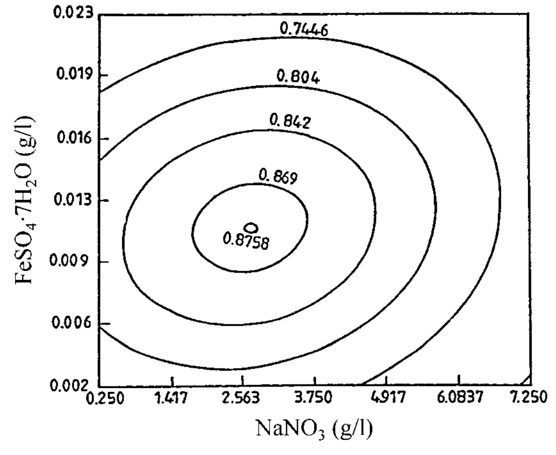


**Fig. 6.** Isoresponse contour plot showing the effect of $NaNO_3$ and $FeSO_4{\cdot}7H_2O$ concentrations on griseofulvin yield (at constant concentrations of sucrose and $K_2HPO_4$).

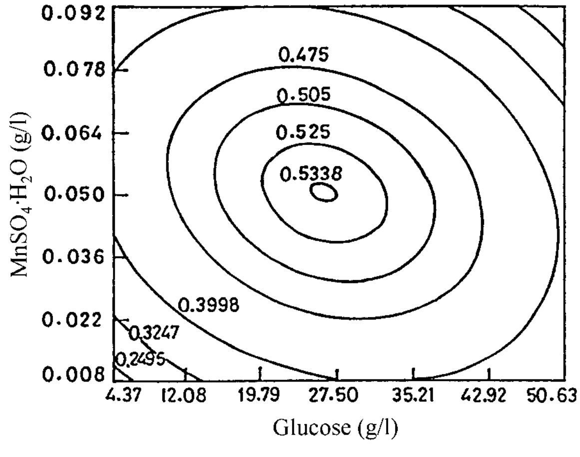


**Fig. 8.** Isoresponse contour plot showing the effect of glucose and $MnSO_4{\cdot}H_2O$ concentrations on griseofulvin yield (at constant concentration of lactose).

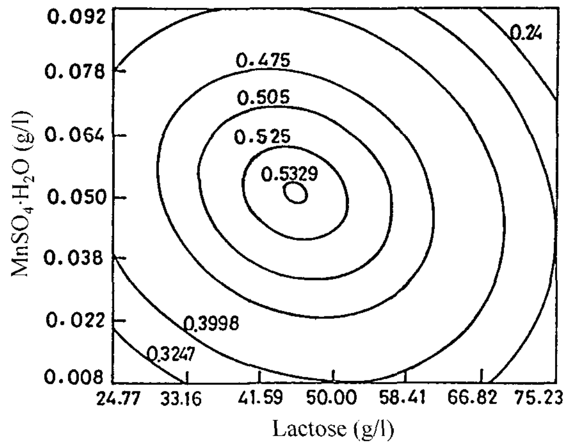


**Fig. 9.** Isoresponse contour plot showing the effect of lactose and $MnSO_4 \cdot H_2O$ concentrations on griseofulvin yield (at constant concentration of glucose).

optimum concentration of the respective constituents. The optimum values drawn from these figures are in close agreement with those obtained by maximizing the regression equations (3) and (4).

Experiments were performed at optimal levels of parameters, and maximum griseofulvin productions of 0.94 g/l and 0.55 g/l were obtained by *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively. The highest level of griseofulvin production before screening and optimization of these medium constituents were 0.74 g/l and 0.40 g/l for *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively.

## CONCLUSIONS

The production of griseofulvin was influenced by the concentrations of medium constituents. Under optimal concentrations of medium constituents, the production of griseofulvin was enhanced by 26.32% and 37.82% by *Penicillium griseofulvum* MTCC 1898 and *Penicillium griseofulvum* MTCC 2004, respectively. Yield of griseofulvin by *Penicillium griseofulvum* MTCC 1898 was 1.71 times higher than that of *Penicillium griseofulvum* MTCC 2004.

## REFERENCES

1. Balamuragam, K., V. Venkata Dasu, and T. Panda. 1999. Propionic acid production by whole cells of *Propionibacterium freudenreichii*. *Bioproc. Eng.* **20:** 109–116.
2. Box, G. E. P. and K. B. Wilson. 1951. On the experimental attainment of optimum conditions. *J. Roy. Statist. Soc.* **B13:** 1–45.
3. Chen, H. C. 1994. Response-surface methodology for optimization of citric acid fermentation by *Aspergillus foetidus*. *Proc. Biochem.* **29:** 339–405.
4. Holbrook, A., F. Bailey, and G. M. Bailey. 1963. A rapid method for the determination of griseofulvin in fermentation broth. *J. Phar. Pharmcol.* **15:** 270–273.
5. Kapat, A., S. K. Rakshit, and T. Panda. 1996. Optimization of carbon and nitrogen sources in the medium and environmental factors for enhanced production of chitinase by *Trichoderma harzianum*. *Bioproc. Eng.* **15:** 13–20.
6. Manta, D., Z. A. Basha, and T. Panda. 1998. Optimization of medium by response surface methodology for the production of tartaric acid by *Gluconobacter suboxydans*. *Bioproc. Eng.* **19:** 285–288.
7. Mason, R. L., R. F. Gunst, and J. L. Hess. 1989. *Statistical Design and Analysis of Experiments - With Applications to Engineering and Science.*, pp. 145–158, John Wiley, New York, U.S.A.
8. Rosenbrock, H. H. 1960. An automatic method for finding the greatest or least value of a function. *Computer J.* **3:** 175–184.
9. Stowe, R. A. and R. P. Mayer. 1966. Efficient screening of process variables. *Ind. Eng. Chem.* **58:** 36–40.
10. Teruel, M. L. A., E. Gontier, C. Bienaime, J. E. N. Saucedo, and J. N. Barbotin. 1997. Response surface analysis of chlortetracycline and tetracycline production with K-carrageenan immobilized *Streptomyces aureofaciens*. *Enzyme Microbiol. Technol.* **21:** 314–320.
11. Venkata Dasu, V., T. Panda, and M. Chidambarm, 2002. Development of medium for griseofulvin production. Part I. Screening of medium constituents using Plackett-Burman experimental design. *J. Microbiol. Biotechnol.* **12:** 355–359.
12. Venkata Dasu, V. and T. Panda. 2000. Optimization of microbiological parameters for enhanced griseofulvin production using response surface methodology. *Bioproc. Eng.* **22:** 45–49.