Neomycin production of *Streptomyces fradiae* NCIM 2418 was optimized by using response surface methodology (RSM), which is a powerful mathematical approach comprehensively applied in the optimization of solid state fermentation processes. In the first step of optimization, with Placket-Burman design, ammonium chloride, sodium nitrate, L-histidine, and ammonium nitrate were established to be the crucial nutritional factors affecting neomycin production significantly. In the second step, a $2^4$ full factorial central composite design and RSM were applied to determine the optimal concentration of significant variable. A second-order polynomial was determined by the multiple regression analysis of the experimental data. The optimum values for the important nutrients for the maximum were obtained as follows: ammonium chloride 2.00%, sodium nitrate 1.50%, L-histidine 0.250%, and ammonium nitrate 0.250% with a predicted value of maximum neomycin production of 20,000 g kg$^{-1}$ dry coconut oil cake. Under the optimal condition, the practical neomycin production was 19,642 g kg$^{-1}$ dry coconut oil cake. The determination coefficient ($R^2$) was 0.9232, which ensures an acceptable admissibility of the model.

1. Introduction

Neomycin is a topical antibacterial antibiotic with low toxicity. This antibiotic is employed for treating a variety of bacterial infections including diseases of skin, wound injury, and tuberculosis. This antibiotic is also used in veterinary practice, in storage tanks of petroleum fuels where it prevents the formation of sludge, and in rubber tree plantations where it increases the flow of latex by preventing bacterial infection of tapping wounds [1]. Neomycin is a bacteriostatic compound active against Gram-positive, Gram-negative, and acid-fast bacteria [2]. Neomycin was first isolated from *Streptomyces fradiae* [2]. Extensive literature is available on neomycin production by *Actinomycetes* spp. [3]; however, *Streptomyces fradiae* was established to be the best strain for neomycin production [4, 5].

Traditionally, neomycin has been produced by submerged fermentation (SmF). In recent years, however, the solid-state fermentation (SSF) processes have been increasingly applied for the production of this antibiotic [6]. SSF compared to SmF is more simple, requires lower capital, has superior productivity, reduced energy requirement, simpler fermentation media, and absence of rigorous control of fermentation parameters, uses less water and produces lower wastewater, has easier control of bacterial contamination, and requires low cost for downstream processing [7, 8]. In the SSF process, the solid substrate not only supplies the nutrients to the culture, but also serves as harborage for the production strains. The moisture content of the medium changes during fermentation as a result of evaporation and metabolic activities and thus optimum moisture level of the substrate is therefore most important [9, 10].
One of the largest oil cakes abundantly produced in India is coconut oil cake from the edible oil extraction industries. Coconut oil cake is dried and marketed as a component of animal feed, but since the selling price of the products is low and the evaporation of water from this waste consumes large amounts of heat, the production of dried coconut oil cake feed is valuable only as a waste disposal method, with marginal amounts of heat, the production of dried coconut oil cake feed and the evaporation of water from this waste consumes large animal feed, but since the selling price of the product is low is coconut oil cake from the edible oil extraction industries.

2. Materials and Methods

2.1. Organisms. *Streptomyces fradiae* NCIM 2418 was obtained from the National Collection of Industrial Microorganisms, Pune, India.

2.2. Culture Maintenance. *Streptomyces fradiae* NCIM 2418 was maintained on agar slants containing (g/L): glucose 4, yeast extract 5, peptone 10. The organism was subcultured every month and preserved at 4 ± 1°C.

2.3. Seed Culture Medium. The composition of the medium used for the development of seed culture was the following (g/L): soluble starch 20, tryptone soy broth 20, yeast extract 3, CaCO\(_3\), KH\(_2\)PO\(_4\), and MgSO\(_4\) 7H\(_2\)O 0.025. 500 mL Erlenmeyer flasks containing 100 mL of inoculum medium were autoclaved at 121°C for 20 min. The initial pH of the media was adjusted to 7.2 before sterilization. The seed culture medium was inoculated with 1 mL of spore's suspension (in sterile distilled water) containing 18 x 10\(^6\) spores from a 9-day slant. The culture was incubated for 4 days on a rotatory shaker at 160 rev per min and at 30°C. The age of the organism in the slant growth, seed age, and inoculum levels were maintained as described previously [5].

2.4. Coconut Oil Cake. Coconut oil cake was obtained from Kollam, Kerala, India, and was chopped by a chopper into small pieces, dried, and ground in a hammer mill. The ground materials were then separated by sieves into particles of desired size (moderate coarse size) and were used in media. Dry weight and moisture content of the substrates were determined gravimetrically after drying the samples at 60°C.

2.5. Neomycin Production by Solid-State Fermentation (SSF) and Antibiotic Extraction. Neomycin production was carried out using coconut oil cake as the basic solid substrate unless otherwise stated. *Streptomyces fradiae* NCIM 2418 was grown in 500 mL Erlenmeyer flasks containing 10 g of each substrate and 0.2 g yeast extract, and moisture content was 45%. The initial pH of the growth media was pH 7.2 before sterilization. Both initial pH and moisture content were measured in preliminary experiments. All flasks were sterilized at 121°C for 20 min. To each flask, 2.0 mL of spore suspension was inoculated. The cultures were incubated statically at 30°C for 8 days.

After suitable periods of growth time, the neomycin was extracted from the fermented solid medium with 10-fold phosphate buffer by shaking (200 rpm) at 30°C on a magnetic shaker for 30 min. The suspended materials and fungal biomass were separated by centrifugation (10,000 x g for 15 min) and the clarified supernatant was used as the source of crude antibiotics.

2.6. Estimation of Neomycin by HPLC. Neomycin was estimated by HPLC-ELSD (evaporative light scattering detection) method reported by Nikolaos and Michael [19]. Walters HPLC system fitted with a Waters ODS-2 C\(_18\) Spherisorb column at evaporation temperature of 45°C was used. The optimized mobile phase was water-acetone (50:50), containing 11.6 mM heptfluorobutyric acid, in an isocratic mode at a rate of 1.0 mL/min. Neomycin was eluted at 4.9 min, with asymmetry factor 1.3. Logarithmic calibration curve was obtained from 2 to 50 μg/mL (r > 0.9997). Limit of detection (LOD) was 0.6 μg/mL and R.S.D. = 1.7% (n = 3, 5, and 10)
3.3 μg/mL). Concentration of neomycin was expressed in g kg⁻¹ of substrate.

2.7. Experimental Design and Data Analysis

2.7.1. Determination of Optimal Minerals, Amino Acids, Carbon and Inorganic Nitrogen Sources. To select the appropriate minerals, amino acids, carbon, and inorganic nitrogen source, in introductory step of optimization, four minerals (zinc sulphate, manganese sulphate, ferrous sulphate, and copper sulphate), four amino acids (L-histidine, L-glutamic acid, L-asparatic acid, and L-arginine), four carbon sources (sucrose, soluble starch, maltose, and glucose) and four inorganic nitrogen sources (sodium nitrate, ammonium nitrate, ammonium hypophosphate, and ammonium chloride) were evaluated (Figures 1–4). These nutrients were, respectively, added in to the flask with coconut oil cake as a basal medium. Initial moisture content of the media was familiarized to 40%. After sterilization and cooling to ambient temperature, 10 mL inoculum was inoculated in the flasks. The final moisture then was well kept at 140%. The neomycin production of Streptomyces fradiae then was well kept at 140%. The neomycin production of NCIM 2418 by SSE. The experiment was designed by SAS package, trial version 9.1. The various levels of variables and central composite design matrix were conferred in Tables 3 and 4. The experiments were performed in duplicate and the mean values are taken for analysis.

A second-order polynomial, (1), which admits all interaction terms, was used to calculate the predicted response:

\[ Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i^2 + \sum \beta_{ij} x_i x_j, \]  

where \( Y \) represents response (dependent) variable, \( \beta_0 \) is the intercept coefficient, \( \beta_i \) is coefficient of the linear effect, \( \beta_{ij} \) is the coefficient of the quadratic effect, and \( \beta_{ij} \) is the coefficient of the interaction effect. Where \( x_i \) and \( x_j \) express the coded level of variables \( X_i \) and \( X_j \) investigated in experiments.

The variable \( X_i \) was coded as \( x_i \) according to the following:

\[ x_i = \frac{X_i - X_0}{\Delta X_i}, \]

where \( x_i \) is (dimensionless) coded value of the variable \( X_i \), \( X_0 \) is the actual value of \( X_i \) at the center point (zero) level, and the \( \Delta X_i \) is the step change value.

Media containing 10 g coconut oil cake as a basal medium from same batch was dispensed into a 500 mL Erlenmeyer flask and supplemented with the nutrients for optimization. The neomycin production of Streptomyces fradiae NCIM 2418 was verbalized in g kg⁻¹ dry coconut oil cake. An SAS package, trial version 9.1, was used for multiple regression analysis of the experimental data obtained.

The F-test was occupied to calculate the statistical significance of the quadratic polynomial. The multiple coefficient of correlation \( R \) and the determination coefficient of correlation \( R^2 \) were calculated to estimate the production of the regression equation.

3. Results

3.1. Determination of Optimal Minerals, Amino Acids, Carbon, and Inorganic Nitrogen Sources. In the introductory step of optimization, the preferred nutrients were added to coconut oil cake separately. The complementary nutrient really advances concentration of neomycin of Streptomyces fradiae NCIM 2418.

The effect of supplementation with various minerals, amino acids, carbon, and inorganic nitrogen sources on neomycin production by Streptomyces fradiae NCIM 2418 is shown in (Figures 1–4). Minerals, amino acids, carbon, and inorganic nitrogen sources in the basal medium were at a level of 1% (w/w).

The effects of supplementation with minerals were studied at a level of 1% w/w and were generally found to have
Table 1: Nutrients and test levels for Plackett-Burman experiment.

| Factors | Name                  | Units | Low level (−1) | High level (+1) |
|---------|-----------------------|-------|----------------|-----------------|
| X1      | Soluble starch        | % w/w | 0.5            | 1.0             |
| X2      | Maltose               | % w/w | 0.5            | 1.0             |
| X3      | Glucose               | % w/w | 0.5            | 1.0             |
| X4      | Copper sulphate       | % w/w | 0.5            | 1.0             |
| X5      | Zinc sulphate         | % w/w | 0.5            | 1.0             |
| X6      | L-asparatic acid      | % w/w | 0.5            | 1.0             |
| X7      | L-glutamic acid       | % w/w | 0.5            | 1.0             |
| X8      | L-Histidine           | % w/w | 0.5            | 1.0             |
| X9      | Sodium Nitrate        | % w/w | 0.5            | 1.0             |
| X10     | Ammonium chloride     | % w/w | 0.5            | 1.0             |
| X11     | Ammonium nitrate      | % w/w | 0.5            | 1.0             |

Table 2: Plackett-Burman experimental design for the evaluation of nutritional factors affecting neomycin production by Streptomyces fradiae NCIM 2418.

| RUN | X1 | X2 | X3 | X4 | X5 | X6 | X7 | X8 | X9 | X10 | X11 | Y (Neomycin g kg⁻¹) |
|-----|----|----|----|----|----|----|----|----|----|-----|-----|---------------------|
| 1   | 1  | 1  | -1 | 1  | -1 | -1 | 1  | 1  | -1 | 1   | 1   | 2345                |
| 2   | 1  | 1  | -1 | 1  | -1 | -1 | 1  | 1  | -1 | 1   | 1   | 11896               |
| 3   | -1 | 1  | 1  | -1 | 1  | -1 | -1 | 1  | 1  | 1   | 1   | 17835               |
| 4   | 1  | -1 | 1  | -1 | 1  | -1 | -1 | 1  | 1  | 1   | 1   | 8756                |
| 5   | 1  | 1  | -1 | 1  | -1 | 1  | -1 | 1  | 1  | -1  | 1   | 2453                |
| 6   | 1  | 1  | -1 | 1  | -1 | 1  | -1 | -1 | 1  | -1  | -1  | 7854                |
| 7   | -1 | 1  | 1  | 1  | 1  | -1 | 1  | 1  | -1 | 1   | -1  | 14673               |
| 8   | -1 | -1 | 1  | 1  | 1  | -1 | 1  | 1  | -1 | 1   | -1  | 8676                |
| 9   | -1 | -1 | -1 | 1  | 1  | -1 | 1  | 1  | -1 | 1   | -1  | 5345                |
| 10  | 1  | -1 | -1 | -1 | 1  | -1 | 1  | 1  | 1   | -1  | -1  | 16965               |
| 11  | -1 | 1  | -1 | -1 | -1 | 1  | 1  | 1  | -1 | 1   | 1   | 7453                |
| 12  | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | -1 | 1   | 1   | 7658                |

a moderate effect on the production of neomycin (Figure 1). Copper sulphate and zinc sulphate supplementation showed the highest production of neomycin at 13423 and 12687 g kg⁻¹ dry coconut oil cake at eighth day of incubation. The effect of the supplementation of organic nitrogen was studied by adding various amino acids at a concentration of 1% w/w. Results indicated that they almost completely stimulated the production of neomycin at eighth day of incubation. L-arginine did not affect neomycin yield (Figure 2). Almost all the carbon sources tested increased the neomycin production at eighth day of incubation. Sucrose reduced the production of neomycin (Figure 3).

Supplementation with various inorganic nitrogen sources gave a significantly higher neomycin yield at day 8 of incubation period. The addition of sodium nitrate, ammonium chloride, and ammonium nitrate at 1% w/w showed the highest yield of 14679, 13138, and 12314 g kg⁻¹ dry coconut oil cake at eighth day of incubation (Figure 4).

Based on the above experiments, soluble starch, maltose, glucose, copper sulphate, zinc sulphate, L-asparatic acid, L-glutamic acid, L-histidine, sodium nitrate, ammonium chloride, and ammonium nitrate were selected for statistical optimization.

3.2. Evaluation of the Nutritional Factors Affecting Neomycin Productivity by Plackett-Burman Design. Eleven different nutritional factors including soluble starch, maltose, glucose, copper sulphate, zinc sulphate, L-asparatic acid, L-glutamic acid, L-histidine, sodium nitrate, ammonium chloride, and ammonium nitrate were sheltered for their effect on neomycin production using the Plackett-Burman design. The independent variables examined and their settings are shown in Table 1. The design plan and the averages of neomycin production for the different trials are given in g kg⁻¹ dry coconut oil cake and shown in Table 2. The main effect of each variable was estimated as the difference between both averages of measurements made at the high level (+1) and at the low level (−1) of that nutritional factor. The data in Table 2 show a broad change from 2345 to 17835 g kg⁻¹ dry coconut oil cake of neomycin production. This change reflects
Figure 1: Effect of minerals (1% w/w) on neomycin production by *Streptomyces fradiae* NCIM 2418 using coconut oil cake under solid-state fermentation.

Figure 2: Effect of amino acids (1% w/w) on neomycin production by *Streptomyces fradiae* NCIM 2418 using coconut oil cake under solid-state fermentation.

Figure 3: Effect of carbon sources (1% w/w) on neomycin production by *Streptomyces fradiae* NCIM 2418 using coconut oil cake under solid-state fermentation.

Figure 4: Effect of inorganic nitrogen sources (1% w/w) on neomycin production by *Streptomyces fradiae* NCIM 2418 using coconut oil cake under solid-state fermentation.
the account of medium optimization to accomplish higher productivity. The analysis of the data from the Plackett-Burman experiments involved a first-order (main effects) model. The main effects of the nutritional examined factors on the neomycin production were measured and conferred graphically in Figure 5.

On the basis of the analysis of the estimate of the eleven variables after day 8 of incubation, ammonium chloride and sodium nitrate showed large positive effect on neomycin production. L-histidine and ammonium nitrate showed large negative effect on neomycin production. Soluble starch, glucose, sucrose, maltose, zinc sulphate, manganese sulphate, ferrous sulphate, copper sulphate, L-arginine, L-aspartic acid, and L-glutamic acid have a slight effect on neomycin productivity. Figure 5 shows the ranking of nutritional factor estimate in a Pareto chart. The Pareto chart displays the magnitude of each nutritional factor estimate and it is a convenient way to view the results of a Plackett-Burman design.

3.3. Central Composite Design and Response Surface Method. According to Plackett-Burman design experiment, four significant variables X10 (ammonium chloride), X9 (sodium nitrate), X8 (L-histidine), and X11 (ammonium nitrate) were preferred for advance optimization using central composite design RSM to calculate the several optimal values. The coded values of the variables at various levels in central composite design (RSM) analysis were given in Table 3.

Response results shown in Table 4 were analyzed using SAS package, trial version 9.1, software. The t-test and P values were used to determine the effect of each nutritional factor on neomycin production (Table 6). Ammonium chloride, sodium nitrate, L-histidine, and ammonium nitrate and the interaction of the four preferred variables had a significant effect on neomycin yield (P < 0.05), as well as the quadratic terms of ammonium chloride, sodium nitrate, L-histidine, and ammonium nitrate. The fit of the model was checked by the coefficient of determination $R^2$, which was measured to be 0.9232, indicating that 92.32% of the changeability in the response can be explained by the model. The value of adjusted determination coefficient ($R^2_{Adj} = 0.8287$) was also high enough to denote the significance of the model. The model can be shown as follows:

$$Y \left( \text{g kg}^{-1} \right) = 38034.31 + 336.2 \times (\text{BLOCK} = '1') - 5604.3 \times (\text{BLOCK} = '2') - X10 \ 999.87 + X9 \ 3278.04 - X8 \ 2514.37 + X11 \ 413.95 + X10 \times X10 \ 95.90 + X10 \times X9 \ 438.31 + X10 \times X8 \ 2550.93 - X10 \times X11 \ 2645.31 - X9 \times X9 \ 1563.46 + X9 \times X8 \ 2163.43 + X9 \times X11 \ 1436.93$$

where Y is the response, that is, neomycin concentration, and X10, X9, X8, and X11 are the coded values of ammonium chloride, sodium nitrate, L-histidine, and ammonium nitrate, respectively.

The significance of each estimate was determined by P values which are listed in Table 5. Condition for neomycin production had significant model because the value of $P > F$ for the model was less than 0.05. Among the model terms, the linear effects of sodium nitrate and L-histidine were more significant than other nutritional factors. These suggest that the concentrations of sodium nitrate and L-histidine were a direct relationship with the neomycin production. The estimate for quadratic effect of sodium nitrate was more significant neomycin production. The interaction between ammonium chloride and L-histidine, ammonium chloride and ammonium nitrate, sodium nitrate and L-histidine, and L-histidine and ammonium nitrate had significant influence on neomycin production.

Table 6 presents Fisher’s F-test of analysis of variance (ANOVA), which also proves that this regression was statistically significant ($P < 0.0001$) at 95% of confidence level. Generally, the measured F value should be several times greater than tabulated F value if the model is admirable prediction of experimental results and the estimated nutritional factors effects are actual. In this case, the ANOVA of the regression model demonstrates that the model is highly significant, as is observable from the measured F value (=9.769752) and a very low probability value ($P > F = 0.0001$). Consequently, these results indicate a good adequacy in the models for neomycin yield. In order to achieve an advanced appreciation of the effects of the variables on the production of neomycin, the predicted model was presented as 3D response surface graphs (Figures 6–9).

The 3D surface plots of the neomycin production with respect to the concentrations of ammonium chloride and L-histidine are shown in Figure 6. The neomycin production increased with the increase concentration of ammonium chloride and L-histidine when sodium nitrate and ammonium nitrate were held at zero level. Figure 7 showed effect of sodium nitrate concentration and L-histidine concentration on neomycin production when ammonium chloride and ammonium nitrate were held at zero level. It can be observed that an increase of neomycin production with increase in concentration of L-histidine and further increase in concentration leads to decrease neomycin production and increase in concentration of sodium nitrate with increases the neomycin production and further increase in concentration leads to decrease neomycin production. The three-dimensional response surfaces at various concentration of L-histidine and ammonium nitrate concentration were plotted in Figure 8. From the graph it was observed that the content of neomycin increases sharply when the concentration of L-histidine decreases below 0.5% (w/w). However the content of neomycin increased with the concentration of ammonium...
Table 3: Coded and actual values of factors in central composite design.

| Factor | Name        | Units  | Axial (−2) | Low (−1) | Levels | High (+1) | Axial (+2) |
|--------|-------------|--------|------------|----------|--------|-----------|------------|
| X10    | Ammonium chloride | % w/w | 1.25       | 1.5      | Center (0) | 1.75      | 2.0        | 2.25       |
| X9     | Sodium nitrate   | % w/w | 1.25       | 1.5      | Center (0) | 1.75      | 2.0        | 2.25       |
| X8     | L-Histidine     | % w/w | 0.125      | 0.25     | Center (0) | 0.375     | 0.50       | 0.625      |
| X11    | Ammonium nitrate | % w/w | 0.125      | 0.25     | Center (0) | 0.375     | 0.50       | 0.625      |

Figure 5: Pareto chart rationalizing the estimate of each variable on the neomycin yield (g kg⁻¹) produced by Streptomyces fradiae NCIM 2418.

Figure 6: Three-dimensional surface plot of neomycin production as function of ammonium chloride (X10) and L-histidine (X8) (ammonium nitrate and sodium nitrate were kept at zero level).

Figure 7: Three-dimensional surface plot of neomycin production as function of sodium nitrate (X9) and L-histidine (X10) (ammonium nitrate and ammonium chloride were kept at zero level).

Figure 8: Three-dimensional surface plot of neomycin production as function of ammonium nitrate (X11) and L-histidine (X8) (ammonium chloride and sodium nitrate were kept at zero level).

Figure 9: Three-dimensional surface plot of neomycin production as function of ammonium nitrate (X11) and sodium nitrate (X9) (ammonium chloride and L-histidine were kept at zero level).

The predicted optimum levels of the tried variables, namely, ammonium chloride, sodium nitrate, L-histidine, and ammonium nitrate, were obtained by applying regression analysis of (3) by using SAS package, trial version 9.1. The optimal levels were as follows: ammonium chloride = 2.00% (w/w), sodium nitrate 1.50% (w/w), L-histidine 0.250%(w/w), and ammonium nitrate 0.250% (w/w) with corresponding...
Table 4: Central composite design arrangement and responses.

| RUN | BLOCK | X10 | X9 | X8  | X11 | Y (Neomycin g kg\(^{-1}\)) |
|-----|-------|-----|----|-----|-----|--------------------------|
| 1   | 1     | 1.50| 1.50| 0.250| 0.500| 18567                   |
| 2   | 1     | 1.50| 1.50| 0.500| 0.250| 1765                    |
| 3   | 1     | 1.50| 2.00| 0.250| 0.250| 18643                   |
| 4   | 1     | 1.50| 2.00| 0.500| 0.500| 18347                   |
| 5   | 1     | 2.00| 1.50| 0.250| 0.250| 19642                   |
| 6   | 1     | 2.00| 1.50| 0.500| 0.500| 4567                    |
| 7   | 1     | 2.00| 2.00| 0.250| 0.500| 10754                   |
| 8   | 1     | 2.00| 2.00| 0.500| 0.250| 17643                   |
| 9   | 1     | 1.75| 1.75| 0.375| 0.375| 0.250                   |
| 10  | 1     | 1.75| 1.75| 0.375| 0.375| 15865                   |
| 11  | 2     | 1.50| 1.50| 0.250| 0.250| 14678                   |
| 12  | 2     | 1.50| 1.50| 0.500| 0.500| 1654                    |
| 13  | 2     | 1.50| 2.00| 0.250| 0.500| 17543                   |
| 14  | 2     | 1.50| 2.00| 0.500| 0.250| 763                     |
| 15  | 2     | 2.00| 1.50| 0.250| 0.250| 765                     |
| 16  | 2     | 2.00| 1.50| 0.500| 0.500| 1864                    |
| 17  | 2     | 2.00| 2.00| 0.250| 0.250| 11543                   |
| 18  | 2     | 2.00| 2.00| 0.500| 0.500| 12543                   |
| 19  | 2     | 1.75| 1.75| 0.375| 0.375| 7843                    |
| 20  | 2     | 1.75| 1.75| 0.375| 0.375| 12356                   |
| 21  | 3     | 1.25| 1.75| 0.375| 0.375| 18532                   |
| 22  | 3     | 2.25| 1.75| 0.375| 0.375| 12853                   |
| 23  | 3     | 1.75| 1.25| 0.375| 0.375| 456                     |
| 24  | 3     | 1.75| 2.25| 0.375| 0.375| 17654                   |
| 25  | 3     | 1.75| 1.75| 0.125| 0.375| 15432                   |
| 26  | 3     | 1.75| 1.75| 0.625| 0.375| 11754                   |
| 27  | 3     | 1.75| 1.75| 0.375| 0.125| 12875                   |
| 28  | 3     | 1.75| 1.75| 0.375| 0.625| 18743                   |
| 29  | 3     | 1.75| 1.75| 0.375| 0.375| 11754                   |
| 30  | 3     | 1.75| 1.75| 0.375| 0.375| 17542                   |

Table 5: Effect estimates for the second-order polynomial model.

| Term          | Estimate | Std. Err. | t   | Pr > |t  |
|---------------|----------|-----------|-----|------|---|
| BLOCK = "T"   | 336.2    | 1200.378  | 0.280078 | 0.783824 |
| BLOCK = "2"   | -5604.3  | 1200.378  | -4.66878 | 0.000439 |
| X10           | -999.875 | 547.8951  | -1.82494 | 0.091066 |
| X9            | 3278.0417| 547.8951  | 5.982973 | 0.0001 |
| X8            | -2514.375| 547.8951  | -4.58915 | 0.000508 |
| X11           | 413.95833| 547.8951  | 0.755543 | 0.463394 |
| X10 * X10     | 95.90625 | 512.5089  | 0.187131 | 0.854447 |
| X10 * X9      | 438.3125 | 671.0317  | 0.653192 | 0.525017 |
| X10 * X8      | 2550.9375| 671.0317  | 3.801516 | 0.002201 |
| X10 * X11     | -2645.313| 671.0317  | -3.94216 | 0.001686 |
| X9 * X9       | -1563.469| 512.5089  | -3.05062 | 0.009289 |
| X9 * X8       | 2163.4375| 671.0317  | 3.224047 | 0.006652 |
| X9 * X11      | 1436.9375| 671.0317  | 2.141385 | 0.051764 |
| X8 * X8       | -428.9688| 512.5089  | -0.837   | 0.417715 |
| X8 * X11      | 1997.0625| 671.0317  | 2.976108 | 0.01072 |
| X11 * X11     | 125.03125| 512.5089  | 0.243959 | 0.81107 |
Table 6: ANOVA for response surface quadratic model.

| Source       | DF | SS    | MS    | F      | Pr > F |
|--------------|----|-------|-------|--------|--------|
| Model        | 16 | 1.12  | 7038  | 9.76   | 0.0001 |
| Pure error   | 3  | 2717  | 9059  |        |        |
| Lack of fit  | 10 | 6647  | 6647  |        |        |
| Total        | 29 | 1.21  |       |        |        |

DF: degree of freedom; SS: sum of square; MS: mean square.

$R^2$-square = 0.9232; Adj. $R^2$-square = 0.8287.

**Figure 8:** Three-dimensional surface plot of neomycin production as function of L-histidine (X8) and ammonium nitrate (X11) (sodium nitrate and ammonium chloride were kept at zero level).

**Figure 9:** Three-dimensional surface plot of neomycin production as function of ammonium chloride (X10) and ammonium nitrate (X11) (L-histidine and sodium nitrate were kept at zero level).

The practical comparable response was 19642 g kg$^{-1}$ dry coconut oil cake. Verification of the predicted values was guided by using optimal concentration of nutrients in fermentation experiments. The result verified the weight and effectiveness of this model.

### 4. Discussions

The renewed interest in producing antibiotics from various production strains has spawned increased interest in solid state fermentation. Previous results indicated that optimal fermentation conditions in solid substrate cultivation must be selected experimentally for each organism/substrate combination [23]. Some general conclusions can be drawn in addition to the specific recommendations for cultivating *Streptomyces fradiae* NCIM 2418 on coconut oil cake. Mahalaxmi et al. [24] reported that the solid substrate composition plays a vital role in the production of the antibiotics. In the present study the neomycin produced by the *Streptomyces fradiae* NCIM 2418 shows much higher than the literature reports. The optimal conditions for the production of neomycin by *Streptomyces marinensis* NUV-5 were determined in earlier work [1, 6] in which a dextrin, raspberry seed powder, and concentrated mineral (1% and 10% w/w) were used as nutrients. Production strain *Streptomyces fradiae* is a well-known neomycin production strain [4, 5, 25] and, therefore, enough of nutrients are required for cell growth and metabolism. Keeping this in view, several nutrients were screened (introductory step of optimization) for neomycin production. The data indicated that neomycin production pattern varied with the coconut oil cake.

The formal method (one factor at a time) for medium optimization is time consuming and cannot depict the combined effect of multiple processes parameters involved [26]. The Plackett-Burman method is comprehensively used for the identification of significant variables as well as their significance levels [27]. From the studied nutrient variables different ingredients have different effects on neomycin production. Ammonium chloride, sodium nitrate, L-histidine, and ammonium nitrate were significant nutritional factors, whereas others were not significant nutritional factors.

Response surface methodology (RSM) is a crucial statistical technique for disclosure interactions among the variables and screening the optimum processes parameters for beneficial responses [28]. As shown in the results above, RSM are powerful tools for identifying the significant nutritional factors and their values for neomycin production. The graphical representations of the regression (3), called the response surfaces plots, were obtained using SAS trial version 9.1, and
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