Chapter – III

THEORETICAL CONSIDERATION

To meet the water quality standards consistent with environmental protection laws, industrial wastewater needs the simultaneous removal of many contaminants. The wastewater containing heavy metals is treated by the convectional methods to meet the pollution board standards and to recover the metals. These methods may be used individually or in combination.

3.1. Convectional methods

The discharge of heavy metals into aquatic ecosystems is a concern over the last few decades. The most important concerns among the world environment is the removal of heavy metals from aqueous waste effectively [1]. The most common remediation (convectional) methods are chemical precipitation, ion-exchange, reverse osmosis, filtration, solvent extraction and adsorption [2, 3, 4].

Chemical precipitation

Precipitation involves the conversion of soluble heavy metals to insoluble salts that will precipitate. The most common precipitation methods are lime precipitation and sulphide precipitation.

Lime precipitation

Lime is generally used due to its cost and easy to control pH in the range of 8-10. For the precipitation of fluoride quick lime can also be used [5] and the excess lime acts as adsorbent [6]. The disadvantages of this method are generation of large volume of sludge, not effective in the case of mixed metal waste.
Sulphide precipitation

Excellent metal removal can be attained by sulphide precipitation since most of the heavy metals form stable sulphide. [7] developed a method for the removal of chromium from electroplating effluents by sulphide-hydroxide precipitation. The disadvantages are high costs and potential formation of toxic H₂S gas.

Ion-exchange

Ion exchange operations are essentially chemical reactions between an electrolyte in waste water and an insoluble electrolyte with which the waste water is contacted. In this process, metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. Ion exchange reactions are stoichiometric and reversible, For example:

$$M^{2+} + 2RH \leftrightarrow R2M + 2H^+$$

where, $R =$ aliphatic portion of cation exchanger.

The disadvantages are high cost and partial removal of certain ions.

Reverse osmosis

The separation process uses pressure to force a solvent through a membrane that retains the solute on one side and allows the pure to pass to the other side. The process however is expensive i.e., both operating and capital costs. The membranes commonly used are

- Cellulose acetate
- Aromatic polyamide
- NS 100
Reverse osmosis process could be effectively applied for denitrification and desalination of water in rural areas, [8] and for the removal of Cr, Pb, Fe, Ni, Cu and Zn from vehicle wash-rack water [9].

The process is expensive, i.e., both capital and operating costs are high.

**Electrodialysis**

This is a membrane separation process in which electric field is applied across a series of membranes, which are inorganic in nature. Two types of membranes are placed alternatively in the electrodialysis cell:

- Cation exchange
- Anion exchange

These membranes are placed at the ends of the cell. Raw wastewater is fed continuously into the concentrating compartments and treated wastewater withdrawn continuously from the alternate compartments.

Like Reverse osmosis, fouling of membrane and concentration polarization are the common problems which affect the performance of electrodialysis unit.

**Ultrafiltration**

These are pressure driven membrane operations that use porous membranes for the removal of heavy metals. Ultrafiltration separate mainly by size exclusion of solute(s). Ultrafiltration retains particles of submicron size by ultra microporous membranes. Concentration polarization effects in ultrafiltration are small at low pressure, low-solute concentrations, and a high velocity on the retentate side of the membrane. Fouling in ultrafiltration occurs by adsorption of solutes on the upstream surface, and/or deposition of solutes within the membrane pores. Fouling can be reversed by periodic cleaning. Types of membrane materials:
cellulose acetate, polyamides, polysulfon. The main disadvantage of this process is generation of sludge.

**Evaporation**

Evaporation is the process in which energy source is used to vaporize a liquid form from a solution. This process consists of evaporating metal plate in rinse water to drive off the vapor and thus concentrate the chromic acid for recovery and reuse. For molecules of a liquid to evaporate, they must be located near the surface, be moving in the proper direction, and have sufficient kinetic energy to overcome liquid-phase intermolecular forces. Only a small proportion of the molecules meet these criteria, so the rate of evaporation is limited. Since the kinetic energy of a molecule is proportional to its temperature, evaporation proceeds more quickly at higher temperature. As the faster-moving molecules escape, the remaining molecules have lower average kinetic energy and the temperature of the liquid thus decreases. This phenomenon is also called evaporative cooling. This is why evaporating sweat cools the human body.

Evaporation is an easy, reliable and maintenance-free process. High energy consumption and undesirable constituents in the recycled bath are the main disadvantages.

**Adsorption**

The process of use of solids for removing substances from either gaseous or liquids solutions is known as adsorption. This involves the preferential partitioning of substances from the gaseous or liquid phase onto the surface of a solid substrate. The material that is being adsorbed or concentrated is called adsorbate. The solid or liquid, which adsorbs is called adsorbent. Most of the
heavy metals are efficiently removed by adsorption method [10]. Adsorption can be classified depending on the type of bonding involved.

**Physical sorption**

In physisorption, the only binding is by weak Vanderwaal’s forces. Hence it is called Vanderwaal’s adsorption. This is due to the fluctuating dipole moments on the interacting adsorbate and adsorbent. These are analogous to those between molecules in liquids. Physisorption is a reversible process and very effective. Physisorption occurs at lower temperature or close to the critical temperature of an adsorbed substance or fluid. Heat of adsorption is 1 kcal/mole.

**Chemical sorption**

In chemisorption, the binding is by a chemical bond, which is a substantial rearrangement of electron density that is formed between the adsorbate and adsorbent. The nature of this bond may lie anywhere between the extremes of virtually complete ionic or covalent character. The strength may vary considerably. Chemical sorption is an irreversible process because of strong electrostatic force of interaction between the adsorbent and adsorbate. Chemical sorption occurs at temperatures much higher than the critical temperature of an adsorbed substance or fluid. Heat of adsorption is 50-100 kcal/mole.

**3.2. Alternative to Convectional methods**

With respect to environmental and economical considerations, removal of heavy metals is important. The removal of these heavy metals by conventional methods
may result in high capital investment and running cost, incomplete metal removal. Most of these methods are ineffective when the heavy metal concentration is in the range of 10-100 mg/L and the permissible concentration is less than 1 mg/L [11]. Hence, there is a growing interest for the development of technique which can easily implemented, economical and effective.

Alternative to these methods is biosorption, which have been extensively studied during several decades.

Biosorption is a process that utilizes inexpensive biomass to remove toxic heavy metals from industrial effluents [12, 13]. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from waste water through metabolically mediated or physico-chemical pathways of uptake [14]. Biosorption can be performed by both living and dead cells [15, 16], offers advantages such as low operating cost, high efficiency in detoxifying low concentration heavy metal from waste water, no production of secondary toxic compounds, short operation time and no nutrients requirements [17, 18]. For developing full-scale biosorption processes, these advantages are the primary incentives [19].

For the efficient removal of heavy metal ions, biosorption uses low-cost, easily available and environment friendly biological materials called biosorbents.

**Biosorbents**

Biosorbents used in present study are given below:

**Syzigium cumini seeds**

*Syzigium cumini*, evergreen tree is native of Indian subcontinent, belongs to family of Myrtaceae. It is a fast growing tree with height of 30 m and can live more than 100 years. It starts bearing fruits from 4 to 7 years. Leaves are smooth,
glossy and oblong opposite leaves with a turpentine smell. The tree starts flowering from March to April and fruits are developed by May or June. The flowers are fragrant and small, about 5 mm diameter. Fruits are oblong, egg shaped, starts green and turns pink to shining crimson black as it matures, and each fruit contains a single seed of 1 to 2 cm long. The whole seed is enclosed in a cream colored coriaceous covering, smooth and oval. All the parts of tree can be medicinally used and it has long tradition in alternative medicine. The seeds are shown below in fig. 3(a).

![Fig. 3 (a) Syzgium cumini seeds](image)

**Caulerpa taxifolia**

*Caulerpa taxifolia*, a green algae (fig 3-b) is native to the Indian Ocean, belongs to family of *Caulerpaceace*. Their roots, which are rhizoids, grow into the ocean floor absorbs water and nutrients. Fronds which are leaf like structure has many feather like projects called pinnules. These fronds are interconnected by stolons, stem like structures that grow about 3-10 cm from main stream, 1-2 mm in diameter, from which rhizoids and new fronds emerge. It has the ability to grow on rocks, sand and mud. It has rapid growth of 1 cm/day.
3.3. ISOTHERMS FOR BIOSORPTION

Freundlich isotherm

The Freundlich [20] relationship is an empirical equation. It does not indicate a finite uptake capacity of the biosorbent and can thus only be applied in case of low and intermediate concentration ranges.

The Freundlich isotherm is given by

\[ Q_e = K_f C_e^n \]  

(3.1)

we get

\[ \log q_e = \log K_f + n \log C_e \]  

(3.2)

where ‘\( K_f \)’ and ‘\( n \)’ are known as Freundlich constants obtainable from the plots of \( \log q_e \) versus \( \log C_e \) on the basis of the linear form of the eq. (3.2).

Langmuir isotherm

Since the chemical forces fall off very rapidly with distance, it is probable that chemisorption does not extend beyond a single layer of biosorbate on the surface
of the solid. It can be anticipated as first pointed out by Langmuir [21] that chemisorbed biosorbate layers may be only one molecule thick. The Langmuir isotherm is the most widely used two-parameter equation. The relationship is of a hyperbolic type form:

\[
\frac{q_e}{q_m} = \frac{bC_e \text{ } (1 + bC_e)}{1 + bC_e} \text{ } (3.3)
\]

where \(C_e\) is the concentration of the biosorbate at equilibrium

\(q_e\) is the amount adsorbed at equilibrium per unit mass of the biosorbent

\(q_m\) is the maximum amount adsorbed per unit mass of type biosorbent

\(b\) is the coefficient related to affinity

Eq. (3.3) can be rearranged as

\[
\frac{(C_e/q_e)}{q_e} = \frac{1}{bq_m} + \frac{C_e}{q_m} \text{ } (3.4)
\]

From the plots between \((C_e/q_e)\) and \(C_e\), we can calculate the slope \((1/q_m)\) and the intercept \((1/b)\). Further analysis of the Langmuir equation is made on the basis of separation factor, \(R_L\) defined as

\[
R_L = \frac{1}{1 + bC_e} \text{ } (3.5)
\]

\(0 < R_L < 1\) indicates Favourable biosorption

\(R_L > 1\) indicates Unfavourable biosorption

\(R_L = 1\) indicates Linear biosorption

\(R_L = 0\) indicates Irreversible biosorption

**Temkin isotherm**

Unlike the Langmuir and Freundlich equation, the Temkin isotherm takes into account the interactions between adsorbents and metal ions to be adsorbed and is based on the assumption that the free energy of sorption is a function of the surface coverage [22].
\[
q_e = \frac{RT}{b_T} \ln A_T + \frac{RT}{b_T} \ln C_e
\]  
(3.6)

where \( A_T \) is the equilibrium binding constant corresponding to the maximum binding energy, \( b_T \) is the Temkin isotherm constant, \( T \) is the temperature (K), and \( R \) is the ideal gas constant (8.315 J/mol/K).

### 3.4. Kinetics of biosorption

Kinetics plays a vital role in biosorption. The kinetics describes the order of the reaction. Among various kinetic models proposed by different authors, Lagergren’s first order model [23] finds extensive application. Most of the biosorption systems using different biosorbents for metal removal are found to satisfy this model. On the other hand, pseudo second order kinetics can also describe these interactions very well in certain specific cases.

The Lagregren’s equation is:

\[
(dq/dt) = K_{ad} (q_e - q_t)
\]

(3.7)

where \( q_e \) and \( q_t \) are the amounts biosorbed at equilibrium and at time \( t \)

\( K_{ad} \) is the rate constant

Integrating on both sides

\[
\int (dq/dt) = \int K_{ad} (q_e - q_t)
\]

\[
\int (dq/ (q_e - q_t)) = \int K_{ad} \ dt
\]

(3.8)

After applying the initial condition \( q_t = 0 \) at \( t = 0 \), we get

\[
\log (q_e - q_t) = \log q_e - (K_{ad}/ 2.303) t
\]

(3.9)

If the plot of \( \log (q_e - q_t) \) vs ‘t’ gives a straight line, it indicates that the first order kinetics better describes the biosorption. Then the rate constant (slope), \( K_{ad} \) can be
calculated from eq. 3.9. If the experimental results do not follow eq. 3.9 they differ in two important aspects:

- \( K_{ad}(q_e-q_t) \) does not represent the number of biosorption sites
- \( \log q_e \) is not equal to the intercept of the plot of \( \log (q_e-q_t) \) against \( t \).

In such cases, the second order rate equation is given by

\[
\frac{dq_t}{dt} = K (q_e-q_t)^2
\]

Equation (3.10) can be written as

\[
\frac{dq_t}{(q_e-q_t)^2} = K \, dt
\]

Let \( q_e-q_t = x \) and differentiation

\[-dq_t = dx\]

Then eq. (3.11) gives \((-dx/x^2) = K \, dt\), this on integration

\[
\int\left(-\frac{dx}{x^2}\right) = \int K \, dt
\]

\[
\frac{1}{x} = Kt + C
\]

\( C = 1/q_e \) at \( t = 0 \) and \( x = q_e \)

Substituting in eq. (3.11), we obtain

\[
\frac{1}{(q_e-q_t)} = Kt + \left(\frac{1}{q_e}\right)
\]

Rearranging the above eq., we get the linear form as

\[
\left(\frac{t}{q_t}\right) = \left(\frac{1}{K \, q_e}\right) + \left(\frac{1}{q_e}\right) \, t
\]

If the plot of \( (t/q_t) \) vs \( t \) yields a straight line, the biosorption is described by the second order kinetics and then the values of \( q_e \) and \( K \) are calculated.

### 3.5. Thermodynamics of biosorption

Biosorption is temperature dependent. In general, the temperature is associated with three thermodynamics parameters namely change in enthalpy (\( \Delta H \)), change in entropy (\( \Delta S \)) and change in Gibbs free energy (\( \Delta G \)).
Change in enthalpy is an important thermodynamic function whose value defines the nature of the biosorption. The positive value of enthalpy change indicates endothermic while the negative value indicates exothermic nature. Jacodus Henricus Van’t Hoff expressed a relation that relates the change in biosorption affinity with change in temperature.

The Van’t Hoff equation is

$$\log \left( \frac{q_e}{C_e} \right) = -\frac{\Delta H}{2.303 \, RT} + \frac{\Delta S}{2.303 \, R}$$  \hspace{1cm} (3.14)

where \( \frac{q_e}{C_e} \) is called the adsorption affinity and is the ratio of the amount per unit mass at equilibrium concentration of the adsorbate \( q_e \) to the equilibrium concentration \( C_e \).

\( \Delta H \) and \( \Delta S \) values are calculated from the plots between \( \log \left( \frac{q_e}{C_e} \right) \) and \( \frac{1}{T} \)

From the plots

Slope = - \( \frac{\Delta H}{2.303 \, R} \) and

Intercept = \( \frac{\Delta S}{2.303 \, R} \)

Change in Gibbs free energy \( \Delta G \) is related to change in enthalpy \( \Delta H \) and change in entropy \( \Delta S \) as

\[ \Delta G = \Delta H - T \Delta S \]

If the value of \( \Delta S \) is less than zero, it indicates that the process is highly reversible. If \( \Delta S \) is more than or equal to zero, it indicates the irreversibility of the process. The negative value for \( \Delta G \) indicates the spontaneity of the biosorption, whereas the positive value indicates the biosorption is not spontaneous.

3.6. Response Surface Methodology (RSM)

Optimization refers to improving the performance of a process in order to obtain the maximum benefit from it. Traditionally, optimization in biosorption process
has been carried out by monitoring the influence of one factor at a time on an experimental response, keeping the others at constant levels. This optimization technique is called ‘one-variable at a time’. This technique used for optimizing a multivariable system is not only time consuming but also often easily misses the interactions between the components. Also, this method requires a large number of experiments to determine the optimum levels when the interactions are significant [24].

In order to overcome this problem, the optimization process has been carried out by using multivariate statistical techniques. Among the most relevant multivariate techniques used in optimization process is the Response Surface Methodology (RSM). RSM is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data taken within the feasible operable region of process variables. It can be well applied when a response (dependent variable), or a set of responses of interest are influenced by several independent process variables. The objective is to simultaneously optimize the levels of these process variables to attain the best system performance. A detailed account of this technique has been outlined [25]. Basically, this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response, and checking the adequacy of the model.

Before applying the RSM methodology, it is necessary to choose an experimental design that will define the number of experimental runs that should be carried out in the experimental feasible region. Several experimental designs exist that reduce the number of experiments. Thus, if it is desired to detect the significant variables
which influence the response, first-order experimental design like Factorial
design, or Plackett-Burman design can be used. On the other hand, to maximize a
response function or to optimize a process, second-order experimental designs
such as three level factorial design, Box-Behnken design, or Central Composite
design can be used.

The present chapter discusses the exploitation of experimental designs for
identifying the significant variables and then application of RSM using different
experimental designs for second order models for optimizing the parameters in
biosorption process, laying emphasis on Central Composite design.

Theory

RSM consists of a group of mathematical and statistical techniques that are based
on the fit of empirical models to the experimental data obtained in relation to
experimental design. Towards this objective, linear or square polynomial
functions are employed to describe the system studied and, consequently, to
explore experimental conditions for its optimization.

The stages in the application of RSM as an optimization technique are as follows:
(1) the selection of significant independent variables either through screening
studies, or by the experience of the researcher, (2) the choice of the experimental
design and carrying out the experiments according to the selected experimental
design, (3) the mathematical and statistical treatment of the obtained experimental
data for fitting a polynomial function, (4) the evaluation of the model’s fitness,
(5) the verification of the model and the necessity and possibility of performing
displacement experiments in the direction to the optimal region, and (6) obtaining
the optimum values for each variable to get the maximum response of the system.
Screening of important variables

Numerous variables may affect the response of the system studied, and it is practically impossible to identify and control the small contributions from each one. Therefore, it is necessary to select those variables which significantly affect the process. Hence screening designs should be carried out to determine which of the several experimental variables and their interactions present more significant effects. Full, or fractional two-level Factorial design, or Plackett-Burman design may be used towards this objective [26].

Choice of the experimental design

The simplest model which can be used in RSM is based on a linear function. For its application, it is necessary that the responses obtained are well fitted by the following equation:

\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \varepsilon \]  

(3.15)

where \( k \) is the number of variables, \( \beta_0 \) is the constant term, \( \beta_i \) represents the coefficients of the linear parameters, \( x_i \) represents the variables, and \( \varepsilon \) is the error associated with the experiments.

Because of linear nature, the responses should not present any curvature. To evaluate curvature, a second-order model must be used. Two-level factorial designs are used in the estimation of first-order effects, but they fail when additional effects, such as second-order effects are significant. So, a central point in two-level factorial designs can be used for evaluating curvature. The next level of the polynomial model should contain additional terms, which describe the interaction between the different experimental variables. Therefore, a model for a second-order interaction presents the following terms:
\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i,j=1}^{k} \beta_{ij} x_i x_j + \epsilon \] (3.16)

where \( \beta_{ij} \) represents the coefficients of the interaction terms.

In order to determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the equation presented below:

\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_i x_i^2 + \sum_{i,j=1}^{k} \beta_{ij} x_i x_j + \epsilon \] (3.17)

where \( \beta_{ii} \) represents the coefficients of the quadratic terms.

To estimate the parameters in Eq. (3.17), it is necessary to study the variables in at least three levels. Many second order symmetrical experimental designs are available and the more known designs are the three-level Factorial design, Box-Behnken design, Central Composite design, and Doehlert design. These designs differ from one another with respect to their selection of experimental points, number of levels for variables, and number of experimental runs.

**Coding of the levels of the variables**

Coding of the levels of the variables consists of transforming the real value of the variables into coordinates inside a scale with dimensionless values, which must be proportional at its localization in the experimental space. For three-level Factorial, Box–Behnken or Central Composite Designs, the following relation is used:

\[ x_i = \frac{X_i - \bar{X}}{\Delta X} \] (3.18)
where $x_i$ is the coded value of the $i^{th}$ variable, $X_i$ is the real value, $\bar{X}_i$ is the value at the center point and $\Delta X_i$ is step change value which is the difference between the real value in the central point and the superior level of the variable.

**Evaluation of the fitted model**

The more reliable way to evaluate the quality of the model fitted is by the application of **Analysis of Variance (ANOVA)**. The central idea of ANOVA is to compare the variation due to the treatment (change in the combination of variable levels) with the variation due to random errors inherent in the measurement of the generated responses. From this comparison, it is possible to evaluate the significance of the regression used to foresee responses considering the sources of experimental variance.

In ANOVA, the evaluation of data set variation is made by studying its dispersion. The ANOVA for the full quadratic model is given in table 3.1. The evaluation of the deviation is represented by its square which is presented in Eq. (3.19):

$$d_i^2 = (y_{ij} - \bar{y})^2$$ (3.19)

where $d_i$ is the deviation at each observation, $y_{ij}$ are the replicates performed in each individual level, $\bar{y}_i$ is the mean of replicates performed in the same set of experimental conditions.

The sum of the squares for all experimental deviations in relation to the mean is called the total sum of the squares ($SS_{tot}$). It can be obtained by adding the sum of the squares due to the fitted mathematical model ($SS_{reg}$), and the sum of the squares due to residuals generated by the model ($SS_{res}$), as shown below:

$$SS_{tot} = SS_{reg} + SS_{res}$$ (3.20)
As replicates of the central point are made, it is possible to estimate the pure error associated with repetitions. Thus, the sum of the squares for residuals can be obtained by adding the sum of the squares due to pure error ($SS_{pe}$) and the sum of the squares due to the lack of fit ($SS_{lof}$), as shown below:

$$SS_{res} = SS_{pe} + SS_{lof}$$  \hspace{1cm} (3.21)

For each source of variation (total, regression, residual, lack of fit, and pure error), the mean square ($MS$) is obtained by dividing the sum of the squares by its respective number of degrees of freedom. The number of degrees of freedom for these sources of variation is calculated by the expressions presented in the third column of table 3.1, where $p$ represents the number of coefficients of the mathematical model, $n$ represents the number of experimental runs, and $m$ represents the number of experimental runs at central point used in the investigation. Equations related to the source of variations for the calculation of $SS$ and $MS$ are also presented in table 3.1.

The significance of regression can be evaluated by the Fisher distribution ($F$ test) which is the ratio between the mean square regression ($MS_{reg}$) and the mean square residuals ($MS_{res}$), i.e.

$$F = \frac{MS_{reg}}{MS_{res}}$$  \hspace{1cm} (3.22)

Thus, a statistically significant value for this ratio must be higher than the tabulated value for $F$ given in the statistical tables. This is an indication that the mathematical model is well fitted to the experimental data.
Table 3.1
Analysis of variance for fitted mathematical model to an experimental data
set using multiple regressions

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares (SS)</th>
<th>Degrees of Freedom</th>
<th>Mean square (MS)</th>
<th>F –value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>$SS_{\text{reg}} = \sum_i \sum_j (\hat{y}_i - \bar{y})^2$</td>
<td>$p - 1$</td>
<td>$MS_{\text{reg}} = \frac{SS_{\text{reg}}}{p - 1}$</td>
<td>$F = \frac{MS_{\text{reg}}}{MS_{\text{res}}}$</td>
</tr>
<tr>
<td>Residuals</td>
<td>$SS_{\text{res}} = \sum_i \sum_j (y_{ij} - \hat{y}_i)^2$</td>
<td>$n - p$</td>
<td>$MS_{\text{res}} = \frac{SS_{\text{res}}}{n - p}$</td>
<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>$SS_{\text{lof}} = \sum_i \sum_j (\hat{y}_i - \bar{y}_i)^2$</td>
<td>$n - p - m + 1$</td>
<td>$MS_{\text{lof}} = \frac{SS_{\text{lof}}}{n - p - m + 1}$</td>
<td></td>
</tr>
<tr>
<td>Pure error</td>
<td>$SS_{\text{pe}} = \sum_i \sum_j (y_{ij} - \bar{y}_i)^2$</td>
<td>$m - 1$</td>
<td>$MS_{\text{pe}} = \frac{SS_{\text{pe}}}{m - 1}$</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$SS_{\text{tot}} = \sum_i \sum_j (y_{ij} - \bar{y})^2$</td>
<td>$n - 1$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$n = \text{number of experimental runs},$

$m = \text{number of experimental runs at central value},$

$p = \text{number of coefficients of the model},$

$\hat{y}_i = \text{estimated value by the model},$

$\bar{y} = \text{overall mean},$

$y_{ij} = \text{replicates performed in each individual level},$

$\bar{y}_i = \text{mean of replicates performed in the same set of experimental conditions}.$

**Determination of the critical values**

For quadratic models, the critical point can be characterized as maximum, minimum, or saddle. It is possible to calculate the coordinates of the critical point.
through the first derivative of the mathematical function, which describes the
response surface and equating it to zero.

The quadratic function obtained for three variables as described below is used to
illustrate the following example:

\[ Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \]

(3.23)

\[ \frac{\partial Y}{\partial X_1} = b_1 + 2b_{11}X_1 + b_{12}X_2 + b_{13}X_3 = 0 \]

(3.24)

\[ \frac{\partial Y}{\partial X_2} = b_2 + 2b_{22}X_2 + b_{12}X_1 + b_{23}X_3 = 0 \]

(3.25)

\[ \frac{\partial Y}{\partial X_3} = b_3 + 2b_{33}X_3 + b_{23}X_2 + b_{13}X_1 = 0 \]

(3.26)

Thus, to calculate the coordinates of the critical point, it is necessary to solve the
first order system formed by equations (3.24), (3.25), and (3.26).

**Second-order experimental designs**

**Full three level-Factorial design**

The number of experiments required for the FFD is calculated by expression
\[ N=3^k \], where \( N \) is the number of experiments and \( k \) is the number of variables.

FFD is an experimental matrix that has limited application in RSM when the
number of variables is more than two, because the number of experiments to be
conducted is large, thereby losing its efficiency in the modeling of quadratic
functions.
Fig. 3.1 shows the representation of the three-level full Factorial designs for the optimization of three variables. Table 3.2 shows some of the applications of the three-level full factorial design.

**Table 3.2**

**Some applications of Full three-level factorial design**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Biosorbent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron and Zinc</td>
<td>Typha domingensis</td>
<td>[27]</td>
</tr>
<tr>
<td>Chromium</td>
<td><em>Sargassum</em> sp.</td>
<td>[28]</td>
</tr>
<tr>
<td>Copper</td>
<td><em>Ascothyllum nodosum</em></td>
<td>[29]</td>
</tr>
<tr>
<td>Chromium</td>
<td>Araucaria angustifolia wastes</td>
<td>[30]</td>
</tr>
<tr>
<td>Lead</td>
<td>Wastes of olive oil production</td>
<td>[31]</td>
</tr>
<tr>
<td>Chromium</td>
<td>Activated carbons from plant precursors</td>
<td>[32]</td>
</tr>
</tbody>
</table>
Box-Behnken design (BBD)

Box and Behnken [33] suggested selecting some of the points from the three-level factorial arrangement, which allow the efficient estimation of the first- and second-order coefficients of the mathematical model. These designs, in this way are, more efficient and economical than their corresponding $3^k$ designs, which require a large number of experiments when the number of variables is more than two.

In Box-Behnken designs [34], the experimental points are located on a hypersphere equidistant from the central point, as exemplified for a three variable design in fig. 3.2. Its principal characteristics are:

1. The number of experiments required is given by $N= 2k (k−1) + c_p$, where $k$ is the number of variables and $(c_p)$ is the number of the central points;

2. All variable levels have to be adjusted only at three levels $(-1, 0, +1)$, with equal intervals between these levels.

Fig. 3.2. BBD for three variables
Fig. 3.2 presents the Box–Behnken design for three-variable optimization with its 13 experimental points. In comparison with the original $3^3$ design with 27 experiments (fig. 3.1), it is noted that this design is more economical and efficient. This experimental design has been applied for the optimization of several chemical and physical processes. Table 3.3 shows some of the applications of the Box–Behnken design.

**Table 3.3**  
Some applications of Box-Behnken design

<table>
<thead>
<tr>
<th>Metal</th>
<th>Biosorbent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>Spent cyanobacterial biomass</td>
<td>[35]</td>
</tr>
<tr>
<td>Copper</td>
<td>Powdered waste sludge</td>
<td>[36]</td>
</tr>
<tr>
<td>Cr (VI), Ni (II) and Zn (II)</td>
<td><em>Bacillus brevis</em></td>
<td>[37]</td>
</tr>
<tr>
<td>Lead</td>
<td><em>Pistacia vera</em> L.</td>
<td>[38]</td>
</tr>
<tr>
<td>Flouride</td>
<td><em>Apatitic tricalcium phosphate</em></td>
<td>[39]</td>
</tr>
</tbody>
</table>

**Central Composite Design (CCD)**

The central composite design (CCD) was presented by Box and Wilson [40]. The advantages of CCD over BBD are that the levels of the variables are five which one more than those in BBD which are three. The full CCD, based on three basic principles of an ideal experimental design, primarily consists of (1) a complete $2^n$ factorial design, where $n$ is the number of test variables, (2) $n_0$ center points ($n_0 \geq 1$) and (3) two axial points on the axis of each design variable at a distance of $2^{n/4}$ from the design center. Hence, the total number of design points, $N = 2^n + 2n + n_0$ and the five levels of the variables are $(-2^{n/4}, -1, 0, +1, +2^{n/4})$. 
Figure 3.3 shows representation of Central Composite design for optimization of three-variables. Table 3.4 shows some of the applications of the Central Composite design

<table>
<thead>
<tr>
<th>Metal</th>
<th>Biosorbent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td><em>Aeromonas hydrophila</em></td>
<td>[41]</td>
</tr>
<tr>
<td>Copper</td>
<td><em>Rhizopus arrhizus</em></td>
<td>[42]</td>
</tr>
<tr>
<td>Copper</td>
<td><em>Enteromorpha prolifera</em></td>
<td>[43]</td>
</tr>
<tr>
<td>Cadmium</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>[44]</td>
</tr>
<tr>
<td>Cadmium</td>
<td><em>Aspergillus niger</em></td>
<td>[45]</td>
</tr>
<tr>
<td>Cadmium and Nickel</td>
<td><em>Saccharomyces cerevisiae</em></td>
<td>[46]</td>
</tr>
</tbody>
</table>

The optimization of process parameters can be simplified through RSM by adapting various experimental designs like full three-level Factorial design, Box-
Behnken design and Central Composite Design. Among them the CCD is a good design for Response Surface Methodology because it permits:

- Minimum number of experiments to be conducted
- Flexibility of assigning more levels for a variable with stronger effect
- Estimation of the parameters of the quadratic model
- Building of sequential designs
- Detection of lack of fit of the model

Hence CCD can be applied with great confidence for the optimization of process parameters in biosorption studies.
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