### Abbreviations and Units of Measurements

1. AACC  
   American Association of Cereal Chemistry
2. ANOVA  
   Analysis Of Variance
3. AOAC  
   Association Of Analytical Chemists
4. APPCB  
   Andhra Pradesh Pollution Control Board
5. BSA  
   Bovine Serum Albumin
6. CCD  
   Central Composite Design
7. Cm  
   Centimeter
8. gm  
   Gram = $10^{-3}$ Kg
9. DNS  
   3,5, Di-Nitro Salicylic Acid
10. EDTA  
   Ethylene Diamine Tetraacetic Acid
11. FAO  
   Food and Drug Administration
12. l or L  
   Litre
13. LSF  
   Liquid State Fermentation
14. SSF  
   Solid State Fermentation
15. SmF  
   Submerged Fermentation
16. RSM  
   Response Surface Methodology
17. µg  
   Microgram = $10^{-6}$ gm = $10^{-9}$ Kg
18. µl  
   Microlitre = $10^{-3}$ cm$^3$
19. *S. cerevisiae*  
   *Saccharomyces cerevisiae*
20. UV  
   Ultraviolet
21. v/v  
   Volume of Solute per Volume of Solution
22. w/v  
   Weight of Solute per Volume of solution
23. ppm  
   parts per million
24. MTCC  
   Microbial Type Culture Collection
25. NCIM  
   National Collection of Industrial Microorganisms
26. ATCC  
   American Type Culture Collection
27. HCL  
   Hydrochloric Acid
28. m  
   Meter
29. mg  
   Milligram = $10^{-3}$
30. GYMP  
   Glucose, Yeast extract, Maltose, Peptone
31. Min  
   Minute
32. ml  
   Milliliter = cm$^3$ = $10^{-6}$ m$^3$
33. t  
   Time
34. A.O.R  
   Aerobic Organic Reactions
35. An.O.R  
   Anaerobic Organic Reactions
36. MFE  
   Mahua Flower Extract
37. MFM  
   Mahua Flower Medium
38. Kal  
   Calories
39. °C Temperature (Kelvin) K
40. Molarity Gram-Molecular weight/litre of solution
41. N Normality = Gram equivalent weight/litre of solution
42. ATP Adenosine Tri Phosphate
43. ADP Adenosine Di Phosphate
44. D Dilution
45. RPM Rotations Per Minute
46. P Productivity = g.l⁻¹.h⁻¹
47. % Percentage
48. pH -log of H⁺ ion
49. wt Weight
50. Å Angstrum = 10⁻¹⁰ m
51. nm Nanometer = 10⁻⁹ m = 10⁻² Å
52. V Volume
53. EtOH Ethyl alcohol or Bioethanol or Ethanol
54. λ Wavelength
55. Y Yield
56. Rt Retention time
57. mol Mole
58. hr Hour
59. O.D Optical Density
60. MB Methylene Blue
61. ADH Alcohol Dehydrogenase
62. NaOH Sodium Hydroxide
63. DF Degree of Freedom
64. MS Mean Squares
65. SS Sum of Squares
66. CFU Colony Forming Units
67. Std Standard
68. MPNG Ministry of Petroleum and Natural Gas
69. Mol wt Molecular weight
70. μ Micron
71. Sci Science
72. Tech Technology
73. U Units
74. PS Polysaccharide
75. BOD Biological Oxygen Demand
76. COD Chemical Oxygen Demand
77. Yi Predicted Response
78. APHA American Public Health Association
## Chemical compounds used in bioethanol fermentation process

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical Compound</th>
<th>Molecular formula</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Magnesium chloride</td>
<td>MgCl₂·6H₂O</td>
<td>Merk India Pvt Ltd, India</td>
</tr>
<tr>
<td>2</td>
<td>Calcium chloride</td>
<td>CaCl₂·2H₂O</td>
<td>Loba Chemicals company, India</td>
</tr>
<tr>
<td>3</td>
<td>Potassium phosphate</td>
<td>K₂HPO₄</td>
<td>Merk India Pvt Ltd, India</td>
</tr>
<tr>
<td>4</td>
<td>Manganese chloride</td>
<td>MnCl₂·4H₂O</td>
<td>Loba Chemicals company, India</td>
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<tr>
<td>5</td>
<td>Copper chloride</td>
<td>CuCl₂</td>
<td>Merk India Pvt Ltd, India</td>
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<tr>
<td>6</td>
<td>Zinc sulphate</td>
<td>ZnSO₄·7H₂O</td>
<td>Loba Chemicals company, India</td>
</tr>
<tr>
<td>7</td>
<td>Cobalt chloride</td>
<td>COCl₃</td>
<td>Merk India Pvt Ltd, India</td>
</tr>
<tr>
<td>8</td>
<td>Sodium hydroxide</td>
<td>NaOH</td>
<td>Loba Chemicals company, India</td>
</tr>
<tr>
<td>9</td>
<td>Sulphuric acid</td>
<td>H₂SO₄</td>
<td>Loba Chemicals company., India</td>
</tr>
<tr>
<td>10</td>
<td>Hydrochloric acid</td>
<td>HCl</td>
<td>Merk India Pvt Ltd., India</td>
</tr>
<tr>
<td>11</td>
<td>Methylene blue</td>
<td>Methylene Blue</td>
<td>Merk India Pvt Ltd., India</td>
</tr>
<tr>
<td>12</td>
<td>Ferrous sulphate</td>
<td>Fe₂(SO₄)₃·H₂O</td>
<td>Merk India Pvt Ltd., India</td>
</tr>
<tr>
<td>14</td>
<td>Biotin</td>
<td>Biotin</td>
<td>Merk India Pvt Ltd., India</td>
</tr>
<tr>
<td>15</td>
<td>Ethyl alcohol</td>
<td>C₂H₅OH</td>
<td>Merk India Pvt., Ltd. India</td>
</tr>
<tr>
<td>16</td>
<td>Agar Agar</td>
<td>Agar</td>
<td>Qualigens, India.</td>
</tr>
<tr>
<td>17</td>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>Loba Chemicals company, India</td>
</tr>
<tr>
<td>18</td>
<td>Ammonium chloride</td>
<td>(NH₄)₂Cl</td>
<td>Loba Chemicals company, India</td>
</tr>
<tr>
<td>19</td>
<td>Ammonium sulphate</td>
<td>(NH₄)₂SO₄</td>
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<tr>
<td>20</td>
<td>Urea</td>
<td>CO(NH₂)₂</td>
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<td>21</td>
<td>Ethylene diamine</td>
<td>C₁₀H₁₆N₂O₈</td>
<td>Loba Chemicals company, India</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Potassium sodium tartrate</td>
<td>KNaC₄H₆O₆·4H₂O</td>
<td>Loba Chemicals company., India</td>
</tr>
<tr>
<td>23</td>
<td>Nutrient agar medium</td>
<td>Medium</td>
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<tr>
<td>24</td>
<td>Oxygen</td>
<td>O₂</td>
<td>Merk India Pvt Ltd., India</td>
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<tr>
<td>25</td>
<td>3,5,Dinitro salicyc acid</td>
<td>C₇H₄N₂O₇</td>
<td>Merk India Pvt Ltd., India</td>
</tr>
<tr>
<td>26</td>
<td>Anthrone</td>
<td>C₁₄H₁₀O</td>
<td>Loba Chemicals company., India</td>
</tr>
<tr>
<td>27</td>
<td>Sodium di-hydrogen phosphate</td>
<td>NaH₂PO₄</td>
<td>Loba Chemicals company., India</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>28</td>
<td>Di-sodium hydrogen phosphate</td>
<td>Na₂HPO₄</td>
<td>Merk India Pvt Ltd., India</td>
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<tr>
<td>29</td>
<td>Proline</td>
<td>C₅H₈NO₂</td>
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<tr>
<td>30</td>
<td>Glycine</td>
<td>C₂H₅NO₃</td>
<td>Loba Chemicals company., India</td>
</tr>
<tr>
<td>31</td>
<td>n-Butanol</td>
<td>CH₃(CH₂)₃OH</td>
<td>BDH, India</td>
</tr>
<tr>
<td>32</td>
<td>Methylene blue</td>
<td>C₁₆H₁₈N₃SCl</td>
<td>Loba Chemicals company., India</td>
</tr>
<tr>
<td>33</td>
<td>Hydrochloric acid</td>
<td>HCL</td>
<td>Loba Chemicals company., India</td>
</tr>
<tr>
<td>34</td>
<td>Sulphuric acid</td>
<td>H₂SO₄</td>
<td>BDH, India</td>
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<td>35</td>
<td>Yeast extract</td>
<td>Nutrient</td>
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<td>36</td>
<td>Peptone</td>
<td>Nutrient</td>
<td>Loba Chemicals company, India</td>
</tr>
<tr>
<td>37</td>
<td>D-glucose</td>
<td>C₆H₁₂O₆</td>
<td>Merk India Pvt Ltd., India</td>
</tr>
</tbody>
</table>
Determination of model coefficients, $\beta$ in a regression model

Determination of model coefficient, $\beta_i$, in regression model is illustrated for the second order polynomial model used for fitting the data obtained in the optimization of temperature and the pH for bioethanol production employing the central composite experimental design, viz..

$$
Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \epsilon \\
\text{..................D.1}
$$

Where

$Y$ = Observed response, $X_1$, $X_2$, $X_3$ represents the levels of the real value of the variables, $\epsilon$ represents the random error in $Y$, the random errors are assumed to be independently distributed variable with a zero mean and a common variance, $\sigma^2$

In CCD matrix notation the model in equation D.1, over $N$ observations, is

$$
Y = X\beta + \epsilon \text{..................................................D.2}
$$

Where

$$
Y = \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \\ y_N \end{bmatrix} \quad x = \begin{bmatrix} 1 & x_1 & x_1^2 & x_2 & x_1x_2 \\ 1 & x_{11} & x_{12} & x_{13} & x_{14} & x_{15} \\ 1 & x_{21} & x_{22} & x_{23} & x_{24} & x_{25} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 1 & x_{N1} & x_{N2} & x_{N3} & x_{N4} & x_{N5} \end{bmatrix} \\
N \times 1 \quad N \times (5+1)
$$

$$
\beta = \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_{11} \\ \beta_{12} \\ \beta_{22} \end{bmatrix} \quad \epsilon = \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \\ \epsilon_4 \\ \epsilon_5 \\ \epsilon_N \end{bmatrix} \quad \epsilon \text{= normally distributed with zero mean and common variance } \sigma^2
$$

(5+1) x 1 \quad N \times 1
The normal equations are
\[ X'Xb = X'Y \]  
………………………………………………(D.3)

Where \( X' \) is the transpose of matrix \( X \) and \( b \) is the matrix of coefficient estimates.

The solutions to these normal equations are
\[ b = (X'X)^{-1}X'Y \]  
………………………………………………(D.4)

where \((X'X)^{-1}\) is the inverse of \(X'X\). Both \(X'X\) and \((X'X)^{-1}\) are symmetric matrices.

The fitted second-order model in the coded variable is
\[
Y_0 = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \varepsilon 
\]  
……………………………………………..(D.5)

The predicted value of the response under study \(Y\) are obtained by regressing the equation D.5 using MATLAB software.

**D.2 construction of the Analysis of Variance (ANOVA) table :-**

After experimentation, the data are analyzed and the results and the analysis are displayed in a tabular form. The table is called analysis of variance (ANOVA) table. The entire data in the table represents measures of information concerning the separate sources of variation in the data.

The total variation in the set of data is called the total sum of squares (SST). The quantity SST is compared by summing the squares of the deviations of the observed response \(Y_u\) about their average value for \(N\) observations,
\[
\bar{Y} = \left\{ Y_1 + Y_2 + \ldots + Y_N \right\}/N \]  
……………………………………………..(D.6)

\[
SST = \sum_{U=1}^{N} \left[ (Y_{u} - \bar{Y})^2 \right] 
\]

The quantity SST associate with \(N-1\) degrees of freedom since the sum of deviations, \(Y_u - \bar{Y}\) is equal to zero.
Appendix-III

The total sum of squares can be partitioned into two parts, the sum of squares due to regression (or sum of squares explained by the fitted model) and the sum of squares unaccounted for by the fitted model. The formulation for calculating the sum of squares due to regression \((SSR)\) is

\[
SSR = \sum_{u=1}^{n} [(Y_u - \bar{Y})^2] \quad \text{(D.7)}
\]

The deviation \(Y_u - \bar{Y}\) is the difference between the value predicted by the fitted model for the \(u\)th observation and the overall average of the \(Y_u\). If the fitted model contains \(p\) parameters, the number of degrees of freedom associated with SSR is \(p-1\).

The sum of squares unaccounted for by the fitted model \((SSE)\) is

\[
SST = \sum_{u=1}^{n} [(Y_u - \bar{Y})(X_u - \bar{X})]^2 \quad \text{(D.8)}
\]

The quantity SSE is also called the sum of squares of the residual or the sum of squares of the errors. The number of degrees of freedom for SSE is the difference \((N-1)-(p-1) = N-p\).

The usual test of the significance of the regression equation is test of the null hypothesis \(H_0\): all values of \(\beta_1\) (excluding \(\beta_0\)) is not zero.

Assuming normality of the errors, the test of \(H_0\) involves first calculating the value of the F-statistic by the following equation.

\[
F = \frac{\text{Mean square regression}}{\text{Meansquare residual}} = \frac{SSR/(p-1)}{SSE/(N-p)} \quad \text{(D.9)}
\]

If the null hypothesis is true, the F-static in equation D.9 follow an F-distribution with \(p-1\) and \(N-p\) degrees of freedom in the numerator and in the denominator respectively. The second step of the test \(H_0\) is to compare the value of \(F\) in equation D.9 to the table value, \(F_{a,p-1}\), \(N-p\) which is the upper 100 \(\alpha\) percent point of the F distribution with \(p-1\) and \(N-p\) degrees of freedom, respectively. If the value of \(F\) in equation D.9 exceeds \(F_{a,p-1}, N-p\) than the null hypothesis is rejected at the \(\alpha\) level of significance and it is inferred that the variation accounted for by the model is significantly greater than the unexplained variation.

Another accompanying statistic is the multiple coefficient of determination \((R^2)\)
The value of $R^2$ is a measure of the proportion of total variation of the values of $Y_u$ about the mean $Y$ explained by the fitted model. It is often expressed in a percent. The analysis of variance table is shown as table.

**ANalysis Of VAriance (ANOVA) table :-**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degree of freedom (DF)</th>
<th>Sum of squares (SS)</th>
<th>Mean squares (MS)</th>
<th>$F$ value</th>
<th>Probability $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Due to regression</td>
<td>$p-1$</td>
<td>SSR</td>
<td>SSR</td>
<td>MSD/MSE</td>
<td></td>
</tr>
<tr>
<td>Regression (fitted model)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual (error)</td>
<td>$N-p$</td>
<td>SSE</td>
<td>SSE/(N-p)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$N-1$</td>
<td>SST</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
F = \frac{\text{Mean square regression}}{\text{Mean square residual}}
\]