ANNEXURE – IX

Waste Water analysis:

The waste water characterization was done using the methods described in Hand book of Methods in Environmental Studies (S.K.Maitti).

1. pH:

Principle: pH is the measure of the intensity of acidity or alkalinity and measure the concentration of hydrogen ions in water. It is the measurement of activity of hydrogen ions by potentiometric measurement using a standard sensing electrode (glass electrode) and a reference electrode (calomel electrode).

Glass electrode consist of a thin glass bulb containing a fixed concentration of HCL solution into which a Ag-AgCl wire is inserted serving as the electrode with a fixed voltage. When glass electrode is immersed in a solution a potential deference develops between the solution in the glass bulb and sample solution. The potential difference E is formulated by Nernst equation:

\[
E = -\frac{RT}{nF} \log \frac{K}{M^n}
\]

Where, E = Half cell potential
T = Absolute temperature
F = Faraday constant
M = Activity of ion measured
R = Gas constant, n = Valance, K= constant

The E cannot be measured alone. If the glass electrode is placed against a reference electrode the potential difference between two electrodes is measured.

Reference electrode consists of a half cell that provides a constant electrode potential. Commonly used reference electrode is calomel and silver-silver-chloride electrode depending on the concentration of KCl solution used in preparing them.
Reagents:

1) Phthalate buffer (pH of 4 at 25°C)
2) Phosphate buffer (pH of 7 at 25°C)
3) Borax buffer (pH of 9.18 at 25°C)

Procedure:

✓ Calibrate the pH meter the standard buffer solution of pH 4.0, 7.0, and 9.18 as given in operational manual of pH meter.

✓ First wash the electrode with D/W and dip the electrode in the buffer of pH 7.0 and move the temperature knob to the required or room temperature and adjust the pH meter.

✓ Now again wash the electrode with distilled water and dip the electrode in the buffer of pH 4.0 and adjust the temperature by moving the knob, thus adjust the pH meter accordingly.

✓ Again wash the electrode with distilled water and dip the electrode in the buffer of pH 9.18 and adjust the temperature by moving the knob to the required or room temperature and thus adjust the pH meter accordingly.

✓ Now after calibration of pH meter wash the electrode with distilled water and then put the sample of which pH to be measured.

✓ After completion of analysis again wash the electrode distilled water and dip the electrode bulb in the storage solution when not in use.

2. Phosphate (P-PO₄): (Stannous chloride method)

Principle: The Phosphate in the water reacts with ammonium molybdate and form complex molybdophosphoric acid, which gets reduced to a complex of blue color in the presence of SnCl₂. The absorption of light by blue color can be measured at 690 nm to calculate the concentration of phosphates.

Reagents:

A) Ammonium molybdate solution:

a) Dissolve 25 g of ammonium molybdate in 175 ml of distilled water.

b) Add 280 ml of concentrated H₂SO₄ to 400 ml of distilled water and cool. Mix the two solutions (a) and (b) and dilute to 1 L.
B) Stannous Chloride solution: Dissolve 2.5 g of stannous chloride in 100 ml of glycerol by heating on a water bath for rapid dissolution.

C) Standard Phosphate solution: dissolve 4.338 g of dried anhydrous potassium hydrogen phosphate (K₂HPO₄) in distilled water and make the volume to 1 L. Dilute this solution to 100 times (10→1000 ml). This is standard phosphate solution containing 10 mg P / L.

**Procedure:**

**For Sample:**
- Take 50 ml of filtered clear sample in a clean conical flask. If the sample contains color and colloidal impurities, they can be removed by adding a spoonful of activated charcoal and then filtering the sample.
- Add 2 ml of ammonium molybdate followed by 5 drops of SnCl₂ solution.
- A blue color will appear. Take reading at 690 nm on a spectrophotometer using a distilled water blank with the same amount of the chemicals. Take the readings after 5 minutes but before 12 minutes of the addition of the last reagent.
- Find out the concentration with the help of the standard curve.

**Preparation of standard curve:**
- Pipette out appropriate ml of phosphate solution from the standard phosphate solution.
- Add 2 ml of Ammonium molybdate and 5 drops of SnCl₂ solution.
- Take the reading at 690 nm. Plot a graph between Absorbance and Concentration.

3. Sulphate (S-SO₄): (Turbidimetric method)

**Principle:**
Sulphate ion is precipitated in the form of barium sulphate by adding barium chloride in hydrochloric acid medium. The concentration of the sulphate can be determined from the absorbance of the light by barium sulphate and then comparing it with the standard curve.
Interference:
Suspended matter and original color of the sample may interfere with the sulphate
determination. Suspended matter can be removed by filtration. Presence of silica in
excess of 500 mg/L and large quantity of organic matter may affect the satisfactory
precipitation of Barium sulphate.

Reagents:
1. Conditioning reagent: Mix 75 gm of NaCl, 30 ml Conc. HCl, 100 ml 95% ethyl
   or isopropyl alcohol in 300 ml distilled water. Add 50 ml glycerol to this
   solution and mix thoroughly.
2. Standard Sulphate solution: Dissolve 0.479 gm of anhydrous Na₂SO₄ in distilled
   water to make 1 liter of solution. This solution contains 100mg/l of sulphate.
   OR: standard solution can also be prepared by diluting 10.41 ml of 0.02N
   H₂SO₄ to 100 ml with distilled water. It also contains 100 mg/l of sulphate.

Procedure:
• 100 ml of sample (not containing more than 40 mg/l of SO₄) or a suitable
  aliquot diluted to 100 ml in 250 ml conical flask and add 5 ml of conditioning
  reagent.
• Stir the sample on a magnetic stirrer and during stirring; add a spoonful of
  BaCl₂ crystals. Stir for only 1 minute after addition of BaCl₂ crystals.
• Take the reading in a spectrophotometer at 420 nm exactly after 4 minutes
  find out the concentration of sulphate from the standard curve.
• Prepare the standard curve, employing the same procedure as describe above
  for 0.0 to 40 mg/l at the intervals of 5 mg/l

4. Chloride: (Titrametric method)

Principle:
Silver nitrate reacts with chloride to form very slightly soluble white precipitate of
AgCl at the end point when all the chlorides get precipitated, free silver ions reacts
with chromate to form silver chromate of reddish brown color.
Reagents:
1. Standard AgNO₃ solution: (0.0141 N)
2. Standard NaCl solution:
3. Potassium chromate:

Procedure:
Standardization of AgNO₃: Take 25 ml of standard NaCl solution (0.0141 N) in 250 ml conical flask. Add 2-3 Drops of K₂CrO₄ as an indicator an titrate it against 0.0141 N AgNO₃. The end point is yellow to reddish color. Take the burette reading and calculate as follows.

Now, \( N_1V_1 = N_2V_2 \)

- Take 50 ml of sample in 250 ml conical flask. Add 2-3 drops of K₂CrO₄ as an indicator and titrate it against 0.0141 N AgNO₃ solution. The color change from yellow to reddish indicates the end point. Take the burette reading.
- To prepare blank use distilled water. Take 50 ml of distilled water in conical flask add 2-3 drops of K₂CrO₄ and titrate it against 0.0141 N AgNO₃ solution.

Formula: Chloride as Cl⁻ ion = \( \frac{(A-B) \times N \times 35450}{\text{ml of sample}} \)

Where, \( A = \) Sample Reading
\( B = \) Blank Reading
\( N = \) normality of AgNO₃

5. Chemical oxygen demand: (Closed reflux method)

Principle: The organic matter and oxidizable inorganic substances present in water or wastewater get oxidized completely by standard potassium dichromate (K₂Cr₂O₇) in the presence of H₂SO₄ to produce CO₂+H₂O. The excess of potassium dichromate remaining after the reaction is titrated with ferrous ammonium sulphate (FAS). The dichromate consumed gives the O₂ required for oxidation of the organic matter. The contents are refluxed for 2 hours.

\[
2K_2Cr_2O_7 + 8H_2SO_4 \rightarrow 2K_2SO_4 + 2Cr_2(SO_4)_3 + 8H_2O + 3O_2
\]
\[
C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2+6H_2O
\]
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Reagents:
1) Standard potassium dichromate (K₂Cr₂O₇), 0.25 N
2) Standard ferrous ammonium sulphate (FAS), 0.1 N
3) Pure mercuric sulphate powder
4) Pure silver sulphate dissolved in concentrated sulphuric acid
5) Ferroin indicator

Procedure:

Standardization of ferrous ammonium sulphate (FAS):
- Take 10 ml standard potassium dichromate in a conical flask and add 30 ml of concentrated sulphuric acid having dissolve AgSO₄. Cool it to room temperature under tap water.
- Add 3-5 drops of ferroin solution as an indicator and titrate it against 0.1 N ferrous ammonium sulphate (FAS) solution.
- Calculate the normality of FAS as follows
  \[ N \frac{Vi}{V1} = N \frac{V2}{V2} \]

Procedure for sample analysis:
- Take round bottom flask/reflux flask. Add few (4-5) glass beads in each flask and then add approximately 0.4 g HgSO₄ to remove chloride interferences.
- Add 10 ml of standard K₂Cr₂O₇ solution of normality 0.25 N.
- For sample take appropriate sample volume and make up the final volume to 20 ml with distilled water or take 20 ml of sample.
- To this add 30 ml concentrated H₂SO₄ which contain dissolved AgSO₄.
- If the color turns green then either take fresh sample with lesser aliquot or add more dichromate and H₂SO₄.
- Put the flask on a hot plate under water condensation for 2 hours for the digestion. After 2 hours switch off the hot plate and add distilled water from the top of condenser.
- Remove all the flasks from hot plate, cool it under tap water.
- Add 3-5 drops of ferroin solution as an indicator and titrate it against 0.1 N FAS. Sharp change of color from blue green to wine red indicates the end point of titration. Note down the burette reading.
• Perform blank in the same manner using distilled water instead of sample.

Calculation:

\[
\text{COD (mg/l) = } \frac{(B-A) \times N \times 8 \times 1000}{\text{ml of sample}}
\]

Where;  
A = burette reading for sample
B = burette reading for blank
N = Normality of ferrous ammonium sulphate

6. Turbidity:

Preparation of stock standard solution:

Stock standard of 4000 NTU is prepared as per the following procedure:
• Take 5 g of reagent grade “Hydrazine sulphate” and dissolve in 400 ml of distilled water this is solution ‘A’.
• Next dissolve 50 g of pure “Hexamethylene Tetramine (Hexamine)“ in 400 ml distilled water. This is solution ‘B’.
• Mix solution ‘A’ and ‘B’ and make the final volume to one liter by adding distilled water and allow this mixture to settle for 48 hours at normal room temperature.

This is a stock solution of 4000 NTU strength of formazine. This solution is stable for period of 6-8 months and working standard can be prepared as required using following table. Shake stock solution well before dilution.
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<table>
<thead>
<tr>
<th>Volume of stock solution (ml) to make up to 1L with high grade distilled water</th>
<th>Final strength (concentration) of Formazine in diluted stock solution (NTU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td>20 of 500 NTU</td>
<td>10</td>
</tr>
<tr>
<td>10 of 500 NTU</td>
<td>5</td>
</tr>
<tr>
<td>20 of 50 NTU</td>
<td>1</td>
</tr>
</tbody>
</table>

**Operation:**

- Insert three pin plug into approximate 230 VAC mains socket.
- Switch the instrument ON and allow 10-15 minutes warm up.
- Select the approximate range. Set the calibration control to maximum clockwise position.
- Insert the test tube with distilled water into cell holder and cover with light shield.
- Adjust set zero controls to get zero on display.
- Remove test tube and replace with test tube containing standard solution.
- Adjust calibration control such that display indicates as follows:

<table>
<thead>
<tr>
<th>Range</th>
<th>Standard solution</th>
<th>Display</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 NTU</td>
<td>1 NTU</td>
<td>1</td>
</tr>
<tr>
<td>0-10 NTU</td>
<td>10 NTU</td>
<td>10</td>
</tr>
<tr>
<td>0-100 NTU</td>
<td>100 NTU</td>
<td>100</td>
</tr>
<tr>
<td>0-1000 NTU</td>
<td>500 NTU</td>
<td>500</td>
</tr>
</tbody>
</table>

The instrument is now ready for test samples. Insert test tube containing unknown sample in cell holder. The display directly gives turbidity in NTU.
7. **Acidity:**

**Principle:**
Acidity of the water is its capacity to neutralize a strong base to a fixed pH. It is caused by the presence of strong mineral acids, weak acids and hydrolyzing salts of strong acids. However, in natural unpolluted fresh waters, the acidity is mostly due to the presence of free CO$_2$ in the form of carbonic acid.

**Reagents:**
1. Sodium hydroxide, NaOH (0.02 N)
2. Potassium hydrogen phthalate (0.02 N)
3. Phenolphthalein indicator

**Procedure:**
Standardization of Sodium hydroxide (NaOH)
- Take 25 ml of standard potassium hydrogen phthalate (0.02 N) in 250 ml conical flask.
- Add 2-3 drops of phenolphthalein indicator and titrate it against 0.02 N NaOH solution till the pink color appear.
- Calculate the normality of NaOH using following formula.

\[ N_1V_1 = N_2V_2 \]

Procedure for sample analysis:
- Take 50 ml of sample in 250 ml conical flask. Add 2-3 drops of phenolphthalein indicator.
- Titrate the sample against 0.02 N NaOH solution. The color change from colorless to pink indicates the end point of titration.
- Note down the burette reading and calculate acidity by using following formula.

\[
\text{Acidity as CaCO}_3 \text{(mg/l)} = \frac{A \times N \times 50,000}{\text{ml of sample}}
\]
8. Alkalinity:

Principle:
Total alkalinity is the measure of the capacity of the water to neutralize a strong acid. The alkalinity in the waters is generally imparted by the salts of carbonates, bicarbonates, phosphates, nitrates, borates, silicates etc. together with the hydroxyls ions in free State. However, most of the waters are rich in carbonates and bicarbonates with little concentration of other alkalinity imparting ions.

Reagents:

1. Sulphuric acid, H₂SO₄ (0.02 N)
2. Sodium hydroxide, NaOH (0.02 N)
3. Methyl orange indicator and mix indicator

Procedure:

Standardization of sulphuric acid:

- Take 25 ml standard sodium hydroxide, NaOH (0.02 N) in 250 ml conical flask.
- Add 2-3 drops of methyl orange indicator to it and titrate it against 0.02 N sulphuric acid (H₂SO₄) till the color changes from orange to red.
- Calculate the normality sulphuric acid using following formula.

\[ N_1 V_1 = N_2 V_2 \]

Procedure for sample analysis:

- Take 50 ml sample in 250 ml conical flask. Add 2-3 drops of mix indicator.
- The turns to blue. Titrate this against 0.02 N H₂SO₄. The color change from blue pale yellow indicates the end point of titration.
- Note down the burette reading and calculate alkalinity by following formula:
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Alkalinity as CaCO₃ (mg/l) = \[\frac{A \times N \times 50,000}{\text{ml of sample}}\]

Where; \[A = \text{burette reading for sample (ml)}\]
\[N = \text{normality of } H_2SO_4\]

9. Conductivity:

Principle: Conductivity is a numerical expression of the ability of a water sample to carry an electrical current and varies with the number and types of ions the solution contains. Most dissolved inorganic substances in water are in the ionized form and hence contribute to conductance. Conductance \(G\) is defined as the reciprocal of resistance \(R\).

\[G = \frac{L}{R}\]

Conductance of a solution is measured between two spatially fixed chemically inert electrodes. To avoid polarization at the electrode surface the conductance is measured is made with an alternating current signal. The conductance of a solution is directly proportional to the surface area \((A)\) and inversely proportional to the distance between the electrode \((L)\). The constant of proportionality \((k)\) is such that

\[G = k \frac{A}{L}, \ 'k' \text{ is called "conductivity"}\]

Reagents:

- Standard potassium chloride solution (1413 \(\mu\) mhos/cm)

Measurement of conductivity:

- Rinse the conductivity cell with distilled water.

- Immerse the conductivity cell in standard KCl solution and calibrate the instrument.

Now immerse the conductivity cell in sample and note the value display on instrument that gives the conductivity of sample.