APPENDIX - I
IODOMETRIC TITRATION

The Iodometric titration used to determine the decrease in sodium sulfite concentration during sulfite oxidation technique of interfacial area determination is as under:

a) Preparation of 0.1N Iodine Solution
   i. Dissolve 20 gm of Potassium Iodide (KI) in 300 ml distilled water.
   ii. Add 13 gm of ground iodine powder in above prepared 300 ml KI solution.
   iii. Swirl the solution until the powder iodine get dissolved into the KI solution.
   iv. Filter the above prepared solution through glass wool.
   v. Washing the glass wool with distilled water and make the final volume 1000 ml using distilled water.
   vi. The solution thus prepared is approximately 0.1N iodine solution.

b) Analytical Procedure
   i. Collect about 5-6 ml solution from column through sampling valve in a measuring cylinder.
   ii. Take 2 ml solution from above measuring cylinder in a 250 ml conical flask.
   iii. Add about 2-3 drops of 1% starch solution as an indicator in above flask.
   iv. Titrate it against 0.1N iodine solution.
   v. Add 0.1N iodine solution till color changes to dark blue.
   vi. Note down the reading.
   vii. Repeat the above procedure and take other readings.
   viii. Find out the sulfite concentration of the sample by conventional titrimetric calculations.
APPENDIX - II
PREDICTION OF DIFFUSIVITY OF OXYGEN

In the determination of interfacial area determination using sulfite oxidation method, Diffusivity of oxygen (\(D_A\)) in water was estimated by the most comprehensive Wilke-Chang equation as follows:

\[
D_A = 7.4 \times 10^{-8} \frac{(x M_B)^{1/2} \, T}{\eta_B V_A^{0.6}}
\]

Where,

- \(D_A\) is the diffusivity of oxygen, cm\(^2\)/s
- \(M_B\) is the molecular weight of solvent (water) = 18
- \(T\) is temperature = 303 K
- \(x\) is the association factor of solvent (water) = 2.6
- \(\eta_B\) is viscosity of solvent (water) = 1 cP
- \(V_A\) is the molar volume of the solute \((O_2)\) at normal boiling point = 25.6 cm\(^3\)/mol
APPENDIX-III
JUSTIFICATIONS OF ASSUMPTIONS IN DYNAMIC GASSING IN METHOD

The dynamic gassing in technique used in the present investigation for determination of volumetric mass transfer coefficient in draft tube bubble columns, assumed a constant gas phase composition, a “well mixed” liquid and a negligible effect of the dynamics of the dissolved oxygen electrode, justified briefly as below:

- **The constant gas phase composition**

  The positional variations in the gas phase composition can affect the saturation concentration $C^*$ of oxygen in the liquid and thereby influence the apparent $k_L a$. For the transfer of a sparingly soluble gas such as oxygen the gas phase mixing is of negligible importance. However, numerous theoretical and experimental demonstrations of this fact are available (Deckwer et al., 1974; Govindarao, 1975). The reason for this observation is that even at the maximum values of $k_L a$ in pneumatically mixed air – water dispersions (no chemical reaction), less than 0.01% of the oxygen in the total air input of the reactor is transferred to the liquid. For practical purposes, the oxygen partial pressure in the gas phase remains unchanged and the assumption of a constant gas phase composition applies.

- **A “well – mixed” liquid**

  Although no gas-liquid contactor of any significant dimensions can be considered “well– mixed” in liquid, the “well– mixed” assumptions can be justified for the mass transfer work for large vessels containing highly viscous (and relatively poorly mixed) fluids. Because the typical $k_L a$ measurements are reproducible to about ± 10%, agreement of $k_L a$ values from two or more properly located sampling points to within ± 10% may be taken as an indication of fully mixed behavior. This is even more so when the mass transfer rates are relatively low (i.e. $k_L a \leq 0.1 \text{ s}^{-1}$). Many investigators (Siegel and Merchuck, 1987; Verlaan and Tramper, 1987) also regard the insignificant impact of liquid mixing on mass transfer and on $k_L a$ calculations for draft tube bubble columns. The fluid (air-water) mixing and the gas-phase mixing considerations are unimportant
from $k_L\alpha$ calculation viewpoint especially when a sparingly soluble gas such as oxygen is involved and the liquid hydrodynamics are in the range of typical pneumatically agitated reactors. It should be noted, however, that in external loop draft tube bubble columns the liquid flow tends more toward plug flow and the mixing tends to be somewhat poorer than in the internal loop draft tube bubble columns as use in the present investigation.

- The negligible influence of DO electrode dynamics

In the transient technique the dynamics of the oxygen electrode itself may influence the $k_L\alpha$ results. The electrode delays, which are a function of the fluid hydrodynamics near its measuring surface, may be satisfactorily accounted for by the first-order model (Nakanoh and Yoshida, 1980; El-Temtamy et al., 1984; Chisti et al., 1987)

$$\frac{C^* - C_L}{C^* - C_{L0}} = \left(\frac{e^{-t k_L \alpha}}{t_E} - k_L \alpha e^{-\frac{t}{t_E}}\right) \frac{t_E}{1 - t_E k_L \alpha}$$

Where $t_E$ is the electrode time lag. For $t > t_E$ above equation reduces to,

$$\frac{C^* - C_L}{C^* - C_{L0}} = \frac{e^{-t k_L \alpha}}{1 - t_E k_L \alpha}$$

$$\ln \left(\frac{C^* - C_{L0}}{C^* - C_L}\right) = \ln \left(\frac{1 - t_E k_L \alpha}{e^{-t k_L \alpha}}\right)$$

$$\ln \left(\frac{C^* - C_{L0}}{C^* - C_L}\right) = k_L \alpha t + \ln (1 - t_E k_L \alpha)$$

Thus, a plot of $\ln \left(\frac{C^* - C_{L0}}{C^* - C_L}\right)$ against time, $t$ yields a straight line of slope $k_L\alpha$ and intercept $\ln \left(1 - t_E k_L \alpha\right)$ from which the time delay may be obtained.

The maximum response time of the DO electrodes used was almost always under 10 seconds, which was in keeping with the manufacturer’s data. Care should be taken to ensure that the $C_L$ values from $C_L$ Vs $t$ curves were read at least 20 seconds after the start of the initial operation. This will ensure that the complete development of the hydrodynamics, and furthermore, the condition that $t > t_E$ was also satisfied. Nakanoh and Yoshida (1983) found that the $k_L\alpha$ calculated by taking the probe response into account did not differ significantly from the values obtained by assuming instantaneous...
response \((t_E = 0)\) for \(k_{La}\) values \(\leq 0.1\ \text{s}^{-1}\). They subsequently ignored the probe response in their calculation of \(k_{La}\). The same observation was reported by Yagi and Yoshida (1975) in quite viscous Newtonian and non-Newtonian fluids. Van’t Riet (1979) showed that ultimate error in \(k_{La}\) to be < 6\% as long as the DO electrode response time (63\% of full scale) was \(\leq 1/ k_{La}\). Thus, the \(k_{La}\) data determined by taking the electrode dynamics into consideration and that determined by ignoring the probe delays did not differ much, so the effects of dynamics of DO electrodes can be neglected in \(k_{La}\) calculations.
APPENDIX - IV

CALIBRATION OF DO PROBE

The stepwise procedure for calibration of DO Probe in water saturated air, used in the dynamic gassing-in method for determining volumetric mass transfer coefficient in draft tube bubble columns, in the present investigation is as follows: (DO probe with its parts is shown in Fig.3.7 of chapter-3)

1. Remove the membrane protector from the membrane cap. Do not cover the small hole on the protector with fingers during removal of the protector.
2. Hold the membrane cap in a vertical position, open-end up.
3. Fill the membrane cap about 2/3 full with Dissolved Oxygen Electrolyte Filling Solution.
4. While holding the DO probe vertically with the tip pointing down, gently screw the module cap onto the tip. Electrolyte should leak out of the thread (Note: If electrolyte does not leak out of the threads, air may remain inside the module cap. To ensure accurate results, repeat this procedure using more filling solution).
5. Attach the DO probe cable connector to the meter.
6. With the probe in the calibration and storage chamber, observe the mg/L dissolved oxygen concentration after the probe has been polarized for the appropriate period of time. Calibration may be performed when the display is stable for several minutes.
7. Secure the probe cable to the calibration and storage chamber by wrapping cable through the bottom of the chamber lid before filling with water.
8. Prepare the calibration and storage chamber by holding it under water and squeezing it a couple of times to pull a small amount of water into the lower chamber through the inlet. Alternately, open the bottom of the chamber and insert a water-soaked sponge. (Note: Avoid completely filling the lower part of the calibration chamber with water).
9. Insert the DO probe into the calibration and storage chamber. The tip of the probe must not be flooded with water or be holding a drop of water on the membrane.
10. Allow at least ten minutes for the atmosphere in the chamber to reach a steady state. (Note: Gently squeezing the lower chamber a couple of times to force water-saturated air into the probe chamber will speed up stabilization. Avoid
squeezing liquid water into the chamber). (Note: Keep the DO probe at a uniform temperature. When holding the probe, do not touch the metallic button on the side of the probe. The button is a thermistor that senses temperature. An inaccurate calibration will result if the temperature of the thermistor is different from the probe membrane).

11. Press the DO key to put the meter in DO Reading mode.

12. Press the CAL key located in the lower left corner of the keypad to initiate calibration.

13. The display will show 100%. Press the ENTER key. The stabilizing icon will appear while the meter completes the calibration.

14. When the calibration is complete, the meter will return to the reading mode. Press the EXIT key during the calibration sequence to back out of the calibration routine, one screen at a time, without completing a calibration. (Note: If the Cal and ? icons flash after calibration, the calibration failed and needs to be repeated).
APPENDIX-V
MANUAL PHENATE METHOD

The reagents used and the stepwise procedure of the manual phenate method used for nitrogen estimation in wastewaters is discussed below:

- **Reagents:**
  
  i. **Phenol solution:** Mix 11.1 ml liquefied phenol (≥ 89%) with 95% v/v methyl alcohol to a final volume of 100 ml. Prepare this solution weekly. (Caution: Wear gloves and eye protection when handling phenol; use good ventilation to minimize all personnel exposure to this toxic volatile substance).

  ii. **Sodium nitroprusside, 0.5% w/v:** Dissolve 0.5 gm sodium nitroprusside in 100 ml deionized water. Store in amber bottle for up to 1 month.

  iii. **Alkaline citrate:** Dissolve 20 gm trisodium citrate and 1 gm sodium hydroxide in deionized water. Dilute to 100 ml.

  iv. **Sodium hypochlorite, commercial solution, about 5%:** This solution slowly decomposes once the seal on the bottle cap is broken. Replace about every 2 months.

  v. **Oxidizing solution:** Mix 100 ml alkaline citrate solution with 25 ml sodium hypochlorite. Prepare fresh daily.

  vi. **Stock ammonium solution:** Dissolve 4.714 gm of anhydrous (NH₄)₂SO₄ in deionized water and dilute to 1 liter. So, this stock solution is having 1000 mg of NH₃-N/L of solution.

  vii. **Standard ammonium sulfate solution:** For obtaining a calibration curve, prepare a series of standard solutions in the appropriate range of the concentrations of the samples such as 0 (blank), 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg NH₃-N/L of solution by stepwise dilution from stock solution of 1000 mg/L to 100 mg/L to 10 mg/L to 1 mg/L and so on.
- **Procedure:**

1. To a 25-ml sample in a 50-ml erlenmeyer flask, add, with thorough mixing after each addition, 1 ml phenol solution, 1 ml sodium nitroprusside solution, and 2.5 ml oxidizing solution.
2. Cover samples with plastic wrap or paraffin wrapper film. Let color develop at room temperature in subdued light for at least 1 h. An intensely blue compound, Indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside. Color is stable for 24 hr.
3. Measure absorbance at 640 nm wavelength in spectrophotometer (Hach, DR/2400) with a light path of 1 cm or greater using quartz cuvette.
4. Treat all standard solutions the same as samples.
APPENDIX - VI
ASCORBIC ACID METHOD

The reagents used and the stepwise procedure of the ascorbic acid method used for phosphorus estimation in wastewaters is discussed below:

- **Reagents:**
  
  i. *Sulfuric acid, H₂SO₄, 5N*: Dilute 70 ml concentrated H₂SO₄ to 500 ml with distilled water.
  
  
  iii. *Ammonium molybdate solution*: Dissolve 2 gm (NH₄)₆Mo₇O₂₄·4H₂O in 500 ml distilled water. Store in a glass- stoppered bottle.
  
  iv. *Ascorbic acid*: Dissolve 1.76 gm ascorbic acid in 100 ml distilled water. The solution is stable for about 1 week at 4°C.
  
  v. *Combined reagent*: Mix the above reagents in the following proportions for 100 ml of the combined reagent: 50 ml 5N H₂SO₄, 5 ml potassium antimonyl tartrate solution, 15 ml ammonium molybdate solution, and 30 ml ascorbic acid solution. Mix after addition of each reagent. Let all reagents reach room temperature before they are mixed and mixed in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 hr.
  
  vi. *Stock phosphate solution*: Dissolve 0.2195 gm of anhydrous KH₂PO₄ in distilled water and dilute to 1 liter. So, this stock solution contains 50 mg/L of PO₄³⁻ - P (phosphorus as phosphates).
  
  vii. *Standard phosphate solution*: To obtain a calibration curve, prepare a series of standard solutions of 0(blank), 0.2, 0.5, 0.8, 1, 2, 3, 4, 5 and 6 mg/L of P as PO₄³⁻ by appropriate dilution of the stock phosphate solution.
Procedure:

1. Pipet 50 ml sample into a clean, dry test tube or 125-ml erlenmeyer flask. Add 0.05 ml (1 drop) phenolphthalein indicator. If a red color develops add 5N H$_2$SO$_4$ solution dropwise to just discharge the color.

2. Add 8 ml combined reagent and mix thoroughly. Ammonium molybdate and potassium antimonyl tartrate react in acid medium of combined reagent with orthophosphate to form a heteropoly acid - phosphomolybdic acid - that is reduced to intensely colored molybdenum blue by ascorbic acid.

3. After at least 10 min but not more than 30 min, measure absorbance of each sample at 880 nm wavelength in spectrophotometer (Hach, DR/2400) with a light path of 1 cm using quartz cuvette and with reagent blank as reference solution.

4. Treat all standard phosphate solutions the same as samples and use a distilled water blank with the combined reagent to make photometric readings for the calibration curve.

Note: Use acid-washed glassware for determining concentrations of phosphorus by the ascorbic acid method. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.
The stepwise procedure of the Cole’s method used for total sugar estimation in fermentation broth is discussed below:

1. Concentrated hydrochloric acid in the ratio of 1: 20 is added to the supernatant (cell free sample after centrifugation) for inversion of sugar present in it. Keep it on boiling water bath for 20 minutes.
2. Cool it and make up total volume to 21 ml with distilled water.
3. To neutralize the acidity, sodium bicarbonate was added till the effervescence come out from the hydrolyzed sample.
4. Measure 20 ml of 1% w/v aqueous ferricyanide solution and 5 ml of 2.5 N sodium hydroxide solutions in 100 ml flask. Place it on wire gauze over a flame.
5. Heating should be arranged in such a way that the mixture begins to boil within 2 minutes. As soon as the mixture boils, the flame can be lowered. Active boiling should be maintained during the whole titration.
6. Add 2 – 3 drops of 1% methylene blue.
7. Titrate with the hydrolyzed sample. Sugars having free –CO and –CHO groups, when heated in alkaline solution, these keto or aldehyde group is converted to form enediol. This has more reducing power and reduces K\textsubscript{3}Fe(CN\textsubscript{6})\textsuperscript{3} to K\textsubscript{4}Fe(CN\textsubscript{6})\textsuperscript{4} and sugars are oxidized to complex mixture of acids. The amount of ferricyanide reduced depends upon the concentration of sugar.
8. The end point is reached when the solution is decolorized.
9. Note down the titration reading (X ml) and calculate the amount of sugar according to following formula:

\[
\text{Sucrose} = 19.2 + (0.065 \times X) \text{ mg} / X \text{ ml}
\]
10. Divide above value with 10 to get total sugar % in w/v of the sample.