CHAPTER 3

MATERIALS AND METHODS

3.0 Selection of Pharmaceutical Industries for in-depth study

Six pharmaceutical industries (four pharmaceutical formulation industries and two basic drug industries) are selected for extensive study regarding present status of effluent generation (both quantity and quality) and possible application of process modification strategy in respective industries to minimize the quantity of wastewater generation conforming the norms of effluent disposal to surface and subsurface water sink.

1. *Four pharmaceutical formulation industries*
   a) Large volume parenterals (LVP) producing industry (South 24 Parganas, West Bengal).
   b) Formulation industry producing tablet and oral liquids (South 24 Parganas, West Bengal).
   c) Formulation industry following the norms of ISO 14001 (Hooghly, West Bengal).
   d) A pharmaceutical industry of medicated ointments studied for low cost effluent treatment (North 24 Parganas, West Bengal).

2. *Two basic drug industries*
   a) A Chlor-alkali bulk drug industry (U.P.)
   b) Isolation and identification of bacteria (*Pseudomonas aeruginosa SSP1*) from the effluent of a bulk drug industry producing sodium citrate and sodium oxalate (Kolkata, West Bengal).

3.1 Formulation Industries

The present study was conducted in three pharmaceutical formulation industries, one producing Tablets, second one producing Oral liquid and third one manufacturing Injectables in the form of generic and patented finished drugs. The selected drug plants were studied in three subsequent years (2010, 2011 and 2012). In the first year, the in-plant processes were studied extensively. Thorough study of the in-plant processes revealed that there was enough scope of process changes to achieve waste minimization, material saving and accordingly some suggestions were put forward to the management of the industry by the present investigator. The suggestions were followed entirely. Then in next two years, similar observations were made regarding
improvements in material savings and waste minimization. Some of the important recommendations were the following.

1. Main test of injectables are sterility test and endotoxin test. Dextrose injections are tested for bacterial endotoxin by Limulus Amebocyte Lysate (LAL) reagent purchased from Charls Liver Endosafe. As per Indian Pharmacopea, 2010 limit of BET is 0.5EU/ml. Fate of a 3500 litre batch comprising 7000 x 500 ml bottles depends on this test. Batch after batch are rejected as the limit of 0.5EU/ml is crossed. Then present investigator suggested conducting Bacterial Endotoxin Test (BET) for all input materials, starting from Raw materials, Potable water, Purified water, Water for injection and Polyethynene granules. For large volume parenterals (LVP) production, raw materials used are Dextrose anhydrous, Sodium chloride, Sodium lactate, Calcium chloride, Potassium chloride, Sodium acetate, Sodium metabisulphate, Sodium sulphite, Dibasic Potassium phosphate, Magnesium Chloride, Ammonium chloride, Metronidazole Ciprofloxacine monolactate, Pefloxacin, Mannitol, Fructose, Glycerin, Tinidazole and the Water for Injection. Among these raw materials, pharmacopeia specifies only the limit of bacterial endotoxin for Water for Injection. Indian Pharmacopea, 2010 specifies the limit of 0.5 EU/ml bacterial endotoxin as for 5% Dextrose Injection, 10% Dextrose Injection 0.9% Sodium Chloride injection, 5% Dextrose and 0.9% Sodium Chloride injection and for other injections.

2. Tablet film coating are done for antibiotic Ofloxacin tablet, antitrichomonial Metronidazole tablet and antiprotozoal Tinidazole tablet by using organic solvent Isopropyl alcohol and acetone. Investigator stops this operation by substituting coating operation by aqueous base coating solution. This is implemented and results of resource savings are shown in Table 3 and Table 4.

3. A tablet formulation had been followed with a fixed formula for long period. As per present investigator’s suggestion the quantities of filler and binder were reduced as shown in Table 6, Table 7 and Table 8.

4. A suspension of Paracetamol is a regular product. Reduction of inactive raw materials in Paracetamol suspension formulation is suggested, implemented and final achievement is shown in Table 5.

3.2 Monitoring and Evaluating Results of Waste Minimisation

After implementation, the results should be evaluated to assess the impact of the WM solution/option. This may cover the estimation of the following aspects:
- Net savings
- Reduction in consumption of input components and
- Reduction in generation of pollution load.

Pharma companies should look for minimization of waste first and if any waste is produced that should be recycled as far as possible. Pharma companies should look for profit by selling waste products.

Pharma companies are scouting for customers to sell the waste products including solvents and pigments, generated in their companies.

These products are used as raw materials in other industries, which buy them at reduced costs, sometimes as much as 50%.

Solvent reduction is a good plan to start waste minimization

Solvent reduction is the sweet spot for greening any pharma process. About 80 percent of pharma waste results from solvent use, with the remainder related to reagents and raw materials. There are many adverse effect of solvent on environment as well as on human health. On the environment side there is risk of fire hazard due to lower flash point of the solvent. Higher vapour pressure of the solvent in the air increases the likelihood of breathing of solvent vapours by the people close to it. As this solvents are highly toxic so replacement of this solvents are required.

After the compression of tablets, a sugar coating is done in case of some categories of tablets. Here organic solvents are required. Here aqueous film coating is used now a days to replace solvent sugar coating.

Green chemistry is the use of chemistry for prevention of air and water pollution. In the manufacture of Ranitidine HCl newer catalyst is developed, thereby reducing the cost of production by 20%.


After adoption of Environment Management System, ISO: 14001 with regular self assessment / internal auditing for waste management in a pharmaceutical formulation industry, development of road map for cleaner production has been achieved. The water consumption has been reduced significantly. Powder spillage during sampling and weighing of raw materials (RM), contamination of air and land affecting human lungs, heavy sound generation during trolley movement carrying RM in production floor causing adverse effects on human ear, powder, broken pieces of tablets, torn strips, torn labels, torn papers on production floors, contamination of land, increasing load on effluent treatment plant (ETP), mixed waste generation after sweeping of floor in oral liquid department, oil leakage from trucks during transferring LDO to boiler tank cause severe problem in the industry. To mitigate all these aspects, effective measures are undertaken and fruitful results are obtained.
3.4 Low cost Treatment System Adopted in a Pharma Formulation Unit

A drug formulation manufacturing company producing medicated ointment dosage forms has an effluent treatment plant, where a detailed study of influent water and effluent water are tested from six points: Raw effluent (T1), Grit filter (T2), Oil and Grease trap (T3), Equalization tank (T4), Aeration tank (T5), Secondary Clarifier (T6). The effluent treatment plant consists of a central sump, where influent water is coming from five production section and one quality control section. From central sump wastewater is coming to grit filter. Large wastes, which are entrapped there, are time to time removed. Now wastewater flows to oil and grease trap. Here oil and grease are separated. Then this wastewater is allowed to go to equalization tank. Here after equalization wastewater is allowed to transfer to aeration tank. Here alum and polyelectrolytes are added and aeration continued. Then after 7 hours flocs are settled. Now the wastewater is transferred to secondary settling tank. After overnight settlement clear water is subjected to membrane filtration, after which the water is drained out.

THE ETP process are running with huge cost involvement, as recurring expense incurred for purchasing of membrane filters regularly, which create a pressure on the small unit, so an alternate route are searched for by the investigator.

Results of analysis of this drained water after the effluent treatment are not satisfactory. pH is acidic, oil and grease, BOD, COD values in the effluent are all high and do not conform the standards prescribed for disposal either to land or to surface water.

The present researcher observed the following points:

1. A flash mixing arrangement is absent in the process which is necessary for optimum coagulation.
2. 25 kg alum and 20 kg polyelectrolytes are used.

Measures taken to counteract this problem

Investigator suggested some steps to overcome this problem, as below

1. First a chemical reaction unit is introduced in ETP process line. Here commercial hydrochloric acid is added to break the cellulose gum present in the influents.
2. This process is changed to 12 kg alum are dissolved in 300 litres water in a barrel, so the amount of alum is reduced and lime is introduced as 8 kg lime are dissolved in another 300 litres barrel. In each barrel at the bottom an outlet with stopcock is arranged.
3. A flash mixer is introduced.
4. Now after wastewater is coming to equalization tank, aeration starts from an air compressor. Then after stabilization wastewater are transferred by a 2HP pump to the flash mixer.
5. Both barrel of alum solution and lime solution are set side by side just above the flash mixer.
6. Heavy duty stirrer of Flash mixer are switched on, stopcock of both barrel are made open, so 2.66% lime and 4% alum solution are dripping out simultaneously to the wastewater of flash mixer.

7. Flash mixer is kept above the level of coagulation tank. Two outlet valve of flash mixer are made open. Coagulant mixed wastewater is now coming out by gravity to the coagulation tank / aeration tank. Air compressor switch are made on for Aeration line going to coagulation tank.

8. After 10 hours coagulation treated water are transferred to secondary settling tank.

9. Overnight settling is allowed.

10. Then membrane filtration are removed, instead treated water are transferred to oxidation pond.

11. After keeping in oxidation pond for 5 days holding time water are analysed and effective result are found as shown in table 3.3. Then the water is reused in gardening. An attempt is also made for toilet flashing.

3.5 Chlor-alkali Industry

Chlor-alkali industry is a red category industry with respect to environment management system, approximately one lakh metric ton of brine sludge is generated from all units in India. It is, therefore, essential to find alternate use of brine sludge instead of storing on land. Fly ash generated in captive power plants of Chlor-alkali industry is required to be used. Waste reduction is required with all possible means throughout the production process. The investigator tried to do the study with that possible aim.

The objective of the present study is to emphasize on waste minimization in a basic drug plant producing sodium hydroxide and to find prospective measures to be undertaken to meet the need of proper environmental management. Corrective actions are taken to reduce wastage and to improve characteristics of wastewater by process modifications.

The present study was conducted in a bulk drug industry (Chlor-alkali plant) producing chlorine (Cl₂) and sodium hydroxide (NaOH). The selected Chlor-alkali plant was visited in two subsequent years (2011 and 2012). In the first visit, extensive studies of the in-plant processes were made. Samples were collected from the final effluent stream and analyzed for the parameters pH, TSS, TDS, COD, BOD, Mercury, and Free Chlorine. Thorough study of the in-plant processes revealed that there was enough scope of process changes to minimize waste generation and accordingly some suggestions were put forward to the management of the industry by the present investigator. The suggestions were followed in toto. After one year, similar observations were made regarding the quantity and quality of the effluent generated from the same industry. Some of the important recommendations were the following.

1. Most of the equipments in the primary brine like settler, purifier are kept open. A fraction of solution is evaporated and dropping out causing process losses and increase wastewater load. Suggestion was to cover the equipment to prevent losses.
2. Highly concentrated Sulphuric acid (92-98%) is used to dry chlorine. 20 kg Sulphuric acid is consumed per metric ton of chlorine produced. Spent acids were wasted in wastewater. Suggestion was made either to sell the spent acids to another industry which will use it or to re-concentrate the acid and use it again in the plant process itself by the same industry.

3. The waste gas containing chlorine used to emanate during normal operation and large amount of chlorine gas was emitted to the environment during irregular plant operation and in emergencies. Suggestion was made to use weak caustic soda solution to absorb the same to produce sodium hypochlorite.

4. Present investigator suggested providing suitable dosing of hydrogen peroxide and sodium bisulphate to neutralize and reduce chlorine content in wastewater.

5. The then practice was to bring together wastewater streams from stable bleaching powder and from aluminum chloride and the pH of mixed wastewater would stand at 11-12. This was further neutralized with 26 liter of commercial hydrochloric acid daily. Similarly, wastewater stream from membrane plant and brine purification plant having low pH of 3-4 was neutralized with 32 kg soda ash daily. Present investigator altogether stopped acid/alkali consumption, instead all wastewater streams are suggested to mix in primary ETP for intermixing and automatic neutralization.

6. Present investigator suggests using the sludge generated in the treatment plant as compost, as fertilizer and also using it as a feed material in cement manufacturing, brick manufacturing and road construction.

7. Aim should be for zero emission of hydrogen as using hydrogen to generate DC power within cell house should be explored in hydrogen based fuel cell (77).

A. Scope of Work
The following are the scopes of work identified:

i) Study of the existing ETP based on earlier records;
ii) Assessment of actual functioning of the ETP;
iii) Suggesting preliminary modifications and alternatives for trial
iv) Preparation of final proposal for necessary modifications, additions and alterations to the existing system;
v) Preparation of realistic cost estimates for the above.

B. Work Procedure
The modifications have been proposed and work undertaken in the following manner:
i) Preliminary modifications are intimated after plant visit. Certain alternatives are suggested at that time, which need to be implemented on trial basis at the plant by the clients. Observations on these trials are intimated to the present researcher for evaluation of the alternatives and finalization of the proposals.
ii) *Final Proposal* is drawn up after observing the results of the trials. Final proposal also contains the approximate market price for implementation of modifications, additions and alterations suggested.

Testing facilities for monitoring of effluent parameters for various trials are provided by the management of the industry. All data pertaining to the ETP are made available to the present researcher. This includes the specifications, drawings and details of the existing plant as well as the records, data of its performance since its initial commissioning and details of the records of operation and maintenance of the existing ETP. Necessary assistance during field visit and the data of input effluent parameters are also provided. Guidance regarding the manufacturing process for identification of source of specific effluent parameters is also made available.

The objective of the present investigation is to study on waste minimization in a basic drug plant producing sodium hydroxide and prospective measures to be undertaken to meet the need of proper environmental management. Corrective actions are taken to reduce wastage and to improve characteristics of wastewater by process modifications.

The results of analysis of the wastewater as furnished by the authority of a bulk drug industry show wide variations. After proper statistical analysis the following figures are considered for further study and improvement.

**Table 3.1 Performance Study of ETP of a Bulk Drug Industry**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inlet of ETP</td>
</tr>
<tr>
<td>Volume (m³/h)</td>
<td>35 – 55</td>
</tr>
<tr>
<td>pH</td>
<td>8 – 12</td>
</tr>
<tr>
<td>TSS (mg/l)</td>
<td>2500</td>
</tr>
<tr>
<td>TDS (mg/l)</td>
<td>7500</td>
</tr>
<tr>
<td>COD (mg/l)</td>
<td>250 – 300</td>
</tr>
<tr>
<td>BOD (mg/l)</td>
<td>35 – 55</td>
</tr>
<tr>
<td>Mercury (mg/l)</td>
<td>0.001</td>
</tr>
<tr>
<td>Chlorides (mg/l)</td>
<td>5000</td>
</tr>
<tr>
<td>Free Chlorine (mg/l)</td>
<td>1000</td>
</tr>
</tbody>
</table>

The following points are considered in order to achieve substantial improvement of effluent quality with the existing system itself.
a) Out of many parameters evaluated the values of TSS, TDS, chloride and free chlorine are shown to be high, which makes the effluent unsuitable for use either for horticulture or for cooling purpose, the proposed end use.

b) Expected TSS removal in an ETP consisting of chemical treatment followed by secondary treatment is about 85-90%, whereas the removal of TSS in the treatment plant of the bulk drug industry under consideration is found to be about 40% only (reduction in the order of 1000 mg/l), which leads to the conclusion that certain inadequacies exist in the chemical treatment (chemical precipitation), which needs to be modified for improvement.

c) The manufacturing process contributes chlorides (the major constituent of TDS) and free chlorine. The possibility of suitable control in the manufacturing process to restrict ingress of the two constituents needs to be reviewed.

d) The existing ETP is provided with a tertiary treatment unit consisting of pressure sand filter, activated carbon filter and an ion-exchange resin bed for softening purpose. In spite of this tertiary treatment the high values of TDS, chloride and free chlorine has led the present investigator to conclude that probably this tertiary treatment system is ineffective.

e) Minor modifications and fine tuning of the existing system are found to be necessary to make the effluent suitable for intended purposes.

3.6 Isolation of Microorganism from Wastewater of Bulk Drug Industry

Table 3.2 presents the source of microorganisms used for comparative study of the role of standard microorganism (*Pseudomonas aeruginosa* ATCC 27853) and the microorganism isolated from the effluent of a bulk drug industry.

**Table 3.2: Source of Microorganisms**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em> ATCC 27853</td>
<td>Prof.S.G.Dastidar, Herbicure Healthcare Bio-Herbal Research Foundation, Metro Garden City, Pailan, D.H.Road, Kolkata-700104, India.</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> SSP1.</td>
<td>Isolated from effluent drain of a bulk drug manufacturing unit</td>
</tr>
</tbody>
</table>
Microorganism isolated is Pseudomonas aeruginosa SSP1

A basic drug manufacturing company in Kolkata producing Citric Acid, an inoculums are collected from the out let point, through which wastewater are going to the outside drain. These inoculums are cultured and a microorganism are isolated and identified as Pseudomonas aeruginosa named as Pseudomonas aeruginosa SSP1.

It is understood that the organism Pseudomonas aeruginosa SSP1 grow by utilizing Citric Acid as a carbon source, so it is thought for further study of its utilization activity on other Active Pharmaceutical Ingredients. Those are as sixteen Antibiotics as below.

**Antibiotics**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin (Pc)</td>
<td>Streptomycin (Sm)</td>
</tr>
<tr>
<td>Ciprofloxacin (Cf)</td>
<td>Norfloxacin (NF)</td>
</tr>
<tr>
<td>Piperacillin (Pp)</td>
<td>Amikacin (Ak)</td>
</tr>
<tr>
<td>Meropenem (Mp)</td>
<td>Ceftazidime (Cd)</td>
</tr>
</tbody>
</table>

**Non – Antibiotics**

<table>
<thead>
<tr>
<th>Non – Antibiotics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioridazine (TZ)</td>
<td>Chlorpromazine (Cpz)</td>
</tr>
</tbody>
</table>

**Preservation of cultures**

All the strains of Pseudomonas aeruginosa were preserved in freeze dried ampoules and also in stab agar media. Regular sub-culturing of the bacteria on bromothymol blue lactose agar((Sen, Chakraborty, Sen and Ghosh, 1968) (89) was done. To ensure purity of cultures, they were always identified before following standard protocol.

**Tests for identification of the bacteria** - the following methods were carried out for the identification of different organisms.

**Morphology**

Smear were made on glass slide and stained according to Preston and Morrell’s (1962) (78) modification of gram staining method as described by Duguid (1966) (82)

**Motility:** This was performed by hanging drop technique (Collee et.al. 1996) (79)

**Biological characterization tests** include the following tests:

**Fermentation of carbohydrates:** Fermentation of different carbohydrates like glucose, lactose, sucrose, mannitol, dulcitol, salicin adonitol, inositol, sorbitol, arabinose, raffinose, rhamnose, maltose, xylose and mannose were tested according to Edwards and Ewing (1972), Farmer et al. (1985)(83), Brown et.at.(1996)(80) and the results were recorded daily upto 96 hours and again on the 7th day of incubation.

**Oxidation – Fermentation test:** The Oxidation – Fermentation test of Hugh and Leifson ( 1993 )(81) was done as described by Edward and Ewing ( 1972 ).
**Methyl – Red test:** This test was performed following the method of by Edward and Ewing (1972).

**Acetyl – Methyl carbinol production test:** The Voges–Proskaur reaction was demonstrated by the method of Barritt (1936) (84) as elaborated according to Collee et.al. (1996)(81).

**Gluconase test:** This was carried out by following Collee et.al. (1996)(81).

**Citrate utilization test:** To demonstrate the ability to utilize citric acid as the sole source of carbon, this test was performed in Simmon’s citrate medium Collee et.al. (1996)(81).

**Malonate test:** This was done following the method described in Collee et.al. (1996)(79).

**Hydrogen sulphide test in Tripple Sugar Iodine (TSI) test:** This was carried out by following Collee et.al. (1996)(79).

**Indole production test:** This test was done according to Kovacs (1956)(85) and Cappuccino and Sherman (1983).

**Phenyldeamine test:** Falkow’s method (1958), a modification of Moeller’s(1955) method as described by Sanyal et.al.(1968)(86) was followed.

**Catalase test:** The modified method as described by Edward and Ewing (1972) and Hickman and Farmer (1978)(83) was followed. A small amount of bacterial culture from nutrient agar slant was rubbed on a clean slide, a drop of hydrogen peroxide was added to it and looked for immediate effervescence of gas bubbles from the culture, for positive reaction.

**Oxidase test:** This was done by filter paper method, described by Kovacs (1956)(85) and Collee et.al. (1996)(79).

**Urease test:** The ability to metabolite urea was tested by using Christensen’s medium as directed by Brown et.at.(1996)(80).

**Phosphatase test:** The method followed for this test is as per Baird (1996).

**Potassium Cyanide (KCN) test:** The test is performed as per Braun and Guggenheim (1932) and Farmer et.al.(1985) (83).

**Coagulase test:** The method of Baird (1996), which is modified from Gillespie (1943), was followed for this test.

**Tween 80 test:** Capacity to hydrolyse Tween 80 was tested by applying a standardized inoculum of the test bacterium on agar (1.5%, Difco) medium containing tryptic soy broth (1.5%, Difco) plus Tween 80 (1 ml v/v) and noting opacity reactions as described by Chakraborty et.al.(1970)(87).
**Bile solubility test:** Here 0.1 ml of a 10% solution of Sodium desoxycholate was added to 5 ml of a broth culture of bacteria, pH < 6.8, kept at 37°C; in case of positive test lysis of cells occurred within 15 minutes.

**Media preparation:** The media used for this study were primarily of two types comprising liquid and solid media as described below:

**Peptone water**

<table>
<thead>
<tr>
<th>Component</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriological peptone (Oxoid)</td>
<td>1%</td>
</tr>
<tr>
<td>Sodium chloride (Analar)</td>
<td>0.5%</td>
</tr>
<tr>
<td>Purified Water</td>
<td>q.s.</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 – 7.4</td>
</tr>
</tbody>
</table>

**Nutrient Broth**

<table>
<thead>
<tr>
<th>Component</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract (Oxoid)</td>
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</tr>
</tbody>
</table>

**Mueller Hinton Broth** *(Mueller and Hinton, 1941 (88); Krogstad and Moellering, 1980 (89))*

<table>
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<tr>
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<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract (Oxoid)</td>
<td>1%</td>
</tr>
<tr>
<td>Casein hydrolysate (Oxoid)</td>
<td>1.75%</td>
</tr>
<tr>
<td>Starch (E.Merck)</td>
<td>1.5%</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>

**Solid Culture Media**

**Peptone Agar**

<table>
<thead>
<tr>
<th>Component</th>
<th>Conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar (Oxoid)</td>
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</tr>
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<td>7.2 – 7.4</td>
</tr>
</tbody>
</table>

These media was used as highly reproducible medium of low nutritional value for standardized assay tests of antibiotics and non-antibiotics with respect to Pseudomonas aeruginosa bacterial strain.

**Nutrient Agar**

<table>
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<td>7.2 – 7.4</td>
</tr>
</tbody>
</table>
**Bromothymol blue lactose agar**

This medium consisted of nutrient agar base and 1.25ml of a 0.2% solution of Bromothymol blue indicator, which was added to this medium to make it 100ml at a pH of 7.4; after autoclaving for 15 minutes at 15 lbs pressure, 1% lactose was added and the medium was steamed again for half an hour before being dispersed in petridishes (Sen, Chakraborty, Sen and Ghosh, 1968) (90).

### 3.7 Test Procedures of Physico-chemical Parameters

The following test procedures are followed for determination of different parametric concentration of wastewater and treated effluent.

**Total Suspended Solids (TSS)**

1. Item analysed: Effluent
2. Range of the Test: Not Specified
4. Name of the method: Gravimetric
5. Equipment Used: Suction Pump and Filtering Apparatus
6. Reference Standard Used: N.A
7. Environmental Condition
   a) Required for Stabilization / storage - Room Temperature
   b) Required for test - Room Temperature
8. Procedure:
   Take initial weight ($W_1$) in grams of a previously dried and desiccated glass microfibre filter (GF/C 47mm) paper. Place it into the filtering apparatus. After that take 100 ml (more or less according to the load of TSS) well-shaken sample and pass it through the filter paper by suction pump. Wash the filter paper four times by distilled water. Take out the filter paper containing suspended solids and dried for four hours at 103°C–105°C followed by desiccation for cooling. Then take the final weight ($W_2$) in grams of the filter paper.

9. Calculation:
   For an aliquot of 100 ml sample
   $$\text{Total Suspended Solids (mg/l)} = \frac{(W_2 - W_1) \times 1000 \times 1000}{\text{Volume of sample taken in ml}}$$

10. Safety Requirement: - Filtration apparatus should be properly fitted so that there are no leaks.
**Total Dissolved Solids (TDS)**

1. **Items to be analysed** - Water
2. **Range of the Test** - Not Specified
4. **Name of the method** - Gravimetric
5. **Equipment Used** - Water Bath, glass basin (100 ml capacity)
6. **Reference Standard Used** - N.A
7. **Environmental Condition**
   - a) Required for Stabilization / storage: Room Temperature
   - b) Required for test: Room Temperature
8. **Procedure**
   
   Weigh a glass basin (100 ml capacity), previously dried at 180°C for one hour and cooled in a desiccator for initial weight \( W_1 \) in grams. Then pour 50 ml filtered sample in it. Then place it on the water bath till dry. After drying remove it from the water bath and let it dry at 103°C – 105°C for two hours in an oven. Cool it through desiccator and take final weight \( W_2 \) in grams until constant weight.

9. **Calculation**

\[
\text{Total Dissolved Solids (mg/l)} = \frac{(W_2 - W_1) \times 1000 \times 1000}{\text{Volume of sample taken in ml}}
\]

10. **Safety Requirement**: - Water level of water bath should be maintained at adequate level.
**pH**

1. Item analysed: Effluent
2. Range of the Test: 0 – 14
5. Equipment Used: pH Meter
6. Reference Standard Used: Standard Buffer solutions of pH 4.0, 7.0 & 9.0
7. Environmental Condition:
   a) Required for Stabilization / storage - At Room Temperature
   b) Required for test - Room Temperature.

   a) Buffer 4.0 - Take one buffer tablet of 4.0 pH in 100 ml distilled water. Let it stand for 24 hours for complete mixing. Shake before use.
   b) Buffer 7.0 - Take one buffer tablet of 7.0 pH in 100 ml distilled water. Let it stand for 24 hours for complete mixing. Shake before use.
   c) Buffer 9.2 - Take one buffer tablet of 9.2 pH in 100 ml distilled water. Let it stand for 24 hours for complete mixing. Shake before use.

9. Procedure - Rinse the glass electrode with distilled water and gently wipe it with a soft filter paper. The determination may now be made by pH meter with glass electrode at room temperature after standardizing the pH meter with buffers 4.0, 7.0 and 9.0/9.2 and directly reading the digital pH reading.

10. Safety Requirement - Handle the electrode with care without touching the tip and keep the electrode always dipped into distilled water, when not in use.

11. Retention Period - Samples are analyzed immediately or within 24 hours when kept in Refrigerator.
**Oil & Grease**

1. Item analysed: Effluent  
2. Range of the Test: > 2 ppm  
5. Equipment Used: Separating Funnel and Distillation apparatus  
6. Reference Standard Used: N.A  
7. Environmental Condition:  
   a) Required for Stabilization / storage - Room Temperature  
   b) Required for test - Room Temperature  
8. Reagents:  
   a) Magnesium Sulphate solution - Dissolve 1 gm Magnesium Sulphate heptahydrate in 100 ml distilled water.  
   b) Milk of Lime - Mix 2gm of anhydrous calcium oxide (CaO) with distilled water into a paste and dilute the suspension to 100 ml with distilled water.  
   c) n - Hexane  
   d) Anhydrous Sodium sulphate  
   e) Hydrochloric acid (1:3)  
9. Procedure:  
Take 1000 ml well mixed sample in a 1000 ml beaker. Add 5 ml magnesium sulphate solution followed by stirring. Add slowly small amounts of milk of lime with stirring until flocculation occurs. Allow to settle for a few minutes and decant the supernatant liquid in a separating funnel. Dissolve the precipitate in a beaker with 1:3 HCl and pour the solution into the previous separating funnel, taking care not to transfer any adventitious solids like twigs, leaves etc. Rinse the beaker and the stirrer with about 50ml petroleum ether. Then extract the oil from the sample by adding 50ml petroleum ether followed by rejecting the lower layer. Then wash the oil – petrol ether layer with distilled water till it is free from acid. Then pass the oil – petrol ether layer through anhydrous sodium sulphate, wash the filter paper with some petroleum-ether, and collect it in a previously dried and weighed flask in grams (W₁). Distill off the petrol – ether from oil – petrol -ether mixture and again weigh the flask after sufficient drying and cooling until constant weight in grams (W₂).  
10. Calculation:  
\[
\text{Oil and grease (mg/l)} = \frac{(W₂ - W₁) \times 10^6}{\text{Sample Volume}}
\]
11. Retention Period: Add H₂SO₄ to PH<2 & store from 28 days.
**Biochemical Oxygen Demand (BOD) for 3 days at 27°C**

1. **Item analysed**
   - Effluent
2. **Range of the Test**
   - > 5 ppm
3. **Specification of the Test**
   - IS 3025 (Part-44) 1993, Reaffirmed 2003
4. **Name of the method**
   - Incubation method
5. **Equipment Used**
   - B.O.D. Incubator
6. **Reference Standard Used**
   - Glassware – Glutamic Acid Solution.
7. **Environmental Condition**
   a. Required for Stabilization / storage :- Below 4°C
   b. Required for test :- Room Temperature
8. **Reagents**
   a) **Phosphate Buffer solution**
      - Dissolve 8.5 gm KH₂PO₄, 21.75 gm K₂HPO₄, 33.4 gm Na₂HPO₄.7H₂O and 1.7 gm NH₄Cl in about 500 ml distilled water and dilute to 1 litre. The pH should be 7.2.
   b) **Magnesium Sulphate solution**
      - Dissolve 22.5 gm MgSO₄.7H₂O in distilled water and dilute to 1 litre.
   c) **CaCl₂ solution**
      - Dissolve 27.5 gm CaCl₂ in distilled water and dilute to 1 litre.
   d) **FeCl₃ solution**
      - Dissolve 0.25 gm FeCl₃, 6H₂O in distilled water and dilute to 1 litre.
   e) **Acid and alkali solution (1N) for neutralization of caustic or acidic waste samples.**
      - Acid – Slowly and while stirring add 28 ml Conc. H₂SO₄ to distilled water and dilute to 1 litre. Alkali - Dissolve 40 gm NaOH in 1 litre distilled water.
   f) **Manganous sulphate solution**
      - Dissolve 480 gm Manganous sulphate tetrahydrate in distilled water. Filter if not clear and make up to 1 litre.
   g) **Alkaline Azide solution**
      - Dissolve 500 gm NaOH in 500 ml distilled water and allow to cool. Dissolve separately 150 gm of KI in a small quantity of freshly boiled and cooled distilled water and add this solution to the caustic solution. Dissolve 10 gm of Sodium Azide in 40 ml of distilled water and add to the above solution. Make up the volume up to 950 ml.
   h) **Potassium Fluoride solution**
      - Dissolve 40 gm of Potassium Fluoride (KF,2H₂O ) in 100 ml distilled water.
   i) **H₂SO₄ (1:1).**
   j) **Starch indicator solution**
      - Triturate 5 gm Starch and 0.01 gm of HgI with 30 ml cold water and slowly pour it with stirring into 1 litre of boiling water. Boil for 3 minutes. Allow the solution to cool and decant off supernatant clear liquid.
      - Or Dissolve 2g of starch and 0.2 g of salicylic acid as preservative, in 100ml of hot distilled water.
   k) **Standard Sodium Thiosulphate working solution**
      - Exactly 0.25 N.
   l) **Use a mixture of 150mg glucose and 150mg glutamic acid per litre as a standard check**
solution. Determine the 3 days 27°C BOD of 2 percent dilution of the glucose – glutamic acid standard check solution. The BOD of which is 200± 37mg/l after 3 days at 27°C.

9. Procedure

a) Neutralize the sample to pH about 7.0 by adding acid or alkali solution whichever is needed.

b) Preparation of Dilution water:
   Take 2 litre good quality of distilled or DM water, aerate for 8hrs and settle for 4hrs. Then add nutrients one ml per litre dilution water each.

c) Seeding of Dilution Water 0.4 % settled sewage is added in dilution water for seeding. (e.g. 0.4%= 0.4 ml settled sewage + 99.6 ml of dilution water)

d) Dilution of sample with seeded dilution water
   Siphon carefully the seeded dilution water into a graduated 1000ml measuring cylinder and fill upto 500ml. Then add required percentage of sample in it and make up upto 1000ml with the seeded dilution water through siphoning. Then mix gently with a glass rod, so that no air will enter. Siphon the above the diluted sample into two BOD bottles and fill completely and allow the stopper. In the same way siphon the dilution water into two BOD bottles as Blank. Incubate one bottle from blank and one from samples for 3 days at 27°C. Measure the initial dissolved oxygen (See RVB/SOP/03/36) both for sample and the blank. After 72 hours incubation period at 27°C, again measure the final dissolved oxygen both for sample and the blank.

10. Calculation

\[
\text{Biochemical Oxygen Demand (BOD) (mg/l)} = \frac{(D_1-D_2) - (C_1-C_2) \times F}{P}
\]

Where, \(D_1=\)Initial D.O. for dilute sample.
\(D_2=\)Final D.O. for dilute sample.
\(C_1=\)Initial D.O. for seeded dilution water.
\(C_2=\)Final D.O. for seeded dilution water.
\(F=\)Ratio of the seed in the sample to that in blank.
\(P=\)Decimal fraction of the sample used.

11. Retention Period: Refrigerator for 48 hours.
**Chemical Oxygen Demand**

1. Items to be analysed: Effluent
2. Range of the Test: -
4. Name of the method: Open Reflux Method
5. Equipment Used: Reflux System
7. Environmental Condition:
   a) Required for Stabilization / storage: Room Temperature
   b) Required for test: Room Temperature

8. Reagents:
   a) Standard Potassium Dichromate solution - Dissolved 12.259 gm Potassium Dichromate, primary standard grade, previously dried at 150°C. For 2 hours, in distilled water and dilute to 1000 ml. This is 0.25 N solution.
   b) Ferroin indicator solution - Dissolve 1.485 gm 1,10 Phenanthroline Monohydrate and 695 mg FeSO₄·7H₂O in distilled water and dilute to 100 ml.
   c) Standard 0.25 (M) Ferrous ammonium sulphate (Mohr salt solution) - Dissolve 98 gm of Ferrous ammonium sulphate in distilled water at 20 ml Conc. H₂SO₄ and cool and diluted to 1000 ml.
   d) Mercuric sulphate crystals or powder
   e) Standard Potassium Hydrogen phthalate solution - Dissolve 425 mg of (previously dried at 110°C for one hour) Potassium Hydrogen phthalate in distilled water and dilute to 1000 ml. This solution has a theoretical C.O.D of 500 mg/L.
   f) Sulphamic Acid.
   g) Standardization of Mohr salt - dilute 25.00ml standard K₂Cr₂O₇ to about 100ml. Add 30ml conc H₂SO₄ and cool. Titrate with FAS titrant using 0.10 to 0.15 ml ferroin indicator.
   h) Sulphuric Acid reagent - Add AGSO₄ (reagent or technical grade) to convert H₂SO₄ at the rate of 5.5gm/kg H₂SO₄ let stand to dissolve.

9. Procedure:

Take 20ml sample in an Erlenmeyer flask (or sample make up to 20ml). In another beaker take about 0.4 gm of silver sulphate and 75ml conc. H₂SO₄ and mix well. Then pour 5.0ml of this mixture in the sample containing flask in cold condition to avoid the possible loss of volatile material by local heat. Then add 10.0 ml of Standard 0.25 (N) K₂Cr₂O₇ solution followed by 30.0ml of H₂SO₄ - silver sulphate mixture in cold condition. Then reflux it for two hours. After two hours cool and dilute to 200.0ml with distilled water. Titrate it with standard Mohr salt solution using ferroin indicator. The blank will be prepared in the same way taking 20.0 ml distilled water. A monthly check of the system must be done by preparing a fresh solution of potassium hydrogen phthalate standard which will give a theoretical COD of 500 mgms/litre.
10. Calculation:

Chemical Oxygen Demand (mg/l) = \frac{(V_B-V_S) \times N \times 8000}{V}

Where, 

- $V_B$ = Titre value of the blank in ml.
- $V_S$ = Titre value of the sample in ml.
- $N$ = Normality of Mohr salt solution.

$V$ = **Volume of sample taken for the test in ml.**

Note: If nitrites present in the sample, 10 mg of sulphamic acid is reserved for each 1mg of $\text{NO}_2^-\text{N}$ present in the sample volume used to overcome nitrite interference.

11. Retention Period: Add H$_2$SO$_4$ to PH<2 & store for 7 days.