CHAPTER 2

CHARACTERISATION OF SKIM SERUM EFFLUENT

2.1. Introduction

Centrifuging involves separation of field latex into two fractions, one containing the concentrated latex of more than 60% of dry rubber and the other containing 4-6 % dry rubber, using a centrifuging machine. To recover the rubber from the skim latex containing ammonia, latex is coagulated using 98% sulphuric acid and the serum left out is drained off. This is known as skim serum effluent. It is collected in skim rubber traps for recovery of rubber before going to wastewater treatment. Apart from the skim serum, the water used for cleaning the latex storage tanks once a week, washing the barrels and floor and that for washing the bowls of the centrifuging machine twice in a shift form part of the effluent. The washings are collected in separate rubber trap for rubber recovery. The water requirement in centrifuging process is in the range of 4-6 litres per kg of dry rubber as latex concentrate. Since the serum left out after coagulation of the skim latex is discharged, together with the water used in the processing, the quantity of effluent discharged is much higher than the quantity of water consumed for the processing\(^1\). The skim serum effluent contains significant amount of non-rubber particles like proteins, sugars, lipids, carotenoids and organic and inorganic salts originating from the latex and very little amount of uncoagulated latex\(^2\). These constituents are excellent substrates for the proliferation of micro organisms, which generate high BOD and obnoxious odour.
To develop an effective treatment scheme, it is necessary to have an exhaustive knowledge about the basics of physico-chemical, biochemical and bacteriological characteristics of the effluent. This chapter delineates the evaluation of the characteristics of skim serum effluent. The methods used for the characterisation are also included in this chapter.

2.2. Experimental

The samples used for the study and the methods followed for analyses are explained in the following section.

2.2. A. Sample collection

The skim serum effluent samples were collected from a centrifuge latex concentration unit in Kottayam district of Central Kerala. The sources of effluents from a centrifuging unit are shown in Fig.1.4

Ammoniated field latex containing 30 to 33% rubber is concentrated by centrifuging to 60% rubber and skim latex containing 4-6 percent rubber. High speed centrifuges are used in this process. The concentrated latex is stored in drums and marketed. The skim latex, which contains about 0.8 per cent ammonia, is coagulated with 98% sulphuric acid to recover rubber. The skim serum produced after coagulation of rubber is stored in a separate trap. Samples of the serum were collected at periodical intervals for the study.

2.2.B. Physico-chemical parameters of the effluent

The physico-chemical characteristics of the effluent samples were analysed as per standard methods\(^5\) for the following parameters
1. pH
2. Turbidity
3. Chemical oxygen demand (COD)
4. Biochemical oxygen demand (BOD)
5. Sulphates
6. Total solids
7. Total dissolved solids (TDS)
8. Total suspended solids (TSS)
9. Total Kjeldahl nitrogen (TKN)
10. Ammoniacal nitrogen (AN)
11. Phosphate
12. Volatile fatty acids (VFA)
13. Sulphide
14. Nitrate
15. Free ammonia
16. Oil and grease
17. Chloride

**Procedure**

**Determination of chemical oxygen demand**

The chemical oxygen demand (COD) is a measure of oxygen equivalent of that portion of organic matter in a sample that is susceptible to oxidation by
a strong chemical oxidant. Most types of organic matter are destroyed by boiling the mixture of chromic acid and sulphuric acid. The sample was refluxed with a known amount of potassium dichromate and sulphuric acid along with silver sulphate as catalyst, and the excess dichromate was titrated with ferrous ammonium sulphate using ferroin indicator to get a sharp end point. The straight-chain compounds were oxidized more effectively when silver sulphate was used as catalyst. Mercuric sulphate was added to the samples before refluxing to mask chlorides. The amount of the organic matter, measured as oxygen equivalent, was proportional to the potassium dichromate consumed. A blank (without sample) with distilled water and all other reagents was refluxed in the same manner. The result was calculated from the equation:

\[
\text{COD (mg/L)} = \frac{(a - b)N \times 8000}{v}
\]

where 
- \( a \) = volume in mL of ferrous ammonium sulphate used for blank.
- \( b \) = volume in mL of ferrous ammonium sulphate used for sample.
- \( N \) = normality of ferrous ammonium sulphate, and
- \( v \) = volume in mL of the sample taken for the test

**Determination of biochemical oxygen demand**

The biochemical oxygen demand (BOD) determination is an empirical test in which standardised laboratory procedures are used to determine the relative oxygen requirements for polluted waters.

Amount of oxygen demand in the sample will depend on the degree of dilution. It provides a measure of the dissolved oxygen consumed by the aerobic microbiological oxidation of the sample under defined conditions.
over a specific period. Normally BOD was determined by measuring the loss in dissolved oxygen of the sample by incubating it for five days at 20°C and has been accepted as standard.

The dissolved oxygen oxidises the manganous hydroxide to manganic hydroxide, which in turn oxidises iodide to free iodine in the acid medium. The iodine thus liberated is determined by titrating with standard sodium thiosulphate using starch as indicator. BOD (5 days at 20°C) mg/L = \( \frac{(D_1 - D_2)}{p} \)

where \( D_1 = \) Initial dissolved oxygen content (mg/L)
\( D_2 = \) Dissolved oxygen content after incubation.
\( p = \) decimal fraction of the sample used

**Determination of total solids.**

Total solids (TS) are the materials left in the vessel after evaporation of a sample and its subsequent drying in an oven at defined temperature. Total solids include non filterable solids and filterable solids.

A well mixed sample was evaporated in a weighed dish and dried to constant weight in an oven at 103°C to 105°C. The increase in weight over that of the empty dish represents the total solids.

Total solids (mg/L) = \( \frac{(A - B) \times 1000}{v} \)

where \( A = \) weight in mg of the residue and dish
\( B = \) weight in mg of the dish, and
\( v = \) volume in mL of the sample taken for the test.
Determination of total dissolved solids

Total dissolved solids (TDS) is the material that passes through a standard glass fiber filter and remains after evaporation, and drying to constant weight at 103°C to 105°C.

A well mixed sample was filtered through a standard fiber filter and the filtrate was evaporated to dryness in a weighed dish to constant weight at 103°C to 105°C. The increase in dish weight represents the total dissolved solids.

\[
\text{Dissolved solids (mg/L)} = \frac{(A - B) \times 1000}{v}
\]

where \( A \) = weight in mg of dish with dried residue

\( B \) = weight in mg of the dish, and

\( v \) = volume in mL of sample taken for the test.

Determination of total suspended solids.

The total suspended solids (TSS) are the retained materials on a glass filter after filtration of a well mixed sample. The well mixed sample was filtered through a weighed standard glass fiber filter and the residue retained on the filter was dried to a constant weight at 103°C to 105°C. The increase in weight of the filter represents the total suspended solids.

Total suspended solids (mg/L) = \( \frac{(A - B) \times 1000}{v} \)

where \( A \) = weight in mg of the filter with dried residue

\( B \) = weight in mg of the filter, and

\( v \) = volume in mL of the sample taken for test.
Determination of sulphides
A known quantity of well mixed sample was titrated with an excess amount of acidified iodine solution and the amount of untreated iodine was measured by titrating with a standard thiosulphate solution using starch indicator. A blank (without sample) with distilled water and all other reagents including same quantity of iodine is also to be conducted.

\[
\text{Sulphides (as } \text{H}_2\text{S}) \text{ (mg/L)} = \frac{17000(A - B) \times N}{v}
\]
where 
- \(A\) = volume in mL of standard sodium thiosulphate for blank.
- \(B\) = volume in mL of standard sodium thiosulphate for sample.
- \(N\) = Normality of thiosulphate solution, and
- \(v\) = volume in mL of the sample taken for test.

Determination of phosphates
In a dilute orthophosphate solution, ammonium molybdate reacted under acid conditions to form molybdophosphoric acid. In the presence of vanadium the vanadomolybdophosphoric acid yellow colour was formed. The intensity of the colour was proportional to the phosphate concentration in the solution. The developed colour was measured using spectrophotometer at 470 nm. A blank was conducted with distilled water and all the reagents.

The calibration curve was prepared using standard phosphate solution. Phosphates (as P), (mg/L) = \(\frac{(w \times 1000)}{v}\)

where 
- \(w\) = weight in mg of phosphates (P) as read from the calibration curve, and
- \(v\) = volume in mL of the sample taken for test.
Determination of total Kjeldahl nitrogen

The total kjeldahl nitrogen (TKN) is a measure of organic nitrogen and ammonia.

In the presence of sulphuric acid, potassium sulphate and mercuric sulphate catalyst, amino nitrogen of many organic materials were converted to ammonium sulphate \((\text{NH}_4)_2\text{SO}_4\). Free ammonia and ammonium-nitrogen also were converted to \((\text{NH}_4)_2\text{SO}_4\). During digestion mercury ammonium complex was formed and then decomposed by sodium thiosulphate. After decomposition the ammonia was distilled from an alkaline medium and absorbed in boric acid. The amount of ammonia was determined by titrating with standard mineral acid using methyl red-methylene blue mixed indicator for a sharp end point. A blank (without sample) with all the reagents was also conducted.

Total Kjeldahl nitrogen (as N) mg/L = \(\frac{(A - B) \times N \times 14000}{v}\)

where \(A\) = volume in mL of the acid used for sample.
\(B\) = volume in mL of the acid used for blank,
\(N\) = normality of the acid, and
\(v\) = volume in mL of the sample taken for test

Determination of ammoniacal nitrogen

A well mixed sample was buffered at pH 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds and distilled into solution of boric acid. The ammonia in distilled water was measured by titrating with standard mineral acid using methyl red - methylene blue mixed indicator. A blank (without sample) was also conducted with all the reagents and distilled water
Ammoniacal nitrogen (as N), mg/L = \( \frac{14000}{A - B} \times N \times 14000 \)

where \( A = \) volume in mL of standard sulphuric acid used for sample.
\( B = \) volume in mL of standard sulphuric acid used for blank.
\( N = \) normality of the sulphuric acid, and
\( v = \) volume in mL of the sample taken for the test.

Determination of free ammonia

On heating the free ammonia was released and the same can be got absorbed in boric acid. It was measured by titrating with standard mineral acid using methyl red - methylene blue indicator.

Free ammonia (as N), mg/L = \( \frac{14000}{A - B} \times N \times 14000 \)

where \( A = \) volume in mL of standard acid used for titration.
\( B = \) volume in mL of standard sulphuric acid used for blank.
\( N = \) normality of the acid, and
\( v = \) volume in mL of the sample taken for the test.

Determination of nitrate nitrogen

The effluent sample was treated with sodium salicylate in an acid medium, the mixture was made alkaline and the yellow colour obtained was measured using a spectrophotometer at 420 nm. A blank (without sample) with all the reagents and distilled water was also conducted.

A calibration curve is determined with standard nitrate solution.

Nitrate nitrogen (as N) mg/L = \( \frac{w}{v} \)

\( w = \) weight in mg of nitrogen (as N) as read from the curve, and
\( v = \) volume in mL of the sample taken for test.
Turbidity was measured using digital Nephelo Turbidity Meter (Range1-1000, No.24), pH was determined using digital pH meter 335-systronics and phosphate by using Geneys 20 spectrophotometer (Thermo Electron corporation, Thermospectronic).

Heavy metals were estimated using Atomic Absorption Spectrophotometer (AAS, Model-Avanta., GBC.) at Rubber Research Institute of India (RRII), Kottayam.

2.2. C. Biochemical analysis

(1) Estimation of soluble protein
Protein was estimated by Lowry’s method$^4$

In this method folin ciocalteus phenol reagent is reduced by the copper treated protein giving a blue colour. The colour yield of this reaction is considered to arise mainly from the tyrosine, tryptophan and phenyl alanine and to some extent from the sequence of certain amino acids bearing functional side group such as arginine, glutamic acid and histidine. The method is sensitive and there is negligible interference from non-protein substance.

Reagents.

A: - 2% Na$_2$CO$_3$ in 0.1N NaOH;

B: - 0.5% CuSO$_4$ in 1% Rochelle salt (sodium potassium tartarate)

C:- Alkaline copper reagent was prepared on the day of use by mixing 50 mL A + 1mL B

D:- Folins’ reagent: - 1: 2 diluted
E:- 10 % Trichloro acetic acid (TCA)

BSA: Albumin standard- 10 mg Bovine serum albumin in 20 mL of 0.1N. NaOH

Procedure: 0.2, 0.5 mL aliquots were taken from the sample. An equal volume of 10% TCA was added and kept over night in refrigerator. The tubes were centrifuged at 5000 rpm for 30 minutes, decanted and the precipitate was dissolved in 1 mL of 0.1 N NaOH. 5 mL of reagent C was added to the above solution and kept for 10 minutes. Then 0.5 mL diluted Folins’ reagent was added, and the optical density was measured at 660 nm in a UV spectrophotometer after keeping it for 30 minutes. A blank containing 1mL of 0.1N NaOH, 5mL of reagent C and 0.5 mL diluted Folins’ reagent was also prepared along with the sample. BSA was used as standard.

(2) Estimation of total sugars

This method was suggested by Scott and Melvin

Reagents:

1. 0.1 g Anthrone was dissolved in a mixture of 29 mL water and 100 mL concentrated \( \text{H}_2\text{SO}_4 \) under ice cold conditions and kept in ice bath.

2. Sucrose (standard)

Procedure: To 0.1 mL sample, 0.4 mL 2.5% TCA was added and kept the tubes in ice bath and then added 3 mL cold anthrone reagent. Standards were also treated in the same way. It was heated in boiling water bath for
15 to 20 minutes and cooled in an ice bath. The absorbance was measured at 627 nm in a UV spectrophotometer. Sucrose was used as standard.

(3) Estimation of reducing sugars

Reducing sugar was estimated according to the procedure suggested by Nelson

Reagents:

Alkaline copper reagent: - copper reagent A and B in the ratio 25: 1 by volume

Copper reagent A: - 25 g Na₂CO₃, 25 g sodium potassium tartarate, 20 g NaHCO₃ and 200 g Na₂SO₄ were accurately weighed and dissolved in 800 mL water and made up the volume to 1000 mL.

Copper reagent B: - 15% CuSO₄ in water containing 1 or 2 drops of conc. H₂SO₄. Copper reagent was prepared on the day of use by mixing reagent A and reagent B in the proportion 25:1 by volume

Arsinomolybdate: - 25 g ammonium molybdate in 450 mL water was prepared. Added 21 mL conc.H₂SO₄ and mixed well. Added 3 g sodium arsenate dissolved in 25 mL water and added to the above solution. The solution was mixed well, filtered and kept at 37°C. Glucose was used as standard.

Procedure: - Pipetted out 0.5 mL aliquot into a test tube and evaporated it completely. 0.5mL distilled water and 1mL cupper reagent (freshly prepared 25 mL A and 1mL B) were added to this and heated on boiling water bath for 20 minutes. It was cooled and added 1mL arsenomolybdate and kept for 15 minutes to develop colour and then made up the volume to 12.5 mL. Optical density was measured at 520 nm in a UV spectrophotometer. A blank with
1mL of distilled water and all the reagents were also prepared along with the sample. Concentration of non-reducing sugar was estimated from the difference between total sugar and reducing sugar.

(4) **Estimation of amino acids**

Amino acids were estimated according the procedure described by Moore and Stein.

Ninhydrine method: - When amino acids are heated with ninhydrine, they are quantitatively deammoniated and a blue colour appears. The blue coloured substance (Ruheman’s purple) is formed by the reaction of some of the ninhydrine with its reduction product hydrin and ammonia. Optical density was measured at 570 nm in a UV spectrophotometer. Leucine was taken as the standard.

Reagents

1. 0.2 g of reagent grade SnCl$_2$.2H$_2$O was dissolved in 125 mL citrate buffer. Added this solution to 5 g of ninhydrine dissolved in 125 mL of methyl cellosolve.

2. 0.2 M. citrate buffer - pH 5. 10.507 g of reagent grade citric acid monohydrate was dissolved in 100 mL of 1N NaOH and diluted to 250 mL.

3. Diluent solvent. It was prepared by mixing equal volumes of water and reagent grade n-propanol.

Procedure: - Added 1 mL of ninhydrine reagent to 0.5 mL sample and standard solutions. Mixed well and heated for 20 minutes in boiling water bath. 5 mL of diluent was added to each tubes and the content were mixed and optical density was measured at 570 nm after 15 minutes. A blank was
also prepared along with the sample. The colour is stable for at least one hour.

(5) **Estimation of phenol**

Phenol was estimated according the procedure described by Swain and Hills.\(^8\)

Reagents: 1N Folins’ reagent and saturated Na\(_2\)CO\(_3\).

Procedure: - To 0.5 mL sample 0.5 mL distilled water was added to make the volume to 1 mL. To this solution 0.5 mL 1N Folins’ reagent and 1 mL saturated Na\(_2\)CO\(_3\) were added, made the volume to 5 mL with distilled water. The mixture was incubated for 1 hour and optical density was measured at 725 nm in a UV spectrophotometer. A blank was also prepared along with the sample. Catechol was used as standard.

The Spectrophotometer used for measuring optical density is UV–visible Recording spectrophotometer, UV-240.

(6) **Total lipids**

Total lipids were extracted according the procedure described by Bligh and Dyer.\(^9\) 100 mL of the effluent sample was concentrated by evaporation on a water bath at 100\(^0\)C. To this 25 mL of methanol was added and kept over night. Then 50 mL of chloroform was added and shaken well. It was then filtered to a stoppered conical flask and washed with chloroform and methanol (2:1, 2 mL each) at least three times. To the filtrate distilled water was added (20\% of total volume) and allowed to stand overnight at 4\(^0\)C for phase separation. The aqueous layer was removed with the help of a Pasteur pipette. Bottom layer was evaporated to reduce the volume, transferred it to a previously weighed beaker using 5mL of solvent and
evaporated to dryness. The beaker was kept in a vacuum dessicator over KOH until constant weight was obtained. The difference between the two weights was taken as the weight of total lipids present. The process was duplicated.

2.2. D. Microbiological analysis
The population of total bacteria in the raw effluent was enumerated using appropriate media. The standard serial dilution plate technique of Pramer and Schmidt\textsuperscript{10} was employed for the enumeration of microbiological population. The sample of wastewater to be tested was diluted serially. A small amount of each dilution (1mL) was then mixed with warm agar liquid culture medium poured into a culture dish, allowed to solidify, and incubated under controlled conditions. The separate distinct bacterial colonies formed on the plates after incubation was counted, and the results reported as colony-forming units (cfu) per unit volume of the sample. The total number of bacteria was determined using appropriate dilutions\textsuperscript{11}.

The medium was prepared as follows:- peptone - 5 g, glucose - 5g, NaCl - 5g, beef extract - 3g, agar - 15g, water - 1 litre, pH - 6.8. All these compounds were accurately weighed and dissolved in one litre of water. It was then transferred to (250 mL each) 4 conical flasks, corked and kept in autoclave for 1 hour.

2.3. Results and discussion
2.3. A. Physico-chemical characteristics
Table 2.1 shows the values of physico-chemical characteristics of skim serum effluent collected at various intervals of time and their average values. It is apparent from Table 2.1 that the values in most cases vary widely. The average of these values is shown in column (8).
Table 2.1 Physico-chemical characteristics of skim serum effluent from latex centrifuging units

<table>
<thead>
<tr>
<th>No</th>
<th>Parameters</th>
<th>Effluent 1</th>
<th>Effluent 2</th>
<th>Effluent 3</th>
<th>Effluent 4</th>
<th>Effluent 5</th>
<th>Average values</th>
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</thead>
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<td>(4)</td>
<td>(5)</td>
<td>(6)</td>
<td>(7)</td>
<td>(8)</td>
</tr>
<tr>
<td>1</td>
<td>pH</td>
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<td>4.3</td>
<td>4.7</td>
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<td>Turbidity (NTU)</td>
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<td>1520</td>
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<td>25.00</td>
<td>25.00</td>
<td>14.00</td>
<td>23</td>
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<tr>
<td>15</td>
<td>Nitrate</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
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<td>Trace</td>
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<td>56</td>
<td>69</td>
<td>60</td>
<td>Trace</td>
<td>60.125</td>
</tr>
</tbody>
</table>

(All values except pH and turbidity are expressed in mg/L)
The general trend observed for various physico-chemical parameters of skim serum effluent are discussed below.

(a) **pH**

The pH values of skim serum effluent varied between 3.6 and 4.7 with an average value of 4.11. This shows that serum effluent is highly acidic and the high acidity of the effluent can be attributed to the use of sulphuric acid for the coagulation of skim latex.

(b) **Organic matter**

Organic compounds are normally made up of a combination of carbon, hydrogen, oxygen and nitrogen. COD is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidised chemically using dichromate in acid solution. BOD determination involves the measurement of dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter.

The organic matter in serum effluent was estimated in terms of COD and BOD. The COD and BOD values of serum effluent were in the range of 27000-38800 mg/L and 10500-23280 mg/L respectively. The average values of COD and BOD were 31603 and 16756 respectively. These figures indicated the presence of high concentration of organic compounds in the serum effluent. The high pollutant effect of serum effluent was mainly due to its high organic nature. The relationship between BOD and COD is shown in the Table 2.2. Typical values for the ratio BOD/COD for untreated municipal wastewater are in the range from 0.3 to 0.8. If the BOD/COD ratio for untreated wastewater is 0.5 or greater, the waste is considered to be easily treatable by biological process. If the
ratio is below 0.3 the waste may have some toxic components in it and acclimated microorganism may be required for its stabilization. For the final effluent the ratio should be 0.1 to 0.3\textsuperscript{11}.

**Table 2.2. Relationship between BOD and COD**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOD/COD</td>
<td>0.38</td>
<td>0.49</td>
<td>0.51</td>
<td>0.6</td>
<td>0.6</td>
<td>0.516</td>
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</tbody>
</table>

The average value of BOD/COD ratio of the serum effluent is 0.516 which shows that major pollutant is organic in nature and could be removed by biological methods.

(c) **Solids**
The solid content of the wastewater is expressed in terms of turbidity, total solids, dissolved solids, total suspended solids and volatile suspended solids.

Measurement of turbidity is based on the comparison of the intensity of light scattered by a sample to the light scattered by a reference suspension under the same conditions. The results of turbidity are reported as nephelometric turbidity (NTU) \textsuperscript{11}. The turbidity values of the serum effluent varied between 140 and 170 NTU with an average value of 156 NTU. The turbidity value shows that serum effluent does not contain too much colloidal or residual suspended matter.

TS is obtained by evaporating a sample of wastewater to dryness at 100-105\textdegree C and measuring the mass of residue. Serum effluent contains 59000-70000 mg/L of TS with an average value of 63400 mg/L. TDS by definition, is the solids contained in the filtrate that passes through a filter.
with a nominal pore size of 2.0 µm or less. The TDS content in serum effluent was in the range of 56000-68000 mg/L having an average value of 60600 mg/L. This shows that the serum effluent from the latex concentration unit contains high concentration of dissolved solids and the major fraction of total solids are in the dissolved form which may vary from 92.8 to 98.5 per cent of the total solids showing an average value of 95.5 percent. The high concentration of TDS indicates the presence of high amount of organic matter in the wastewater. TSS is the portion of TS retained on a filter with a specified pore size, measured after drying at a specified temperature of 105°C. It is somewhat arbitrary because it depends on the pore size of the filter paper used for the test. TSS of serum effluent was in the range of 1500-5000 mg/L and the average value was 2880 mg/L. Volatile suspended solids (VSS) are those solids that can be volatilized and burnt off when the TSS are ignited at 500 to 550°C and are considered as organic matter. VSS of serum effluent was in the range of 1470-4170 mg/L and the average value was 2745 mg/L. The major fraction of total suspended solid is considered as organic matter since 95.3 % of total suspended solid is volatile in nature.

(d) Nitrogen

TKN content of serum effluent varied between 7000 mg/L and 11000 mg/L with an average value of 8000 mg/L whereas the AN content varied between 2500 and 5000 mg/L with an average value of 3900 mg/L. At pH levels below 7, ammonium ion is the predominant species whereas at pH above 7, the predominant species is free ammonia. Therefore, ammonia is determined by raising the pH and distilling off the ammonia into a solution of boric acid and estimated titrimetrically with standard sulphuric acid.
The TKN includes ammonia and organic nitrogen but does not include nitrite and nitrate nitrogen. Hence, the TKN content monitored in the samples include the ammoniacal nitrogen and organic nitrogen present in the proteinaceous matter which is a constituent of natural rubber latex. The presence of ammoniacal nitrogen in the effluent is attributed to the fact that the field latex is preserved with ammonia to prevent coagulation and microbial action. From Table 2.1, it is evident that the ammoniacal nitrogen content of the effluents from the skim serum section is very high. The skim which is coagulated with sulphuric acid contains about 0.8 per cent ammonia and this should be the reason for the higher ammoniacal nitrogen content in the skim serum effluent.

(e) **Phosphates**

The usual forms of phosphates that are found in aqueous solutions include orthophosphate, polyphosphate and organic phosphates. Phospholipids in the original latex get hydrolysed into phosphates. Dihydrogen orthophosphate is also added during processing of rubber latex. The total concentration of phosphate in the serum effluent was in the range of 1400 to 4000 mg/L and its average value was 2560 mg/L.

(f) **Volatile fatty acids (VFA)**

Concentration of VFA was in the range of 1200 to 2000 mg/L and the average value is 1520 mg/L. For soluble and easily degradable substrates, such as sugars and soluble starches, the acidogenic reactions can be much faster and may increase the volatile fatty acid and hydrogen concentrations and depress the pH\textsuperscript{11}. 
(g) Sulphates and sulphides

The concentration of sulphate is very high since sulphuric acid is added to coagulate skim latex. Sulphur is also required in the synthesis of proteins and is released during their degradation. Sulphate content of serum varied between 16000-17000 mg/L and with an average value of 16700 mg/L. Sulphate is gradually converted to sulphides in anaerobic conditions which in turn combine with hydrogen to form hydrogen sulphide. Concentration of sulphide in the serum effluent was 14 to 31 mg/L and the average value was 23 mg/L.

(h) Oil and grease

Oil and grease includes fats, oils, waxes and other allied constituents. It was estimated by extracting with petroleum ether. The concentration of oil and grease in serum effluent ranges from trace amount to 24 mg/L. The average value was 16.625 mg/L.

(i) Nitrate and chloride

Concentration level of nitrate was not significant enough to detect. Chloride concentration in the serum effluent varied between trace to 69 mg/L and the average value was 60.125 mg/L. Very small amount of chlorine is associated with natural rubber latex and the rest may originate during processing.

2.3.B. Metallic constituents

Many metals in trace quantities are necessary for the growth of biological life and the absence of sufficient quantities of these could limit biological growth. But higher concentration of these will lead to toxicity. The concentration of metals is high in the effluent compared to the original
serum\textsuperscript{14}. High concentration of these metals may come from the processing of rubber latex since preservatives like TMTD-zinc oxide (Tetramethyl thiuram disulphide) are added in addition to ammonia. Serum effluent contained 7.099 ppm of iron, 0.025 ppm copper, 204.5 ppm zinc, 0.037 ppm manganese, 3.44 ppm calcium and 1.61 ppm magnesium (Table 2.3) when analysed using atomic absorption spectrophotometer (AAS).

Table 2.3 Concentrations of metal ions estimated using AAS

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>7.099</td>
</tr>
<tr>
<td>Cu</td>
<td>0.025</td>
</tr>
<tr>
<td>Zn</td>
<td>204.500</td>
</tr>
<tr>
<td>Mn</td>
<td>0.037</td>
</tr>
<tr>
<td>Ca</td>
<td>3.440</td>
</tr>
<tr>
<td>Mg</td>
<td>1.610</td>
</tr>
</tbody>
</table>

2.3.C. Biochemical constituents

Biochemical analysis of serum effluent showed the presence 865 mg/L of proteins (soluble), 589 mg/L phenol, 1250 mg/L total sugars, 1095 mg/L reducing sugar, 155 mg/L non-reducing sugar, 15952 mg/L free amino acid and 540 mg/L lipids (Table 2.4). The high concentration of proteins, sugars and amino acids contributes to the pollutant effect.
Table 2.4 Biochemical constituents of serum effluent

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration in mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble protein</td>
<td>865</td>
</tr>
<tr>
<td>Phenol</td>
<td>586</td>
</tr>
<tr>
<td>Total sugar</td>
<td>1250</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>1095</td>
</tr>
<tr>
<td>Non-reducing sugar</td>
<td>155</td>
</tr>
<tr>
<td>Free amino acid</td>
<td>15952</td>
</tr>
<tr>
<td>Lipids</td>
<td>540</td>
</tr>
</tbody>
</table>

2.3.D. Bacteriological properties

The total viable bacterial population present in skim serum effluent was determined by pour and spread plate method. This method was used to culture, identify, and enumerate bacteria\textsuperscript{11}. The colony forming units per unit volume (cfu/mL) of the sample is $22 \times 10^4$. The high acidity of the effluent from latex concentrate units adversely affects growth of bacteria and hence the bacterial population is comparatively low\textsuperscript{15}.

2.4. Conclusion

- The skim serum effluent discharged from a latex centrifuging unit is strongly acidic in nature. The average pH value of the effluent is 4.11. The high acidity is due to the use of sulphuric acid in skim latex coagulation.
The average values of COD and BOD are 31603 mg/L and 16756 mg/L respectively. The average value of BOD/COD ratio is 0.516 which shows that the major pollutant in the serum effluent from latex centrifuging unit is organic in nature which could be removed by biological methods.

The turbidity values of the serum effluent (average value of 156 NTU) shows that serum effluent does not contain too much colloidal or residual suspended matter. The average value of TDS content in serum effluent is 60600 mg/L showing that the serum effluent from latex concentration unit contain high amount of dissolved solids. A major fraction of total solids is present in the dissolved form showing an average value of 95.5 per cent of total solids.

The average value of TKN content of serum effluent is 8000 mg/L whereas the AN content is 3900 mg/L. The TKN includes ammonia and organic nitrogen.

Phosphates, VFA, sulphates and sulphides, oil and grease, nitrate and chloride were also estimated. The concentration of sulphates is very high (16700 mg/L) since sulphuric acid is added to coagulate skim latex.

Metallic constituents of serum effluent were also estimated using AAS. It contains 7.099 ppm of iron, 0.025 ppm copper, 204.5 ppm zinc, 0.037 ppm manganese, 3.44 ppm calcium and 1.61 ppm magnesium.

Biochemical analysis of serum effluent shows the presence 865mg/L of soluble protein, 586 mg/L phenol, 1250 mg/L total
sugar, 1095 mg/L reducing sugar, 155 mg/L non-reducing sugar, 15952 mg/L free amino acid and 540 mg/L lipids. High concentration of proteins, sugars and amino acids contributes to its pollutant load.

- The colony forming bacterial units per unit volume (cfu/mL) of the sample is $22 \times 10^4$. The high acidity of the effluent from latex concentrate units adversely affects the growth of bacteria and hence the bacterial population is comparatively low.

References

1. CPCB. Comprehensive Industry Document on Natural Rubber Processing, Central Pollution Control Board, Delhi, (1996).


