2.1. INTRODUCTION

The wastewater composition usually refers to the actual amount of physical, chemical and biological constituents present in the wastewater. The important physical characteristic of an effluent is its total solid content, which is composed of floating matter, suspended matter, colloidal matter and dissolved matter. The other physical parameter such as the color and odour are influenced by age, concentration and chemical nature of the wastewater.

The chemical property of wastewater is characterized by its organic matter and inorganic matter. The organic matter contains proteins, carbohydrates, fats, oils and pesticides. The inorganic parts of wastewater comprises of nitrogen, phosphorous, chloride, sulphate and heavy metals etc.

The biological characteristics of wastewater include microorganisms such as bacteria, fungi, protozoa and macro organisms like vertebrates and invertebrates. In general the presence of colliform bacteria, heterotrophic bacteria, and pathogenic organisms in wastewater provide the basic information regarding the nature of the effluent and its degrading organic matter present in wastewater (Black, 1952).
Discharge of the industrial effluents and sewage into river, stream and lake water alter the physical, chemical and microbial properties of potable water quality. The carbon and nitrogen contents of waste water cause the reversible and irreversible changes in the surface water and ground water quality, through seepage, when discharged into low level areas in streams and land (Apparao et al., 1991). The presence of organic compounds in wastewater, rich in nutrients enhances the growth of the blue green algae, in receiving water bodies (Munwar, 1970). The physico-chemical characteristics of wastewater under seasonal and short term variable conditions are to be regularly analyzed to provide a reliable information for the design of wastewater treatment plants. A good number of reports is available on the nature of the effluents, from paper and pulp mill (Gill and Torr, 1975), sugar mill (Shrivastava and Ranu, 1988), distillery industry (Kothandaram et al., 1974), tannery industry (Bolton and Klain, 1971), palm oil industry (Lee et al., 1981) and sago mill (Subramanian and Sastry, 1988). However, information on physico-chemical properties of sago mill effluent which is similar to the chemical nature of tapioca starch mill effluent (Pescod and Thanh, 1977) is scanty.

Microbial composition of an effluent generally indicates the type of biological treatment required, where the native microbial isolates are used as inoculum (Gowrisankar et al., 1997). Many industrial effluents with rich concentration of various nutrients, organic substrates carry antibiotic resistant microorganisms (Altherr and Kasweck, 1982). Similarly, Murray et al. (1984) have
I studied the presence of microorganisms with resistance to antibiotic or heavy metals in industrial effluents. However, the impact of microorganisms on water quality of an effluents was focussed by Baruah et al. (1993). Hassian et al. (1988) have used a food industrial effluent as a medium for the cultivation of important industrial microorganisms viz., Saccharomyces cerevisiae and Pencillium notatum. Hence, before initiating studies on effluent treatment, it would be appropriate to understand its physical, chemical and microbial status. This information on the native micro-flora would come handy while designing a treatment system, choosing a candidate microbe or microbes for microbial treatment of the effluent as those native flora would be a well adapted one for the target effluent.

In this background, the untreated sizing mill effluent was analyzed for its physio-chemical properties and microbial load. Further, the native heterotrophic bacteria were characterized up to generic level.

2. 2. MATERIALS AND METHODS

2. 2. 1. SAMPLE COLLECTION AND ANALYSIS

M/s Omshakthi sizing mill is situated in Erode with a capacity of 2208 - 4500 ends. The effluent samples were collected from the sizing mill outlet and they were subjected to various analysis. Six sample schedules were carried out over a period of 9 months. Replicates and appropriate controls were maintained in all experiments.
2. 2. 2. PHYSICO-CHEMICAL ANALYSIS OF THE SIZING MILL EFFLUENT

The physico-chemical characteristics of the sizing mill effluent includes BOD, COD, pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total N, Total Sulfate, Phosphate etc., were analyzed adopting the standard and recommended procedures (APHA, 1989). These data were statistically analyzed applying statistical methods viz., ANOVA (one way) and Standard Deviation.

2. 2. 3. MICROBIAL ANALYSIS OF SIZING MILL EFFLUENT

Effluent samples (100ml) was collected in a pre sterilized 300 ml glass bottle with stopper, and it was transported to the laboratory within 24 hrs in an icepack. Before analysis, the samples were brought to room temperature and used.

2. 2. 4. ENUMERATION OF TOTAL HETEROTROPIC BACTERIAL POPULATION

Sizing mill effluents were decimally diluted with sterile saline solution (0.8%). Each sample was diluted serially from $10^1$ - $10^5$. One ml of sample was taken as inoculum and pour plated with sterile nutrient agar medium (Himedia, Mumbai). The replicates and control were incubated at 37°C for 24 hrs - 48 hrs. After incubation the plates were examined for bacterial growth. The number of bacterial colonies were counted and expressed as a Colony Forming Units (CFU/ml).
2. 2. 5. **ISOLATES AND CHARACTERIZATION OF SIZING MILL EFFLUENT NATIVE BACTERIAL ISOLATES**

Morphologically dissimilar colonies from nutrient agar plates were selected and isolated. These colonies were purified using quadrant streaking technique. The isolated pure bacterial colonies were transferred to sterile nutrient agar slants for storage. These pure cultures were identified for bacterial population up to generic level, adopting the methodology of Sumido and Aiso (1962) (Fig. 2. 1).

2. 3. **RESULTS**

Sizing mill effluents were collected from two different sizing capacity of same mill and their physico-chemical properties as described in the methodology. The results are presented in the (Table 2. 1).

The pH of the sizing mill effluent was in acidic range of 4.85 - 5.69, which was lower than the inland standard discharge values of 5.5 - 9.0 and there was no significant difference between, the two samples. The conductivity of the effluent varied from 1347 - 2340 \(\mu\)mhos /cm. The low value was indicative of the presence of low ionic compounds in the effluent. The colloidal matter of the effluent represented the total suspended solids. The TSS values were in the range of 5076 - 6290 mg/l. The dissolved organic substances of the effluent represented the total dissolved solids. The TDS values varied from 13645 - 19350 mg/l. The organic substance of the effluent was measured in terms of COD by chemical oxidation using acidified dichromate solution. The COD values varied from 32750
Fig. 2.1. Scheme for identification of bacterial isolates from environment samples (Simudu and Aiso, 1962)

Gram staining

Positive
- Cocci
  - Micrococcus sp.
  - +ve Motile Corynebacterium sp.

- Small Rods
  - Spore

- Rods

Negative
- Sensitive to penicillin
  - +ve Sensitive
    - Pigmentation
      - Yellow/Orange
      - Flavobacterium sp.
      - Cytophaga sp.

- -ve Resistant
  - Hush & Leifson
    - Fermentative
      - Acid & gas
      - Kovac's oxidase test

- Non-fermentative
  - Pseudomonas sp.
  - Acid & No gas
    - Non luminescent
      - Vibrio sp.
    - Luminescent
      - Photobacterium sp.

- +ve Aeromonas sp.
- -ve Enterobacter sp.
Table 2.1. Physiochemical characteristics of two different sizing mill effluents

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Units</th>
<th>Sample I</th>
<th>Sample II</th>
<th>* I.S.D. values</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>No.</td>
<td>5.69 ± 0.15</td>
<td>4.85 ± 0.4</td>
<td>5.5-9</td>
</tr>
<tr>
<td>Conductivity</td>
<td>μmhos/cm</td>
<td>2340 ± 28.2</td>
<td>1347 ± 52.3</td>
<td>#</td>
</tr>
<tr>
<td>TSS</td>
<td>mg/l</td>
<td>5076 ± 62.2</td>
<td>6290 ± 127.2</td>
<td>100</td>
</tr>
<tr>
<td>TDS</td>
<td>mg/l</td>
<td>13645 ± 332.3</td>
<td>19350 ± 98.9</td>
<td>2100</td>
</tr>
<tr>
<td>Chloride</td>
<td>mg/l</td>
<td>200 ± 28.2</td>
<td>285 ± 7.1</td>
<td>1000</td>
</tr>
<tr>
<td>Sulfate</td>
<td>mg/l</td>
<td>10 ± 1.4</td>
<td>13 ± 3.3</td>
<td>1000</td>
</tr>
<tr>
<td>Oil and grease</td>
<td>mg/l</td>
<td>9 ± 2.1</td>
<td>6.8 ± 0.84</td>
<td>10</td>
</tr>
<tr>
<td>BOD</td>
<td>mg/l</td>
<td>14915 ± 473.7</td>
<td>19955 ± 437.3</td>
<td>30</td>
</tr>
<tr>
<td>COD</td>
<td>mg/l</td>
<td>32750 ± 523.2</td>
<td>42170 ± 240.4</td>
<td>250</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>mg/l</td>
<td>39 ± 4.2</td>
<td>49 ± 4.1</td>
<td>50</td>
</tr>
<tr>
<td>Dissolved phosphate</td>
<td>mg/l</td>
<td>8.7 ± 1.3</td>
<td>11 ± 1.1</td>
<td>10</td>
</tr>
<tr>
<td>Volatile fatty acid</td>
<td>mg/l</td>
<td>256 ± 15.5</td>
<td>318 ± 5.7</td>
<td>#</td>
</tr>
</tbody>
</table>

* Inland Standard discharge values

**Sample I** = Effluent from sizing mill capacity of 2280 (ends).
**Sample II** = Effluent from sizing mill capacity of 4500 (ends).
# = Standard discharge value is not available for the Conductivity parameter and Volatile fatty acid.
The oxidation of organic substances in the effluent were carried out by microorganisms to detect the BOD of the effluent. The BOD values were in the range of 1491 - 19955 mg/l. Both the BOD and COD values were exceptionally high when compared to recommended standards. The source of the nitrogen was limited. The values of the total nitrogen content of the effluent varied between 39 - 49 mg/l. The presence of phosphates was measured in a dissolved condition. The values were in the range of 8.7 - 11.0 mg/l. The sulfate was estimated using gravimetric method. The sulfate content was low in the effluent and the values were in the range of 10 - 13 mg/l. The chloride values varied from 200 - 285 mg/l. The oil and grease contents were estimated using soxhlet distillation method and the values were low (6.8 - 9.0 mg/l). The volatile fatty acid of the effluent was in the range of 250 - 318 mg/l and it was expressed in terms of acetic acid. The sizing mill was enumerated for its total heterotrophic bacterial load. Six cycles of analysis were carried out.

The heterotrophic bacterial load was found to be low and was in the range between 28 and $48 \times 10^4$ CFU/ml. No significant difference was observed between the two sizing mill effluent samples (Table 2. 2). On the basis of morphological, biochemical, and physiological characteristics, bacterial isolates were characterized up to generic level. The bacterial population was restricted to 3 genera, namely *Bacillus* sp., *Pseudomonas* sp., and *Micrococcus* sp. Among these three bacterial genera, *Bacillus* sp., was predominant (65.01%) followed by
Bacterial isolates

1. Bacillus sp. - 65.01%
2. Pseudomonas sp. - 28%
3. Micrococcus sp. - 7%
Table 2. Total heterotrophic bacterial load in sizing mill effluent

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Bacterial load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sample I (x 10^2 CFU / ml)</td>
</tr>
<tr>
<td>1</td>
<td>32.0</td>
</tr>
<tr>
<td>2</td>
<td>28.0</td>
</tr>
<tr>
<td>3</td>
<td>31.0</td>
</tr>
<tr>
<td>4</td>
<td>37.0</td>
</tr>
<tr>
<td>5</td>
<td>40.0</td>
</tr>
<tr>
<td>6</td>
<td>29.0</td>
</tr>
</tbody>
</table>

*Pseudomonas* sp. (28%) and *Micrococcus* sp. (7%) (Fig. 2.2).

2.4. DISCUSSION

Studies on the characteristics of sizing mill effluent revealed that the effluent contains more organic substances indicating the feasibility of biological treatment. The pH of the effluent varied between 4.85 and 5.69. Similar pH range was reported in sago mill effluent (Subramanian *et al.*, 1988) and distillery effluents (Kothandaram *et al.*, 1974). The low pH value of the sizing mill effluent may be due to the presence of short chain organic acids and free carbon dioxide. The conductivity of the sizing mill effluent was low when compared to the total solid content. This indicates the presence of low ionic substances in the effluent.

The total suspended solids of this effluent was high in the range of 5076 - 6290 mg/l. This kind of high suspended particles 410 - 60360 mg/l were reported for the composite effluent of palm oil industry, generated during the digestion process of the palm fruit (Lee *et al.*, 1981). The dissolved solid values of
the sizing mill effluent were also high (13645 - 19350 mg/l), despite low conductivity. The total solid contents observed in tannery effluent by Ganga et al. (1995) ranges between 1513 and 26118 mg/l. In this study the solid content of the sizing mill effluent was ten times higher than the inland discharge standard value, 2100 mg/l laid down by Pollution Control Board. Even though, the sizing mill effluent is heavily loaded with suspended and dissolved solids, in view of their organic nature, they provide an opportunity for biological treatment.

The sizing mill effluent was observed to have phenomenally very high BOD and COD values (Table 2.1). Both the BOD and COD values overshoot the suspended standards by many hundred folds which clearly indicates the absolute necessity for treating this effluent, without which, the ecosystem would be affected. Such alarming high COD values (1,10,000 - 1,3000 mg/l) was reported for the distillery effluent (Lettinga et al., 1980). The COD : BOD ratio of this sizing mill effluent was in the range of 2.18 - 2.48. This ratio was comparable to that of palm industry effluent (2.2 - 2.5), distillery effluent (2.2 - 2.8) and diary farm effluent (2.0 - 2.5). This ratio shows a higher concentration of organic carbon in the effluent which makes it relatively easier to treat biologically.

Further more, the nitrogen content of the effluent (39 - 49 mg/l) is well within the stipulated limits. This optional nitrogen content is beneficial in two ways (i) It provides proportionate nitrogen to the Carbon content of the
effluent for microbes to achieve effective reclamation (ii) As it does not warrant
nitrogen supplement to enhance microbial growth, does not add to the problem
of sludge. Nitrogen content of the effluent is highly unique to that particular
industrial process. This is true as petroleum refinery industry, owing to its higher
hydrocarbon content has very low nitrogen content (3.5 mg/l) (Bhadhuria, 1996).

The phosphate content of the sizing mill effluent (8.7 – 11.0 mg/l) was
more or less similar to the values (4.9 – 12.4 mg/l) of sugar mill effluent. The
chloride content of the effluent was probably contributed due to the water used
for the preparation of the size mixture. Sulfate contents of the sizing mill effluent
was very low (10 – 13 mg/l), and are comparable to that of potato processing
effluent, which has similar chemical characteristics (Laudin et al., 1971). The volatile
fatty acid content of the effluent was high, in the range of 250 – 300 mg/l, which
composed of long and short chain fatty acids, required for the biological treatment.
The volatile fatty acid content was similar to the VFA content of the sago mill
effluent (51 - 815) Subramanian et al. (1988). The oil and grease content of the
sizing mill effluent was low well below the permissible standard.

All the major physico-chemical characteristics of the sizing mill effluent
viz., TSS, TDS, COD, BOD were alarmingly well above the standard discharge
limits except for few inorganic constituents viz., sulfates, chlorates, phosphates.
This clearly indicates the presence of high organic content in the sizing mill effluent.
The statistical analysis, ANOVA (one way) has shown that the $F_{1,11}$ ratio = <1 concerning the variation due to capacity of sizing was insignificant. The tabulated value was greater than the calculated value at 5% level of significance.

Microbial analysis of the sizing mill effluent showed that the heterotrophic acterial populations were very low and restricted to three bacterial genera. Among these, *Bacillus* (65%) was found to predominate followed by *Pseudomonas* and *Micrococcus* (Fig. 2, 2). Earlier Rajannan and Oblisamy (1979) reported, such low bacterial load in paper mill effluent. Gowrisankar *et al.* (1997) has reported the presence of only two bacterial genera (*Psuedomonas* sp., and *Xanthomaonas* sp.,) in a textile mill effluent. The ability of *Bacillus* and *Pseudomonas* to survive in a hostile environment was reported by Gowrisankar *et al.* (1997) in their works with xenobiotic enriched soil ecosystem.

Anuradha and Subburam (1995) have studied the total heterotrophic bacteria in sewage of Coimbatore city and they found the bacterial genera of *Pseudomonas* sp., *Enterobacteria* sp., *Bacillus* sp., *Alcaligenes* sp., *Micrococcus* sp., and *Corynbacterium* sp., *Pseudomonas* sp., and *Bacillus* sp., were predominant among bacterial population. Of all the bacterial genera identified from above studies, it clearly indicated that the *Bacillus* sp. and *Pseudomonas* sp. were the most resistant and metabolically versatile bacterial species. Sasikala *et al.* (1995)
have reported the presence of purple non-sulfur phototropic bacteria in the industrial wastewater. Similarly Kulkarni and Dhawadkar (1997) reported the presence of thermotolerant bacteria in industrial sewer line.

In the present microbial study, the low bacterial density may be due to the toxic nature of the effluent, and lack of nutrient which is clear on the basis of physico-chemical parameters.

In short, this study reveals the highly toxic nature of the effluent which is evident from BOD, COD, TSS and TDS values. Owing to such toxic nature the heterotrophic bacterial load was found to be very low. Nevertheless, several *Bacillus*, *Pseudomonas*, *Micrococcus* are indeed encouraging and are some of the most metabolically versatile organisms and hence provide an opportunity for effective biological treatment of the sizing mill effluent. In view of their toxic nature and higher organic contents, appropriate biological treatment needs to be designed with the native *Bacillus*, *Pseudomonas*, *Micrococcus* isolates.