APPENDIX A: Sugar industry manufacturing, waste generation, disposal and vermi-composting practices.

APPENDIX A1: Present disposal of solid and liquid wastes.

Plate A1: Open dumping of furnace ash.

Plate A2: Open dumping of furnace ash.
Plate A3: Open dumping of filter mud.

Plate A4: Discharge of combined effluents.
### APPENDIX A2: Vermi-processing in industrial units

#### Table A1: List of on going vermi processing of industrial wastes:

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Type of industry</th>
<th>Waste/generation rate/state</th>
<th>Earthworm inoculums</th>
<th>References/Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tanning</td>
<td>Salt, hair, likme, fleshmyrob dust, buffing dust and trimmed waste.</td>
<td><em>Perionyx exavatus</em></td>
<td>Shahul Hameed et al. (2000)</td>
</tr>
<tr>
<td>2</td>
<td>Pepsi Foods Ltd., Channo, Punjab</td>
<td>Potato Peels</td>
<td><em>Pheritima elongata</em></td>
<td>Arora, 1998</td>
</tr>
<tr>
<td>3</td>
<td>Doaba Co-operative Sugar Punjab</td>
<td>Press mud</td>
<td><em>Pheritima elongata</em></td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>4</td>
<td>Indian Aluminium Company Belgaum</td>
<td>Canteen solid waste</td>
<td>*NA</td>
<td>White, 1996</td>
</tr>
<tr>
<td>5</td>
<td>Citric India Ltd</td>
<td>Citric acid effluent</td>
<td>*NA</td>
<td>White, 1996</td>
</tr>
<tr>
<td>6</td>
<td>DWM bauxite mines</td>
<td>Waste land development</td>
<td>*NA</td>
<td>Jamble, 1996</td>
</tr>
<tr>
<td>7</td>
<td>FDC, Ltd., Roha</td>
<td>Soya Residue 3 tons/day</td>
<td>*NA</td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>8</td>
<td>Marmgoa Potr Trust</td>
<td>Canteen waste</td>
<td>*Eisenia fetida</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Hindustan Motors Ltd., Indore</td>
<td>NA</td>
<td></td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>10</td>
<td>Hindustan Lever Ltd., Zahura Ltd</td>
<td>Tomato skin seed</td>
<td><em>Pheritima elongata</em></td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>11</td>
<td>MICO Bangalore</td>
<td>*NA</td>
<td>*NA</td>
<td>Nayak and Rath, 1996</td>
</tr>
<tr>
<td>12</td>
<td>HMT Bangalore</td>
<td>*NA</td>
<td>*NA</td>
<td>Nayak and Rath, 1996</td>
</tr>
<tr>
<td>13</td>
<td>Military Dairy Farm Bangalore</td>
<td>*NA</td>
<td>*NA</td>
<td>Nayak and Rath, 1996</td>
</tr>
<tr>
<td>14</td>
<td>Orient vegetexp Ltd., Nasik</td>
<td>Onion residue</td>
<td>*NA</td>
<td>White, 1996</td>
</tr>
<tr>
<td>15</td>
<td>Sakthi Sugar Ltd.,</td>
<td>Bio methanisation of distillery spent wash</td>
<td>*NA</td>
<td>Singh, 1996</td>
</tr>
<tr>
<td>16</td>
<td>Sesa Goa Ltd.,</td>
<td>Canteen waste/ Land reclamation</td>
<td>*Eisenia fetida</td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>17</td>
<td>Venkateshwar Hatcheries Pune</td>
<td>Poultry residue 4 tons/day</td>
<td>*NA</td>
<td>White, 1996</td>
</tr>
<tr>
<td>18</td>
<td>VST Industries Hyderabad</td>
<td>*NA</td>
<td>*NA</td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>19</td>
<td>Thermax Ltd., Pune</td>
<td>Canteen Waste</td>
<td></td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>20</td>
<td>Spice Board a</td>
<td>*NA</td>
<td>*NA</td>
<td>Nayak and Rath, 1996</td>
</tr>
<tr>
<td>No.</td>
<td>Company/Plant</td>
<td>Product/Resource</td>
<td>Organism/Reference</td>
<td>Year</td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------</td>
<td>-----------------------------------</td>
<td>---------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>21</td>
<td>Central Govt. Org., Rath</td>
<td>Vermi filter for utilization of distillery spent wash</td>
<td>*NA</td>
<td>White, 1996</td>
</tr>
<tr>
<td>22</td>
<td>Jagatjit Industries, Punjab</td>
<td>Distillery Sludge</td>
<td><em>Pheritima elongata</em></td>
<td>Singh, 1997</td>
</tr>
<tr>
<td>23</td>
<td>Electronic Division Nanjangud</td>
<td>Processing Canteen Waste</td>
<td>*NA</td>
<td>Jamble and Mannivannan,1996</td>
</tr>
<tr>
<td>24</td>
<td>M P Glychem Industries Ltd., Indore</td>
<td>Soya Residue</td>
<td>*NA</td>
<td>Singh, 1996</td>
</tr>
<tr>
<td>25</td>
<td>Paper mills Shimoga Karnataka</td>
<td>Punjab, Ropar</td>
<td>*NA</td>
<td>Sudhir Ghatnekar, 1999</td>
</tr>
<tr>
<td>26</td>
<td>Soyabean Oil Extraction Plants</td>
<td>Soya Residue</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>27</td>
<td>Naptha Based Organo Chemicals</td>
<td>*NA</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>28</td>
<td>Dairy and Chese Manufacturing</td>
<td>Dairy waste</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>29</td>
<td>Food and vegetable processing industries</td>
<td>Nasik Maharashtra, Valsad Gujarat</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>30</td>
<td>Slaughter Huse</td>
<td>Slaughter waste</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>31</td>
<td>Coir processing waste, Chiplun</td>
<td>Tenkasi Tamilnadu</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>32</td>
<td>Dye industry, Chiplun</td>
<td>Maharashtra</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>33</td>
<td>Oil Refinery Trivendrum</td>
<td>Kerals</td>
<td>*NA</td>
<td>Sudhir Ghatnekar,1999</td>
</tr>
<tr>
<td>34</td>
<td>Paper /board mill sludge</td>
<td>Board mill sludge Finland</td>
<td>*Lumbricus terristris</td>
<td>Kevian, (2005)</td>
</tr>
</tbody>
</table>

*NA: Details not available
Appendix A3: Organic waste producing industries

Table A2: List of Important Organic Waste Producing Industries with United Nations Classification Codes.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Type of industry</th>
<th>UN classification codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Slaughter houses</td>
<td>3111a</td>
</tr>
<tr>
<td>2.</td>
<td>Packing houses</td>
<td>3111b</td>
</tr>
<tr>
<td>3.</td>
<td>Poultry processing</td>
<td>3111c</td>
</tr>
<tr>
<td>4.</td>
<td>Dairy products</td>
<td>3112</td>
</tr>
<tr>
<td>5.</td>
<td>Canning of fruits and vegetables</td>
<td>3113</td>
</tr>
<tr>
<td>6.</td>
<td>Canning of fish</td>
<td>3114</td>
</tr>
<tr>
<td>7.</td>
<td>Olive oil extraction</td>
<td>3115a</td>
</tr>
<tr>
<td>8.</td>
<td>Vegetable oil refining</td>
<td>3115b</td>
</tr>
<tr>
<td>9.</td>
<td>Grain mills</td>
<td>3116</td>
</tr>
<tr>
<td>10.</td>
<td>Cane sugar factories</td>
<td>3118a</td>
</tr>
<tr>
<td>11.</td>
<td>Beet sugar manufacturing</td>
<td>3118b</td>
</tr>
<tr>
<td>12.</td>
<td>Starch and sugar manufacturing</td>
<td>3121a</td>
</tr>
<tr>
<td>13.</td>
<td>Yeast manufacturing</td>
<td>3121b</td>
</tr>
</tbody>
</table>

WHO Report, 1992
Appendix A4: Waste minimization opportunities

Table A3: Waste minimization opportunities in various Industrial sectors

<table>
<thead>
<tr>
<th>Industrial Sector</th>
<th>Clean Production</th>
<th>Recycling through EOP Treatment</th>
<th>Waste Utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron And Steel</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Metal Planting</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Non-ferrous Metals</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Pulp and Paper</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Textile</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Tannery</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>B</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Distiller</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Food Processing</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Thermal Power</td>
<td>C</td>
<td>B</td>
<td>A</td>
</tr>
</tbody>
</table>

A: High     B: Moderate     C: Low

Source: Khanna, (1996) ; EOP; End of pipe
Appendix A5: Flow diagram for process of sugar manufacturing

Figure A1: Flow Diagram for Sugar Manufacturing
Appendix A6: Sanjeevani sugar factory Dayanand Nagr Goa:

The sugar industry under consideration is the only one in Goa situated near Tisk Goa on NH 4 A. This has a crushing capacity of 1200 tons per day. Generates about 45 ty⁻¹. The disposes the solid wastes openly on land creating environmental problems of odor, huge dump waste, aesthetics' flow diagram of solid waste generation and combined effluents presented in figure

Figure A2: Solid waste generation and combined effluents

A – Washing of filter cloth, B – Boiler House leakage etc, C – Excess condensed water.
D – Pond Overflow, E – Spill over and handling losses

Source: Munnoli, 2002*
APPENDIX B: Physicochemical characteristics and Geotechnical properties

B1: Determination of moisture Content
The moisture contents of the soil and compost samples were determined by the oven drying method. A known weight $W_1$ of the sample was taken. The sample is kept in the oven for 24 hrs at 100 $^\circ$C and weighs $W_2$. The difference in weight gives the water evaporated (w).

Water Content = \[
\frac{W_1 - W_2}{W_2} \times 100
\]

B2: Determination of Lose bulk density
The waste procured s n taken in a container of known volume. The container filled with waste and the empty containers were weighed.

Weight of the container = $X$ gms
Weight container with waste = $Y$ gms
Volume of the container = $V$ cc

Loose bulk density = \[
\frac{Y - X}{V}
\]

B3: Determination of Total solids
Clean silica crucible was heated at 100 $^\circ$C for one hour in a hot air oven; it was cooled in a desiccator's and weighed. Then weigh about 10 Gms of sample in the silica crucible. It was dried in a hot air hot air oven at 1000C to a constant weight for overnight, cooled in desiccators and weighed again

Weight of empty silica crucible = $A$ gms
Weight of crucible + sample = $B$ gms
Weight of crucible + sample after drying at 100 $^\circ$C = $C$ gms

Moisture (%) = \[
\frac{C - B}{B - A} \times 100
\]
Total Solids = \[
\frac{C - A}{B - A} \times 100
\]

The dried samples were retained for further estimations
B4: Determination of organic carbon

Empty and clean silica crucible is heated at 550 °C for 3 hours in a muffle furnace, and then cooled in a desiccators and weigh. Accurately weigh about 2-4g of sample in a silica crucible. The sample was dried in an oven as described above cooled in desiccators and weighed. The crucible is transferred in to a muffle furnace and the sample is ignited at 550 + 50 0C for 3 hours. There after the same is cooled and weighed.

Weight of empty silica crucible = A gms
Weight of crucible + sample = B gms
Weight of crucible + sample after drying at 103 to 105 0C up to constant weight = C gms
Weight of crucible and ash after ignition = D gms

\[
\frac{C - D}{D - A} \times 100 \quad \text{and Ash Content} = \frac{B - A}{B - A}
\]

Organic Carbon is calculated as:

\[
\text{Organic Carbon (°)} = \frac{\text{Volatile solids}}{\text{Total solids}} \times 50 \%
\]

B5: Determination of pH

About 5g of sample is placed in 100 ml distilled water and shaken vigorously. The sample is allowed to settle down for 1 hour. These solutions were used for determination of pH using a digital pH meter.
**B6: Water content determination**

\[ W_1 = \text{empty wt of container} \]

\[ W_2 = \text{empty wt of container} + \text{Vermi-compost} \]

\[ W_3 = \text{empty wt of container} + \text{Vermi compost} + \text{after oven drying for 24 hrs @ 100 °C} \]

\[ \frac{W_3 - W_2}{W_3 - W_1} \times 100 \text{ Expressed as %} \]

**B7: Determination of nitrogen in press mud sample**

**Principle:**

Nitrogen content of plant is converted into \((\text{NH}_4)_2\text{SO}_4\) by digesting with conc. \(\text{H}_2\text{SO}_4\). The acid digest is distilled for \(\text{NH}_3\) by using 40\% \(\text{NaOH}\). The distilled \(\text{NH}_3\) is trapped in boric acid as mixed indicator solution. The amount of ammonia trapped is estimated by titrating against standard acid.

**Reagents**

1. Conc. \(\text{H}_2\text{SO}_4\)
2. Digestion mixture — \(\text{Na}_2\text{SO}_4\) or \(\text{K}_2\text{SO}_4 : \text{CuSO}_4 : \text{selenium powder in 50:10:1 ratio.}\)
3. 40\% \(\text{NaOH}\)
4. 2\% or 4\% Boric acid
5. 0.01\% \(\text{H}_2\text{SO}_4\)
6. Mixed indicator (Bromeresol green (0.1 g) + Methyl red (0.07 g) in 100 ml of 95\% ethanol.

Transfer 15 ml of mixed indicator to 1 liter of boric acid. Adjust the boric acid + mixed indicator solution to the bluish purple mid-color at pH 4.5 by using dil HCL or \(\text{NaOH}\) (0.1N) to get sharp endpoint. This indicator is pink at pH 4.2, bluish purple
mid-color at pH 4.5 and bluish green as the pH rises to 4.9 or above when ammonia is trapped.

Procedure:
1. Transfer 0.5 g powdered dried plant sample to Kjeldhal flask.
2. Add 10 ml of conc. H₂SO₄ and 0.2 to 0.3 g digestion catalytic mixture (Na₂SO₄ or K₂SO₄ helps in increasing boiling point, Cu₂SO₄ and se-powder act as catalysts in quick conversion of organic-N to inorganic N).
3. Digest on low flame initially for 10-15 minutes until frothing stops, pre-digestion is necessary for oil seed crops or seeds.
4. Then digest at high flame (temp.) for 1-1 1/2 hours till the contents of Kjeldhal flask become clear.
5. Cool the flask and transfer the contents of flask quantitatively to 50 ml volumetric flask and make up the volume by adding distilled water. This dilution may be necessary when micro Kjeldhal method is followed (The capacity of distillation flask is about 20 ml. therefore, the volume of digested material + 40% NaOH should be around 20 ml. Moreover when sample handled is less, the loss of NH₃ is avoided.
6. Pipette out 10 ml of acid digest and transfer to micro Kjeldhal distillation assembly.
7. Add sufficient quantity (about 10-15 ml) of 40% NaOH to make the contents distinctly alkaline.
8. Before adding NaOH, boric acid—mixed indicator solution should be kept ready at the receiving end (Better to use higher concentration (4%) and lower volume about 20 ml boric acid). However, neither the volume nor the strength of boric acid is important.
9. Carry out the distillation even after the color changes from bluish purple to bluish green. Continue for some more time to trap all the NH₃ released (Don't take out immediately after the color change).

Litmus test: Test with red litmus after giving sufficient time after color changes (5-10 min). No change in the color of red litmus indicates complete distillation.
10. After the distillation is over, estimate the quantity of NH₃ distilled (trapped in boric acid) by titrating against 0.01 N- H₂SO₄ till color changes to purple.

Reactions
During distillation
\[ \text{H}_3\text{BO}_3 + \text{NH}_3 \rightarrow \text{NH}_4\text{H}_2\text{BO}_3 \quad \text{NH}_4^+ + \text{H}_2\text{BO}_3 \]
Boric acid (Ammonium borate)
(Color changes to bluish green)
During titration
\[ \text{H}_2\text{BO}_3^- + \text{H} \rightarrow \text{H}_3\text{BO}_3 \]
OR
\[ \text{NH}_4\text{H}_2\text{BO}_3 + \text{H} \text{ (from std. H}_2\text{SO}_4) \rightarrow \text{H}_3\text{BO}_3 + \text{NH}_4^+ \]
Calculations
\[ \% \text{N} = \text{Titre value} \times \text{N H}_2\text{SO}_4 \times 0.014 \times \text{dil. Factor (if any)} \times 100 \]
Wt. of pl. sample (g)

**B8: Determination of Phosphorus in plant Sample**

Principle: Orthophosphate (phosphorus) present in the plant digest when reacts with vanadate and molybdate gives a yellow colored complex i.e. phosphovanadomolybdate in acid solution. The yellow color is due to the substitution of oxyvanadium and oxymolybdenum radicals for oxygen of PO₄ to give a heteropoly compound. The intensity of yellow color is measured calorimetrically at 400-490 nm using spectrophotometer.

Reagents:
   Preparation: Solution A: Dissolve 1.25g ammonium metavanadate in 300ml of boiling water. Cool and add 250ml conc. HNO₃ and again cool to room temperature.
Solution B: Dissolve 25g ammonium molybdate in 400ml of distilled water pour
solution A to Solution B mix well and make up the volume to 1 litre with distilled
water.

2. Phosphorus standard solution
Preparation: Prepare 100ppm of P standard stock solution by dissolving 0.2195g of
pure KH2PO4 in 500ml of distilled water.
Working standards of P: Transfer 0,1,2.5,5,7.5 and 10ml of 100ppm P- stock
solution into separate 50 ml volumetric flasks to get 0,2,5,10,15, and 20 ppm of P-
working standards.
Procedure:
1. Transfer 5 ml aliquot of plant digest (Triacid or diacid digested plant samples)
   into 50 ml volumetric flask.
2. Add 10 ml of vanadomolybdate reagent to samples and to each standards and
   mix thoroughly and make up the volume to 50ml with distilled water.
3. After 30 minutes of color development read the intensity of yellow color on a
   spectrophotometer at 470nm (i.e. between 400 – 490nm )
4. Draw the calibration curve (standard graph) of P standard by plotting the P-
   absorbance against P- concentration.
5. Find out the P- content in plant digest sample by referring to the standard
curve.

Calculations
% in plant sample = Graph ppm * Vol. of dilution made x Vol of PL. digest * 100
                    10^6  Aliquot       Wt. of PL. sample

B9: Determination of potassium:
Principle: In flame photometry, also known as flame emission or flame atomic emission,
the sample in solution is sprayed in to flame to vaporize, atomize, and exite the sample.
The exited atoms of the element of interest emit light at certain discrete wavelengths,
which are characteristic of that element. Light of the wavelength of interest is separated
from remainder of emitted radiations and its intensity is measured. The intensity measurement can be related directly to the concentration of the element of interest usually by comparing with the intensities, of standard or series of standards.

Reagents:
1. Potassium standard 100 ppm of K: Dissolve 0.191 g of KCl in some volume of distilled water and then make up the volume to one liter.

Materials required Flame photometer, pipette, volumetric flask, etc.

Procedure:
Preparation of standard curve:
Take 0, 1,2,3,4 and 5ml of 100-ppm K solution in a separate 50 ml volumetric flasks, make up the volume to 50 ml with distilled water and mix well. After adjusting needle of flame photometer to zero by feeding blank, adjust the needle to 100 by feeding maximum concentrated K solution. Then feed the standards to record the flame photometer readings. Plot flame photometer readings verses concentrations of standards and draw the standard curve.

Sample:
Feed the digested sample solution to the flame photometer and record the reading (if dilution is required one should do the dilution before feeding to the instrument). Compare the unknown sample reading with the standard curve to determine the percentage k in the sample.

Calculations:

\[
\% K = \frac{\text{Graph ppm x Volume of digested sample}}{10^6 \times \text{Weight of sample}}
\]

For diluted solutions:

\[
\% K = \frac{\text{Graph ppm x Volume of digested sample x Volume made up}}{10^6 \times \text{Weight of sample x Aliquot taken for dilution}}
\]
B10: Determination of geotechnical properties.
A steel cylindrical core cutter of size 10 cm height and 2.8 cm internal diameter immersed in the compost for taking known weight of vermi-compost from the culture box to evaluate geotechnical properties.

B10.1 Determination of Specific gravit by density bottle

\[
\begin{align*}
M_1 &= \text{empty wt. of density bottle} \\
M_2 &= \text{empty wt} + \text{Vermi compost} \\
M_3 &= \text{empty wt} + \text{Vermi compost} + \text{Water} \\
M_4 &= \text{empty wt} + \text{Water} \\
G &= \frac{(M_2 - M_1)}{(M_2 - M_4) - (M_3 - M_4)}
\end{align*}
\]

B10.2: Determination of bulk density of vermicompost:
A core cutter of known volume is taken; the core cutter is immersed in a vermi bed slowly until it is completely filled with the vermi-compost. The photograph showing different core cutters for the experimentation is placed in photograph 2.1.

\[
\begin{align*}
V &= \text{Volume of core cutter} \\
W_1 &= \text{empty weight of core cutter} \\
W_2 &= \text{empty weight of core cutter} + \text{vermi compost} \\
W &= \text{Weight of vermi compost in the core cutter} = W_1 - W_2 \\
\text{Bulk density} &= \gamma = \frac{W}{V}
\end{align*}
\]

B10.3 Determination of dry density:
The dry density \(\rho_d\) is determined by the relation

\[
\rho_d = \frac{\gamma}{(1+w)}
\]

Where \(\gamma\) = Bulk density
\(W\) = water content
Core cutters used to draw samples of vermi compost

Plate B1: Core cutters
B10.4 Voids ratio 'e': The voids ratio e is calculated from the following relation if the specific gravity G is known.

\[ e = \left( \frac{G \cdot \gamma_w}{\gamma_d} \right) - 1 \]

Where \( e \) = voids ratio
\( G \) = Specific gravity
\( \gamma_w \) = Density of water
\( \gamma_d \) = Dry density

B10.5 Porosity n is obtained by the relation

\[ n = 1 - \frac{\gamma_d}{[G \cdot \gamma_w]} \] Saturated density:

B10.6 Saturated density is obtained by the relation

\[ \gamma_{sat} = \frac{[G + e] \cdot \gamma_w}{[1 + e]} \]

B10.7 Degree of saturation \( S_r \) is obtained from the relation

\[ S_r = \frac{[W \cdot G]}{e} \]

B10.8: Air content \( a_c = 1 - S_r \)

B10.9: Percentage air voids \( n_a \)

\[ n_a = 1 - \left[ \frac{\gamma_d \cdot (1 + W \cdot G)}{G \cdot \gamma_w} \right] \]
B11 Characterization of soils:
Analysis of soil was done in accordance with IS:2720- Indian Standard Methods of Test for soils.

B11.1 Liquid Limit:
Liquid limit test was conducted by the cone penetration method as per IS:2720 (Part V) - 1985.

B11.2 Plastic Limit:
This test was also carried out as per the procedure outlined in IS 272(Part V) -1985.

B11.3 Particle Size Distribution:
Particle size distribution of the soil was determined in accordance with IS: 2720(part IV) - 1985 Using standard set of IS Sieves, Samples were machine sieved and percentage assign in various sieves was obtained.
Based on the test results, a graph representing particle size distribution prepared on a semi log graph paper.

B11.4 Identification of soil type:
Identification of soil type based on the samples obtained from the industry was carried out as per IS: 1498-1970. At first visual examination of soil was made to determine whether it is highly organic, coarse grained or fine grained. There after type of soil was ascertained by running sieve analysis and plotting grain size distribution curve.
B11.5 Determination of permeability:
The test was performed following the method in accordance with IS Code 2720(Part17) - 1986. Constant head method is performed on the soil samples and coefficient of permeability (K) is calculated as

\[ K = \frac{Q}{At} \frac{Kr}{Vr} \]

\[ K_{27} = \frac{K}{V_{27}} \]

Where

- \( Kr \) = Permeability at temperature \( T \) °C
- \( Q \) = Quantity of water
- \( A \) = Area of specimen
- \( i \) = Hydraulic gradient
- \( t \) = Time
- \( V_{T} \) = Coefficient of viscosity at \( T \) °C
- \( V_{27} \) = Coefficient of viscosity at 27 °C

B11.6 Determination of pH

The test was carried out in accordance with IS Code (Part 2720 )_1987 Using digital pH meter. 30g of soil was mixed with 100 ml of distilled water kept stirred continuously and recorded the pH meter reading after half an hour.

B11.7 Water holding capacity: The three-vermi-composts were subjected to a compaction test to evaluate the optimum water content and dry densities using a standard proctor compaction test. A representative soil sample of the industry 3 kg was taken and added press mud of 200g with 60-70% water content and given a compaction of 25 blows with a standard 2.5 kg hammer. The compaction was carried out in three layers and the excess soil trimmed off. The bulk density and water content are determined each case. The experiment repeated with second time 200g of press mud.
Appendix C: Composition of stains Biochemical Medias

1. Nutrient Agar

Peptone 10 g
Beef Extract 3g
Sodium Chloride 5 g
Distilled water 1000ml
Dissolve the ingredients; adjust the pH to 7.2 to 7.4 with 1N NaOH
Agar 20 g
Digest in boiling water bath for ½ hr Dispense in flasks and sterilize at 121°C, 15lb for 20 min.
For ready made 28%/ liter.

2. Nutrient Broth

Same as Nutrient Agar but without agar

3. Sabaraud’s Agar

Peptone 10 g
Sodium Chloride Traces
Glucose 40% 100ml
D/W 900ml
pH 7.6
agar 29-30 g
Glucose should be sterilized separately and added to the melted sterilized medium before use (40g in 100 ml D/W)

4. Wicker hams Agar (Yeasts)

Peptone 5g
Malt Extract 3g
Yeast Extract 3g
Glucose 100ml
D/W 900ml
pH 5.5
Agar 30g
Glucose 10% sterilize separately
5. Normal saline:
0.85 g in 100 ml distilled water and sterilized

6. Skimmed Milk Agar test (protease)
Inoculate milk agar plates and incubated at R.T for 24-48 h. Then examine the plates for the presence or absence of a clear area around the organism. A clear area around the bacterial growth was indicative of positive proteolysis. Un-inoculated agar plate served as the control.

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>0.1g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.02g</td>
</tr>
<tr>
<td>Agar</td>
<td>20g</td>
</tr>
<tr>
<td>Milk</td>
<td>20ml</td>
</tr>
<tr>
<td>D/W</td>
<td>80ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

7. Carboxyl methyl Cellulose agar test (CMC)
Inoculate CMC agar plates and incubated at R.T for 24-48 h. Then examine the plates for the presence or absence of a clear area around the organism. Then flooded the plates with 0.1 % Congo red for 15 min and then rinsed with 1Nacl for 15 min and poured off excess of stain. A clear area around the bacterial growth was indicative of positive cellulose activity. Un-inoculated agar plate served as

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>0.5 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1g</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>0.02g</td>
</tr>
<tr>
<td>Agar</td>
<td>2g</td>
</tr>
<tr>
<td>CMC</td>
<td>10g</td>
</tr>
<tr>
<td>D/w</td>
<td>90ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Cango red: 1g
D/w 100ml
8. Starch Agar (Amylase test)

Inoculated starch agar plates incubated the plates at R.T. for 24 to 48 h. Then flooded the plates with Grams iodine for 1 min and poured off excess of stain. Clear zone surrounding the organism was a positive test. Un-inoculated agar plate served as the control.

Peptone  
Beef extract  
Agar  
Starch  
D/w  
pH  
Grams iodine solution: 
Iodine  
Potassium Iodide  
Distilled water

9. Chitinase activity

Crude chitin of 10g is taken in a concentrated hydrochloric acid kept at 4°C overnight. This preparation is filtered through a glass wool and centrifuged at 7000 rpm and washed with distilled water.

Inoculated chitin agar plates with fresh cultures incubated at R.T for 24-48 h. Following the incubation the clear zones shown taken as positive.

Peptone  
Beef extract  
Chitin  
P\text{H}  
Agar  
Distilled water
10. Lipase test

Inoculated coconut agar by means of spot inoculation and incubated for 24-48 h at R.T. Following the incubation, observed the plates for presence or absence of growth and a clear zone around the organisms. Un inoculated tubes serves as control.

Peptone 10 g
Sodium chloride 5.0
Coconut oil 2 ml (g)
Agar 10g
Distilled water 1000 ml
pH 7.4
Sterilization 121 °C, 15 minutes.

11. Citrate utilization test

Inoculated Simmon’s citrate agar slants by means of stab and streak inoculation and incubated for 24-48 h at R.T. Following the incubation, observed the slant for presence or absence of growth. Citrate utilizes were indicated by the presence of growth (blue color) on the slants. Un inoculated tubes serves as control.

Ammonium di-hydrogen phosphate 1g
Potassium di-hydrogen phosphate 1g
Sodium chloride 5 g
Magnesium Sulphate 0.2g
Sodium Citrate 5g
Distilled water 1000 ml
agar 10 g
Bromothymolblue(0.2%) 0.2g
pH 7.4
Sterilization 121 °C, 15 minutes.

12. Oxidase test

Test reagent: 1 % aqueous solution of tetra methyl – p- phenylenediamine hydrochloride. Streak the inoculums from slants on what-man filter paper soaked in test reagent. Purple in 5 – 10 seconds indicated positive test result.
13. **Catalase test**

Test reagent: hydrogen peroxide

Place 1 ml of hydrogen peroxide in a test tube and add 1 ml broth culture. Effervescences was observed indicated positive test result.

14. **H₂S production and motility test**

Hydrogen sulphide production was checked in SIM agar tubes by stab inoculating the organism and then incubating at R.T. for 24-48 h. After incubation, it was observed for black insoluble ferrous sulphide for a positive indication of test. Following incubation, motile cultures showed diffused growth whereas non-motile cultures grew only along the line. Uninoculated tubes served as the control.

- Peptone: 5g
- Beef extract: 3g
- Ferrous ammonium sulphate: 0.2g
- Sodium thiosulphate: 0.025g
- Agar: 10g
- Distilled water: 1000ml
- pH: 7.4

Dispense 5ml in tubes and sterilize 20 minutes at 121 °C by autoclaving.

15. **Carbohydrate utilization test**

Inoculated sugar broth containing a carbohydrate source with phenol red indicator. Incubate the tubes at R.T for 24-48 h. Then examine the tubes for change in color and presence or absence of gas bubble. Positive reaction is indicated by change in color (red-yellow) of the indicator and appearance of air bubble in the Durham tube.

- Peptone: 10g
- Sodium chloride: 5g
- Carbohydrate solution 10%: 0.5 ml
  (Sterilized and added separately)
- Distilled water: 900 ml
- pH: 7.3
- Phenol red: 50 ml

Tube the medium (5 ml), put inverted Durham’s tube before sterilization and autoclaved at 120 and 15 lbs for 20 minutes. Phenol red preparation 1 g phenol red + 10 ml 0.1 N NaOH + 20 ml distilled water, gentle heating + 10 ml 0.1 HCl make 500 ml by distilled water.
Inoculated young cultures in the medium dispensed into two tubes. After inoculation, the medium of one tube overlaid with sterile liquid paraffin. Growth and color change of indicator was noted in the two tubes. Strict aerobes grow only in aerobic conditions. Facultative anaerobes grow in both aerobic and anaerobic conditions. The anaerobic organisms grow only in anaerobic conditions. Un inoculated tubes served as the control.

Peptone 2.0 g
Sodium chloride 5.0 g
Dipotassium hydrogen phosphate 0.3 g
bromothymol blue (1 %) 0.01g in water 0.3 ml
Distilled water 1000 ml
pH 7.1
Glucose 10 % ( sterile separately )
Sterilization 121 c, 15 lbs
0.5 ml of glucose solution was added in each tube. Sterilized paraffin was added to study the fermentative pathway of carbohydrate utilization.

17. Indole production test
Indole production was checked in SIM agar tubes by stab inoculating the organism and then incubating at R.T. for 24-48 h. After incubation, 10 drops of Kovac's reagent were added and it was observed for coloration. The red reagent layer was a positive indication of indole production. Un inoculated tubes served as the control.

Peptone 5g
Beef extract 3g
Ferrous ammonium sulphate 0.2g
sodium thiosulphate 0.025g
Agar 10g
Distilled water 1000ml
pH 7.4
Dispense 5ml in tubes and sterilize by autoclaving.
kovac's reagent

Amyl or isoamyl alcohol 150ml
p-dimethyl - amino benzaldehyde 10g
Conc. hydrochloric acid 50ml

18. Gelatin hydrolysis test

Inoculated gelatin deep tubes and incubated at R.T. for 24-48h. Following the growth the tubes were refrigerated for 30 minutes and the medium was observed. Liquid medium after refrigeration was positive. Un inoculated tube served as control.

Nutrient broth 1000ml
Gelatin 150g
pH 7.4

Digest dispense in small suspension tubes and sterilize (5ml) by intermittent sterilization at 100 °C for ½ hr for 3 consecutive days.

Spot inoculated the gelatin agar plates incubated at R.T for 24-48 h. following the incubation flood the plates with gelatin precipitating agent. A clear zone around the colony indicates positive result.

Nutrient agar 100ml
Gelatin 0.4%
Gelatin precipitating agent 15% HgCl₂ in 20% (vol/vol) concentrated HCl

19. Urease test

Inoculated test culture heavily over the entire slope surface of urea agar slant tubes and incubated at R.T. for 24-48h. Following the growth, a positive test indicated by change in color from yellow to purple. Un inoculated tube served as control.

Peptone 1g
Sodium chloride 5g
Potassium dihydrogen phosphate 2g
Phenol red 0.2% 6ml
Glucose 10% 10ml
Distilled water 1000ml
Agar 20g
pH 6.8-6.9

digest/ dispense in 5 ml in tubes sterilize by autoclaving sterilize 10% glucose separately.
20. The activity of a deaminase-phenylalanine deaminase test

Inoculated phenylalanine deaminase deep slant tubes and incubated at R.T. for 24-48h. Following the growth, allow few drops of 10% ferric chloride solution to trickle down the surface of the slant. Green color developed in the fluid in the slope is positive test activity of deaminase. Un inoculated tube served as control.

Medium composition

- Yeast extract: 3g
- dl-phenylalanine: 2g
- l-phenylalanine: 1g
- Disodium hydrogen phosphate: 1g
- Sodium chloride: 5g
- Agar: 15g

Distilled water 1000ml. Adjust the pH to 7.4 distribute in test tubes and sterilize by autoclaving at 121°C for 15 minutes. Allow to solidify in tubes as long slopes.

21. Maltonate utilization

Inoculated Sodium maltonate medium tubes by means of stab inoculation and incubated for 48 h at R.T. Following the incubation, observed the culture positive results are indicated by change in color of the indicator from green to blue. Un inoculated tubes serves as control.

- Yeast extract: 1g
- Ammonium sulphate: 2g
- Dipotassium hydrogen phosphate: 0.6g
- Potassium dihydrogen phosphate: 0.4g
- Sodium chloride: 2g
- Sodium maltonate: 3g
- Bromothymol blue: 0.025g
- Distilled water: 1000ml.
23. Triple sugar iron agar medium composition

Inoculated the slants of TSI agar using a straight needle first stab the butt down to the bottom, then streak the surface of the slant. Incubate at 370 for 18-24 hrs. The Yello butt red slant shows glucose has fermented but not lactose and sucrose. Yellow butt — yellow slant is taken as lactose and/ or sucrose has been fermented. Red butt red slant — neither glucose, lactose or sucrose has been fermented. Bubbles in butt/broken agar/ indicated gas production. Blackening of the butt indicates Hydrogen sulphide production.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3g</td>
</tr>
<tr>
<td>East extracts</td>
<td>3g</td>
</tr>
<tr>
<td>Peptone</td>
<td>15g</td>
</tr>
<tr>
<td>Protease peptone</td>
<td>5g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1g</td>
</tr>
<tr>
<td>Ferrous sulphide</td>
<td>0.2g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5g</td>
</tr>
<tr>
<td>Sodium thiosulfate</td>
<td>0.3g</td>
</tr>
<tr>
<td>Agar</td>
<td>15g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Adjust ph to 7.4 if necessary, sterilize by autoclaving at 121 °c for 15 minutes.

24. Methyl red test

Inoculated MR-VP broth and incubated at room temperature. Then added 5-6 drops of methyl red indicator and observed for the color change. A bright red color was indicative of a positive test. Un inoculated tubes served as control.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5g</td>
</tr>
<tr>
<td>Di potassium hydrogen phosphate</td>
<td>5g</td>
</tr>
<tr>
<td>Glucose</td>
<td>5g</td>
</tr>
<tr>
<td>pH</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Dispense 5ml in tubes sterilize glucose separately put 0.25 ml of 10% glucose in each tube.
25. Voges Proskauer
Inoculated MR-VP broth and incubated at R.T. for 24-48 hrs. Fallowing the incubation added 1 ml of 40% potassium hydroxide and 3 ml of 5% solution of alpha-napthol in absolute ethanol. A pink color was indicative of positive result. Un inoculated tubes served as control.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>5g</td>
</tr>
<tr>
<td>Di-potassium hydrogen phosphate</td>
<td>5g</td>
</tr>
<tr>
<td>Glucose 10% solution</td>
<td>50 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

26. Mortality medium
Inoculated nutrient broth and incubated at R.T for 24-48 hrs. Fallowing incubation Motile cultures showed diffused growth where as non motile cultures grew only along the line.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>Agar</td>
<td>4g</td>
</tr>
</tbody>
</table>

Dispense 5ml in tubes and sterilize.

27. Nitrate reduction test
Inoculated nitrate broth and incubated at R.T for 24-48 hrs. Fallowing incubation, added 5 drops of sulfanilic acid and then 5 drops of alpha- napthylamine. Red coloration indicated a positive test while in negative test, red color observed after addition of 5mg of Zinc. Un inoculated tubes served as control.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>3 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>5 g</td>
</tr>
<tr>
<td>sodium chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>1 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Dispense in tubes (5 ml) of autoclave
28. Grams staining:

Reagents

<table>
<thead>
<tr>
<th>Crystal violet solution:</th>
<th>Crystal violet</th>
<th>5g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
<tr>
<td>Grams iodine solution:</td>
<td>Iodine</td>
<td>1g</td>
</tr>
<tr>
<td></td>
<td>Potassium Iodide</td>
<td>2g</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>300 ml</td>
</tr>
<tr>
<td>Counter stain</td>
<td>Safranin 2.5% (wt/vol) in 95% Vol/Vol ethanol. 10 ml</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1000 ml, Filtered through Whatman filter paper No.1.</td>
</tr>
<tr>
<td>Decolorizing agent</td>
<td>Ethanol 95% vol/vol.</td>
<td></td>
</tr>
</tbody>
</table>

Procedure

1. Prepare a heat fixed smear from an 18-24 hour culture.
2. Stain with crystal violet solution for 1-2 minutes.
3. Wash the smear in gentle and direct stream of tap water for 2 seconds.
4. Rinse with Grams iodine solution and allow the iodine to set for 1 minute.
5. Wash the smear in gentle and indirect stream of tap water for 2 seconds.
6. Blot the smear dry with absorbent paper.
7. Flood with 95% ethanol for 30 seconds with gentle agitation.
8. Counter stain with safranin for 2 minutes.
9. Wash the smear until in gentle and indirect stream of water until no color appears.
10. Blot or air dry the slide.
11. Observe / examine under the microscope.

29 Endo-spore staining (Schaeffer and Fulton's method)

1. Air dry or heat fix the bacterial smear with minimum flaming.
2. Place the slide over a beaker of boiling water with the bacterial film on the upper side.
3. Flood the slide with 5% aqueous solution of malachite green and leave it to act for 1 minute, while the water continues to boil.
4. Wash in cold water.
5. Treat with 0.5% safranin.

Endospores appeared green with cells colored red

Enzyme activities

30: Amylase:

The Amylase activity is determined by using starch agar plates. The microorganisms inoculated aseptically kept for incubation for 24 hrs. Following incubation the plates are flooded with grams iodine. The organism showing clear zones were recorded along with zone diameter.

Grams Iodine

31. Protease:

The protease activity is determined by using skimmed milk agar plates. The organisms inoculated aseptically kept for 24 hr incubation. Following the incubation, the plates observed for clear zone formation and the zone diameter recorded.

32 Cellulase:

The cellulose activity is determined by using carboxyl methylcellulose agar plates; the organisms were inoculated aseptically kept for 24 hrs incubation. The plates were flooded with cango-red and the observation for clear zones and the zone diameter were recorded.

Cango red 1g

Distilled water 100ml
33 Lipase:

The lipase activity is determined by using the coconut oil agar plates. Following the incubation the plates observed for halos, the diameters were recorded.

34 Gelatinase:

The gelatinase activity is determined by using 12.5% gelatin in nutrient agar media. This preparation is autoclaved successively for three consecutive days and then the plates are poured. The organisms are inoculated aseptically, following the incubation for 24 hrs the plates were observed for clear zones by adding gelatin precipitation agent.

i.e. 15.5 Hg Cl₂ in 20% (vol/vol) concentrated HCl

35 : Chitinase:

10 g of Crude chitin is kept in 50 ml of concentrated HCL filtered through glass wool and over night in the refrigerator. This preparation centrifuged at 7000 rpm for 10 minutes. The pallet formed is purified by centrifuging with distilled water. Out of this 1g is taken and added to the 100ml media preparation as detailed in appendix V. Following incubation for 24 hrs the clear zones are recorded for their diameters. (Kannan, 1996; Wilkie, 1998; Plummer, 1988)