CHAPTER 7

UTILIZATION OF SPENT RESIDUE
7.1 Biogas from spent Residue (Mentha arvensis/ Cymbopogon martinii var. motia)

7.1.1 Introduction

As stated earlier world production of essential oil from Mentha arvensis and Cymbopogon martinii var. motia is about 30000 and 150 tonnes/year respectively (Vaze, S. V., 2000). India has taken the credit for production of 13,000 and 100 tonnes/year of these essential oils (Gupta, R., 2000, Singh, A. K., 2000) and maximum amount of the same is exported. It has been estimated about 109,05,500 metric tonnes of biomass is left after extraction of mint oil and Cymbopogon oil per year. The same spent residue is generally used as a source of energy to run the distilleries of boiler. A huge amount of CO\(_2\) and other gases are emitted to atmosphere, while burning essential oil crop. Introduction of such a big quantities of CO\(_2\) to atmosphere has tremendous effects on ozone layer and ultimately responsible for green house effect phenomenon (Samet, J. M. et al., 2000). In order to have control over such problem some alternate route has to be finding out to reduce the load of CO\(_2\) emission during steam distillation.

In the concern, biomethanation of mint and Cymbopogon spent residue, is supported to be a promising technology to meet energy demand in steam distillation /fractional distillation of essential oil.

7.1.2 Possible mechanism of production of bio gas from spent residue

Keeping the aforesaid factor in view the present set of experiments were designed to find out suitable technique to use spent residue (obtained in steam distillation) to generate biogas. The amount of cellulose in spent residue of mint & Cymbopogon crop was measured about 31-33% of dry biomass. Beside this, about 20% hemicellulose, 14-16% lignin and 5% protein was noticed in spent residue. These two component i.e. cellulose & hemicellulose are main organic substrate for biogas production in bio-menthanation process (Fanin, K. F. et al., 1983, Habig, C. et al., 1984). So, it is planned to find out the possibility of using spent residue of essential oil bearing crop for biogas production under HRT.

The organic components i.e. cellulose, hemicellulose and carbohydrate & protein of spent residue may undergo microbial hydrolysis (United Nation, Guide book, 1980) and subsequently interacting with acidogenic & menthanogenic bacteria to give methane gas (SPOBD China, 1979, Tumwasron, S., 1981) (Fig-7.1). This means a consortium of microbes (Table-7.1) responsible for production of methane.
Fig:-7.1 Possible Mechanism of Biomethanation of spent residue obtained after steam distillation of Mentha arvensis/Cymbopogon martini var. Motia
<table>
<thead>
<tr>
<th>Type of bio-reaction</th>
<th>Microbes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>Cellulose degrading microbe</td>
</tr>
</tbody>
</table>
|                     | *Acetoribrio sp  
                     |  *Clostridium sp  
                     |  *Ruminococcus sp.  |
| Hemicellulose degrading Microbe | *Cellulomonas sp  
                     |  *Thermoanaerobacter sp  
                     |  *Ruminococcus sp.  
                     |  *Clostridium sp  |
| Starch degrading microbes | *Bacteroides sp  
                     |  *Butyrivibrio sp  
                     |  *Clostridium sp  |
| Acetogenesis        | *Enteric bacteria  
                     |  *Streptococci  
                     |  *Anaerovibrio lepoyctica  
                     |  *Bacteroides amlophilus  
                     |  *Bacteroides ruminocola  
                     |  *Bacteroides succigens  
                     |  *Butyrivibrio fibrisolvens  
                     |  *Clostridium sp  
                     |  *Eubacterium ruminatum  
                     |  *Ruminococcus albus  |
| Methanogenesis      | *Methanobacillus omelianski  
                     |  *Methanobacterium strain M.O.H.  
                     |  *Syntrophobacter Wolinii  
                     |  *Syntrophomonas Wolfii  
                     |  *Methanosarcina Barkeri  
                     |  *Methanococcus mazei  
                     |  *Methanothrix soehnggenii  |

**Table- 7.1 Microbes involve in biogas production**
from organic waste or spent residue obtained from agro-chemical industries (Wilkie, A. et al., 1986, Hobson, P. N., 1973, Innotti, E. L., 1978). Activated sludge of cattle dung based bio-gas generating digester contain such consortium of microbes and can be used to start biomethanation process in *Mentha arvensis/Cymbopogon martinii var. motia* spent residue based bio-gas digester.

7.1.3 Material & Methods

7.1.3a Substrate

a) Cattle Dung

Fresh cattle dung used for digester start up and daily feeding was collected from the local dairy. Composition of the cattle dung varies from season to season due to the change in the feed of the cattle. The chemical analysis represent the average of composition of different batches (Table-7.3) cattle dung was collected twice a week. Sample of cattle dung were used to be kept in cold room to avoid further degradation.

b) Innocula

Innocula for the digester starts up were taken from an active cattle dung digester capable of producing methane. This digester was maintained under laboratory conditions. The slurry was filtered with cheese cloth under continuous nitrogen gassing and filtrate was used to seed the new experimental digester.

c) Spent Residue

Spent residue obtained after steam distillation of mint/ *Cymbopogon* crop was used as substrate for bio-gas production. The spent residue obtained from steam distillation was air dried for 72 hours and subsequently incubated at 75°C, till constant weight. The dry spent residue was crushed to 60 mesh size particle by Ball mill and kept under dehumidified atmosphere for further use.

7.1.3b Operation of Digester

Narrow mouth aspirator bottle having capacity of 2.5 liter were used for bio-gas digester were operated at 33 ± 2°C. The working volume of digester both controls and experimental sets, were kept 1.6 liter. The control set digester was having 9% cattle dung slurry (T.S w/v) and 10% active innocula. For the same purpose in each digester 888.80 gm of fresh cow dung (T.S. 18%) was added following by 160 ml of active innocula. The final working value of each digester was adjusted 1.6 liter by adding tap water.

The experimental digesters were also having cattle dung & innocula as stated above. However some calculated amount of cattle dung was replaced with spent residue. The amount of spent residue varies from 10% to 50%. Table –7.2.
### Table 7.2 Different substitution of cattle dung with spent residue of mint/Cymbopogon crop.

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Cattle dung (fresh) (gm)</th>
<th>Mint /Cymbopogon spent residue (g)</th>
<th>Water (ml)</th>
<th>Innocula (ml)</th>
<th>Total volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle dung 100% (control)</td>
<td>888.80</td>
<td>0</td>
<td>551.2</td>
<td>160</td>
<td>1600</td>
</tr>
<tr>
<td>Spent residue 10% + 90% Cattle dung</td>
<td>800.00</td>
<td>16</td>
<td>624.0</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Spent residue 20% + 80% Cattle dung</td>
<td>711.11</td>
<td>32</td>
<td>696.98</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Spent residue 40% + 60% Cattle dung</td>
<td>533.33</td>
<td>64</td>
<td>915.56</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Spent residue 50% + 50% Cattle dung</td>
<td>444.44</td>
<td>80</td>
<td>988.45</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

7.1.3c Monitoring of simulated digester

As with any biochemical process, anaerobic treatment systems needs to be monitored to ensure successful operation. Gas production rates, gas composition (CH₄ and CO₂ content), pH and reduction in total solids, volatile solids, total carbohydrates, cellulose, hemicellulose, volatile fatty acids profile, total acidity, and alkalinity etc, are some of the several parameters which needs to be monitored together on daily or at definite interval to get complementary information.
7.1.3d Gas analysis

A) Quantitative gas measurement

Total gas was analyzed by water displacement method. The distant end of the gas outlet pipe (PVC tubing) from the digester was inserted in the water filled, graduated narrow mouth glass measuring cylinders inverted in water filled trough. The gas produced in the digester-displaced water in the measuring cylinder, which was measured manually every day.

B) Qualitative gas analysis

The qualitative gas analysis in terms of CH₄ and CO₂ contents was done using airtight gas syringe. To facilitate the withdrawal of gas into the syringe, a butyl rubber cork with a septum was used to cap the mouth of the measuring cylinder. The quality of bio-gas produced was analyzed by using an indigenous gas chromatograph (Nucon, Series 5765) equipped with thermal conductivity detector (TCD) and a 2 meter Porapak Q (80 to 100 Mesh) column. Hydrogen was used as a carrier gas at a flow rate of 20ml/min. The oven, injector and detector temperatures were 60°C, 80°C and 80°C respectively.

Qualitative analysis of bio-gas produced was done following standard gas chromatography (GC) technique. Gas sample gas (0.5ml) was injected into the Porapak Q column, through the injector port with gas tight syringe. The identification of N₂, CH₄ and CO₂ was done using commercial standard gases (SPANCAN, surrey, England). The CO₂ and CH₄ content were expressed as relative percentage (v/v).

7.1.3e Constituent analysis

a) Total solids (TS)

To determine moisture content and total solids, slurry samples were subjected to oven drying following the method as described by AOAC (1975).

A known quantity of sample (g) was added in pre-weighted dry petriplate and was transferred to the oven for drying at 70°-80°C, till a constant weight was observed. The difference between the wet and the dry weight was taken as moisture content and expressed as % moisture content (w/w) or % moisture content as applicable.

Calculation

Weight of dry empty petri plate (g) = x

Weight of petri plate + sample (g) = y

Weight of petri plate + dry sample (g) = z
Photo- 7.1 Temperature controlled Biogas room (37 C with simulated biogas digester)
\[
\frac{\text{Moisture content} = (y - x) - (z - x) \times 100}{(y - x)}
\]

The total solid in the slurry sample was estimated by volumetric method. A known volume of liquid sample was dried in a pre-weighed dry petriplate at 70°-80°C, till the constant weight was observed and expressed as (%, w/v) dry weight:

Weight of dry empty petriplate (g) = \(x\)

Volume of sample (ml) = \(y\)

Weight of petriplate + dry sample (g) = \(z\)

Total solids (%, w/v) = \(\frac{(z - x) \times 100}{y}\)

b) Total volatile solids (VS)

The oven-dried sample after total solids estimation was further analyzed for VS and ash content. Sample (0.5 g) was taken in a clean pre-weighed silica crucible, and ignited in a muffle furnace at 550° C for 8 hrs. The crucible was cooled and weighed to determine ash content. From this value % ash and % VS was calculated.

Weight of dry empty crucible (g) = \(x\)

Weight of crucible + sample (g) = \(y\)

Weight of crucible + sample after ignition (g) = \(z\)

Ash content (%, w/w) = \(\frac{(y - x) - (z - x) \times 100}{(y - x)}\)

VS content (%, w/w) = 100 – Ash content (%, w/w)

The organic carbon was calculated based on VS content using Van Bemmelen factor

\[
\text{Organic carbon} (%) = \frac{\% \text{ organic matter or VS}}{1.724}
\]

c) Total Carbohydrates

Total carbohydrates in the oven-dried sample were estimated according to the method described by Updegraff (1969) for cellulose. Sulphuric acid (10 ml, 67%, v/v) was added to 0.05g of sample.
Photo- 7.2 Simulated laboratory scale semi-continuous digester and gas measurement assembly
After 60 minutes the sample was diluted to 50 ml with distilled water. To a 0.2-ml aliquot of this sample, 1.8 ml distilled water was added. Then 4ml of cold anthrone reagent were added, sample was mixed in cyclo- mixer, and the tubes were transferred to a boiling water bath. However, precautions were taken to see that reflux action takes place without evaporation, for which tubes were covered with glass marbles. To provide uniform temperature to the reaction mixture the level in the water bath was maintained equal to the reaction mixture in the tube. After 16 minutes in the water bath, samples were immediately cooled in ice. Optical density (OD) was read at 620 nm against a reagent blank. The concentration of total carbohydrates in the sample was calculated using glucose standards.

Preparation of standard curve:

Pure dry glucose (0.005g) was dissolved in 1ml H₂SO₄ (67%, v/v) with gentle heat. The solution was then diluted to 50 ml with distilled water in a volumetric flask to contain 100-μg glucose/ml. using this standard stock solution, appropriate dilutions were prepared as under. Colour was developed as before using anthrone reagent. O.D. was read at 620 nm using a spectrophotometer (Hitachi, Tokyo, Japan). A standard curve was obtained and concentration of total carbohydrates (% w/w) present in the sample was determined.

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Concentration (μg)</th>
<th>Water (a) (μl)</th>
<th>Glucose stock (b) (μl)</th>
<th>Total volume (a + b) ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>2000</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>1750</td>
<td>250</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>1500</td>
<td>500</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>1250</td>
<td>750</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>1000</td>
<td>1000</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>125</td>
<td>750</td>
<td>1250</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>150</td>
<td>500</td>
<td>1500</td>
<td>2.0</td>
</tr>
<tr>
<td>8</td>
<td>175</td>
<td>250</td>
<td>1750</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>200</td>
<td>0</td>
<td>2000</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Calculation:-

\[
\text{Total carbohydrates (\%)} = \frac{\text{Conc at 620nm} \times \text{Dilution factor} \times 100}{\text{Sample used for color development (ml) \times Sample (g) \times 10^6}}
\]

Dilution factor = 50

Sample used = 0.2 ml

Sample weight = 0.05 g

d) Cellulose

Cellulose was estimated colourimetrically by the method described by Updegraff (1969). Oven dried sample (0.1 g) was taken and moistened with distilled water. Acetic nitric reagent (5 ml) was added, sample was mixed on a cyclo-mixer and the tubes were then placed in a boiling water-bath for 30 min to digest all the organic matter except cellulose. Tubes were then cooled to room temperature. The sample was then filtered through Millipore glass filter (Advance Micro Device Pvt. Ltd, India) under vacuum using peristaltic pump (Millipore Indiana, USA). The residue was washed two to three times till it become pale yellow or colourless. The filtrate was discarded and the cellulose fibers along with the glass filter were put in a test tube. To the tube 10 ml of H₂SO₄ (67%, v/v) was added and after 60 min sample was diluted to 50ml with distilled water. Colour was developed using an aliquot of the sample (0.2 ml), using cold anthrone reagent in the same way as for the total carbohydrates and cellulose present in the sample was calculated and expressed as cellulose content (%, w/w) using a cellulose standard curve.

Preparation of standard curve:
Standard curve for cellulose was prepared using pure dry Cellulose powder (0.005 g). It was dissolved in 1 ml H₂SO₄ (67%, v/v) with gentle heat. The solution was the diluted to 50 ml with distilled water in a volumetric flask to contain 100 µg cellulose powder/ml. Using this standard stock solution appropriate dilutions were prepared as under.

Colour was developed as before using anthrone reagent. O.D. was read at 620 nm using a spectrophotometer. A standard curve was made and concentration of cellulose (%, w/w) present in the sample was determined.
<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Concentration (µg)</th>
<th>Water (a) (µl)</th>
<th>Sock Cellulose Powder (b) (µl)</th>
<th>Total volume (a + b) ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0</td>
<td>2000</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>2.</td>
<td>25</td>
<td>1750</td>
<td>250</td>
<td>2.0</td>
</tr>
<tr>
<td>3.</td>
<td>50</td>
<td>1500</td>
<td>500</td>
<td>2.0</td>
</tr>
<tr>
<td>4.</td>
<td>75</td>
<td>1250</td>
<td>750</td>
<td>2.0</td>
</tr>
<tr>
<td>5.</td>
<td>100</td>
<td>1000</td>
<td>1000</td>
<td>2.0</td>
</tr>
<tr>
<td>6.</td>
<td>125</td>
<td>750</td>
<td>1250</td>
<td>2.0</td>
</tr>
<tr>
<td>7.</td>
<td>150</td>
<td>500</td>
<td>1500</td>
<td>2.0</td>
</tr>
<tr>
<td>8.</td>
<td>175</td>
<td>250</td>
<td>1750</td>
<td>2.0</td>
</tr>
<tr>
<td>9.</td>
<td>200</td>
<td>0</td>
<td>2000</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Calculation: -

\[
\text{Cellulose} \% = \frac{\text{Conc at 620nm} \times \text{Dilution factor} \times 100}{\text{Sample used for color development (ml)} \times \text{Sample (g)} \times 10^6}
\]

Dilution factor = 50

Sample used = 0.2 ml

Sample weight = 0.100 g

e) Hemicellulose

Hemicellulose was estimated colourimetrically by the method described by Deschatelets and Yu (1986). To oven dried sample (0.1g), 10 ml H₂SO₄ (3%, w/v) was added and the sample was autoclaved at 121° c for 60 min. The solution was then neutralized with 10N KOH and HCl and volume was made to 50 ml with distilled water. To an aliquot (0.2ml) of the diluted
hydrolysate, 1ml p-bromoaniline reagent was added. A blank was prepared in the same manner. One of the sets (sample) was kept in a water bath at 70°C for 10 min, whereas the blanks were kept in dark at room temperature. After incubation at 70°C, the test tubes were cooled rapidly to room temperature and kept in dark for 70 min. The purpose of the blank is to correct for the furfural already present in the sample as well as for possible colouration associated with the sample. OD was read at 520 nm using spectrophotometer Hitachi, of all tests and corresponding blanks using a reagent blank for zero adjustment.

**Preparation of standard curve:**

Pure anhydrous xylose (0.05 g) was dissolved in 100 ml distilled water to prepare stock solution having 1000μg xylose/ml. Standard xylose solution were prepared by suitably diluting the stock solution. Standard curve was prepared to calculate hemicellulose in the sample and expressed as hemicellulose (% w/w).

Calculation:

$$\text{Hemicellulose} \% = \frac{\text{Conc. at 620 nm} \times \text{Dilution factor} \times 100}{\text{Sample used for color development (ml) } \times \text{Sample (g)} \times 10^6}$$

Dilution factor = 50

Sample used = 0.2 ml
Sample weight = 0.100 g

<table>
<thead>
<tr>
<th>Standard No.</th>
<th>Concentration (μg)</th>
<th>Water (μg) (a)</th>
<th>Stock xylose (μl) (b)</th>
<th>Total volume (a + b) ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>200</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>160</td>
<td>40</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>120</td>
<td>80</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>80</td>
<td>120</td>
<td>2.0</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>200</td>
<td>160</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0</td>
<td>200</td>
<td>2.0</td>
</tr>
</tbody>
</table>
f) Phosphorous

Phosphorous in the sample was estimated colourimetrically by the method described by Jackson (1967). Tri-acid mixture (12 ml) was added to 100 mg of the sample. The sample was refluxed at 80°C till it turned white or pale yellow. The sample was then cooled and volume was made to 50 ml with distilled water. The solution was filtered through Whatman filter paper 1 (Whatman International Ltd., England) and the filtrate was used for phosphorous estimation. To an aliquot (5 ml and 10 ml) of the filtrate, 10 ml Burton’s reagent was added. Volume was made to 50 ml with distilled water. After 10-15 min OD was read at 420 nm against reagent blank on a spectrophotometer. The phosphorous present in the sample was then calculating using a phosphorous standard curve and expressed as phosphorous (mg/l).

**Preparation of standard curve:**

Standard stock solution of phosphorous was prepared (Appendix) to contain 50 μg phosphorous/ml. The stock solution was suitably diluted to give standard phosphorous solutions.

<table>
<thead>
<tr>
<th>Volume of stock solution taken (ml)</th>
<th>Concentration obtained when stock solution is diluted to 50 ml final volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Blank</td>
</tr>
<tr>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>
Phosphorous (mg/kg dry matter) =

\[ \text{Volume of digest (ml) } \times 50 \times \text{phosphorous (ml/l)} \] 
\[ \text{Weight of sample (g) } \times \text{volume of digest used to develop colour} \]

g) Kjeldhal Nitrogen

Nitrogen present in the sample was estimated by Kjeldhal’s method as described by Jackson (1967). Nitrogen in organic material is first converted to ammonium sulphate by digestion with concentrated H₂SO₄. The digested solution is made alkaline and the ammonia released by distillation is absorbed in standard boric acid solution. The nitrogen present is determined by back titration with HCl of known normality.

Oven dried homogenized sample (100 mg) was with 5 ml of concentrated H₂SO₄ and kept for digestion at 80°C for 6 to 8 hrs. After digestion the content was cooled to room temperature and digested sample was diluted to 50 ml with distilled water. An aliquot (5 ml) was taken in Kjeldhal apparatus (Widson Scientific works, N. D. India) and 10 ml of 40% (w/v) NaOH was added. 2 drops of mixed indicator were added to 5 ml of 40% boric acid in a conical flask. Ammonia from Kjeldhal apparatus was absorbed in the flask till the volume became about 20 ml. This solution was titrated against 0.01 N HCl solution.

Nitrogen content (% w/v) was estimated as given:

\[ \% \text{N} = \frac{\text{titration value of sample titration/ value of blank}}{\text{N} \times 1.4x} \] 

\[ = \frac{\text{Total volume after digestion/ sample (g) x digested sample taken for distillation (ml)}}{N} \times \text{normality of HCl} \]

h) Proteins

The protein is estimated by multiplying the total Nitrogen with 6.25.

Total protein = N x 6.25

7.1.3f Retention Time (RT)

The duration for which a particular protein of the feed stock added to the digestion system remains in the digester is known as RT.

RT = Total volume of the fluid in the digester
\[ \text{Volume of the fluids passes in to and out of the digester per day.} \]
Anaerobic digestion has a bad reputation for process instability. According to Sham (1984), if, however, the SRT can be made significantly long (100 f) process stability is rather good. The present day technology defines SRT as the most crucial design and operational parameter. The three operational factors affecting the performance of anaerobic digesters are interlinked and can be considered collectively.

The relationship between volume of the digester (U) and retention time (t) has been given as under.

$$U = C(1 + D) \frac{tf}{yd}$$

where

- $C = \text{desired capacity of the plant (cu. ft. of biogas/day)}$
- $d = \text{weight of water added to unit weight of wet dung}$
- $t = \text{temperature of digester content}$
- $f = \text{fermentation period in the days}$
- $y = \text{yield gas/unit weight of dung}$
- $d = \text{density of dung water mixture}$.

Lowrence (1971), and subsequently, Jewell et al. (1976), reported that the minimum solids retention time (SRT) when the bacterial biomass will not be washed out as 7.5 days 35°C and 12 days at 25°C. However, it is always advisable fermentation longer retention periods for complete fermentation.

### 7.1.3g Hydraulic Retention Time (HRT)

HRT is defined as the average time spent by the input slurry inside the digester before it comes out. Rats of biogas generation is initially high and then completion. Thus the time required for 70-80% digestion is considerably less than that needed to achieve cent percent or completely digestion. HRT is chosen so as to achieve 70-80% digestion. HRT varies between 20 to 120 days depending upon the design and operating temperature of digester.

The total solids concentration-loading rate (LR) and HRT are related by following equation (TERI, Manual, 1987)

$$LR = K \frac{TS}{HRT}$$

Per kg cattle dung, LR is expressed in units of kg v.s/day m³. T.S in percentage and HRT in days and K is found to have an approximate value of 7.
7.1.4 Result & Discussion

The chemical analysis of cattle dung and spent residue of mint and *Cymbopogon* crop was given in table-7.3. The total solid of cattle dung was noticed to be 15-18% and volatile solid was about 70.76. The amount of volatile solid was little more in mint and *Cymbopogon* sp. as compared to cattle dung.

Bio-chemical analysis of cattle dung (fresh) and spent residue (mint and *Cymbopogon* sp.) showed that about same amount of organic carbon was present in all samples. Total carbohydrate of cattle dung was significantly higher as compared to spent residue of *Mentha arvensis* and *Cymbopogon sp* crops. The cellulose amount was noticed to be minimum in cattle dung and maximum in spent residue of both mint and *Cymbopogon sp* crop. About 31-33% of cellulose was present in spent residue of *Mentha arvensis* and *Cymbopogon sp* Crop. Both *Mentha arvensis* and *Cymbopogon* sp. contain higher amount (20%) of hemicellulose as compared to cattle dung. The nitrogen C:N ratio, Phosphorous were notice to be more or less same in all the substrates used for biogas production.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cattle dung (fresh)</th>
<th><em>Mentha arvensis</em> spent residue</th>
<th><em>Cymbopogon</em> sp. spent residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solid (% w/w)</td>
<td>18</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Volatile solid (%)</td>
<td>70.76</td>
<td>88.60</td>
<td>89.25</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>49.17</td>
<td>48.46</td>
<td>47.42</td>
</tr>
<tr>
<td>Total carbohydrate (%)</td>
<td>42.84</td>
<td>34.24</td>
<td>36.46</td>
</tr>
<tr>
<td>Cellulose (%)</td>
<td>18.95</td>
<td>32.2</td>
<td>31.11</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>13.24</td>
<td>20.4</td>
<td>20.1</td>
</tr>
<tr>
<td>Lignin</td>
<td>10.727</td>
<td>16.44</td>
<td>14.22</td>
</tr>
<tr>
<td>Nitrogen %</td>
<td>1.727</td>
<td>0.889</td>
<td>0.86</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>28:1</td>
<td>50:1</td>
<td>51:1</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>0.8499</td>
<td>0.941</td>
<td>0.841</td>
</tr>
<tr>
<td>C:P ratio</td>
<td>58:1</td>
<td>71:1</td>
<td>76:1</td>
</tr>
<tr>
<td>Total protein</td>
<td>10.795</td>
<td>5.562</td>
<td>5.375</td>
</tr>
</tbody>
</table>

Table-7.3 Chemical composition of cattle dung, spent residue of *Mentha arvensis* and *Cymbopogon* sp.
Table 7.4 shows about production of total biogas, biogas liter/kg TS added, methane (%) and digestion of organic materials. In cattle dung based digester the amount of total gas was noticed to be 760 ml/day. So these results tally with earlier findings (Sangeeta, 1999). The amount of methane at 30 days \( 1 \approx RT \) was about 55.9% of total biogas. During this period 30.12% organic material was decomposed. By substitution of different amount of spent residue (\textit{Mentha arvensis}), maximum biogas production was noticed to be 900 ml/day and the methane content was about 64.7% of the total biogas. Beyond substitution of spent residue (\textit{Mentha arvensis}) amounting more than 40% caused reduction in production of total biogas and also methane content.

The cattle dung based digester produce maximum biogas (806 ml/day) at 40 days HRT. The percentage of methane was noticed to be about same as observe in case of 30 days HRT. By substitution of spent residue (\textit{Mentha arvensis}), the maximum increase in biogas and methane content was noticed at 50 days HRT. This could have been due to more time taken by the spent residue (\textit{Mentha arvensis}) for its digestion of organic material and subsequently biogas production. These finding are in contrast to earlier finding (Badger, D. M., 1979, Prasad, C. R., 1985, Traor, A. S., 1992, Varshney, A.C., 1987).

Table 7.5 shows about production of total biogas, biogas liter/kg TS added, methane and digestion of organic material of spent residue (\textit{Cymbopogon} sp).

By substitute different amount of spent residue (\textit{Cymbopogon} sp) maximum biogas production was noticed to be 803 ml/day and the Methane content was about 61.42 of the total biogas beyond the substitution of spent residue (\textit{Cymbopogon} sp) amounting more than 40% caused reduction in production of total biogas and also methane content.

The maximum increase in biogas and methane content was noticed at 50 days HRT substitution of spent residue (\textit{Cymbopogon} sp). This could have been due to more time taken by the spent residue (\textit{Cymbopogon} sp) for its digestion of organic material and subsequently biogas production.

Generally fresh cattle dung consists of around 20% of solid & 80% water. Total solid in term consists of 70% volatile solid and 30% fixed solid. Cattle dung (fresh cattle waste) used in the present set of experiment contain about 18% total solid and 8.2% water. The volatile solid present in fresh dung was noticed to be 70%. In 30 days HRT cow dung based digester produced 144 liter/kg T.S. added. However with increase of HRT to 40 days the maximum gas yield was noticed to be 202 liter/kg T.S.
<table>
<thead>
<tr>
<th>Substitution</th>
<th>30 days HRT</th>
<th>40 days HRT</th>
<th>50 days HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total gas ml/day</td>
<td>Bio gas l/kg T.S Added</td>
<td>Digestion of organic material (%)</td>
</tr>
<tr>
<td>1</td>
<td>Control 100% Cattle dung</td>
<td>760±6</td>
<td>144±0.2</td>
</tr>
<tr>
<td>2</td>
<td>Cattle dung 90% + 10% spent residue</td>
<td>806±0.7</td>
<td>152±0.02</td>
</tr>
<tr>
<td>3</td>
<td>Cattle dung 80% + 20% spent residue</td>
<td>858±0.7</td>
<td>169±0.03</td>
</tr>
<tr>
<td>4</td>
<td>Cattle dung 60% + 40% spent residue</td>
<td>900±0.4</td>
<td>169.8±0.02</td>
</tr>
<tr>
<td>5</td>
<td>Cattle dung 50% + 50% spent residue</td>
<td>848±0.5</td>
<td>160.7±0.01</td>
</tr>
</tbody>
</table>

Table- 7.1.4 Bio methanation of spent residue (*Mentha arvensis*) at different HRT
**Table- 7.5 Bio methanation of spent residue (Cymbopogon sp.) at different HRT**

<table>
<thead>
<tr>
<th>S.N</th>
<th>Substitution</th>
<th>30 days HRT</th>
<th>40 days HRT</th>
<th>50 days HRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total gas ml/day</td>
<td>Bio gas l/kg T.S Added</td>
<td>Digestion of organic material (%)</td>
</tr>
<tr>
<td>1</td>
<td>Control 100% Cattle dung</td>
<td>714±0.02</td>
<td>140.4±0.02</td>
<td>30.01±0.13</td>
</tr>
<tr>
<td>2</td>
<td>Cattle dung 90% + 10% spent residue</td>
<td>794±0.05</td>
<td>149.8±0.3</td>
<td>35.16±0.23</td>
</tr>
<tr>
<td>3</td>
<td>Cattle dung 80% + 20% spent residue</td>
<td>803±0.42</td>
<td>151.5±0.4</td>
<td>41.01±0.02</td>
</tr>
<tr>
<td>4</td>
<td>Cattle dung 60% + 40% spent residue</td>
<td>784±0.01</td>
<td>148±0.15</td>
<td>43.03±0.05</td>
</tr>
<tr>
<td>5</td>
<td>Cattle dung 50% + 50% spent residue</td>
<td>781±0.11</td>
<td>147.4±0.1</td>
<td>28.03±0.11</td>
</tr>
</tbody>
</table>
added, during this period the ratio of slurry and water was about 1:1 as reported earlier work (Mittal, K. M., 1996a).

In spent residue based digester (*Mentha arvensis* 40% + 60% cow dung) having so HRT maximum biogas yield was noticed about 290 liter/kg T.S added. During this period the total solid was 9% out of which volatile solid was 88%. This was achieved by making slurry of fresh cattle dung 533.33 gm + 64 gm spent residue (*Mentha arvensis*) + 915.56 ml water.

The spent residue (*Cymbopogon*) 40% + 60% cattle dung based digester at 50 days HRT also produced maximum biogas 262.5 l/kg T.S added.

When the loading of spent residue was too high (60% *Mentha arvensis* + 40% cattle dung /*Cymbopogon* 40% + 60% cattle dung) the pH of the digester fell down to high acidic (4.5-5.0) (SPOBD, 1979). Due to its becoming acidic following mobility of microorganism to biodegradable all feed materials (Waart, J. D., 1980).

The total solid concentration, loading rate and HRT related and expressed in term of (TERI. Manual., 1987).

\[
LR = \frac{K \times TS \text{ (%)}}{\text{HRT}}
\]

where

- LR is loading
- TS total solid in percentage
- HRT Hydraulic Retention Time.

The ‘K’ values of different biogas digester are given in the table-7.6. The cow dung based digester the value of ‘K’ was noticed to be 7.0 and the value is in contrast to earlier finding (Mittal, K. M., 1996b). With increase in volatile solid value in mixed slurry-feeding digester. The gradually increasing in ‘K’ value from 7-7.95 was noticed. Both in *Mentha arvensis* and *Cymbopogon* sp. spent residue bio digester.
<table>
<thead>
<tr>
<th>Substitution</th>
<th>Cattle dung</th>
<th>Spent residue (mint) + Cattle dung</th>
<th>Spent residue (Cymbopogon sp) + cattle dung</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 days HRT</td>
<td>40 days HRT</td>
<td>50 days HRT</td>
</tr>
<tr>
<td>100% Cattle dung</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Cattle dung 90% + 10% spent residue</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cattle dung 80% + 20% spent residue</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cattle dung 60% + 40% spent residue</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cattle dung 50% + 50% spent residue</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

**Table- 7.6** The value of ‘K’ in different substitution of cow dung, spent residue of *Mentha arvensis* and *Cymbopogon* sp at different HRT.
7.1.5 Designing of Commercial Scale bio gas digester

Basing on the data obtained from the laboratory scale digester. It was estimated to design a commercial scale digester having the capacity of 75 m$^3$ gas/day which can be used for steam distillation unit of *Mentha / cymbopogon* crop for obtaining essential oil.

For steam distillation about 0.6 tonnes of dry biomass is needed to distill 3 tonnes of mint /*Cymbopogon* herb. This 0.6 tonnes of waste biomass of mint /*Cymbopogon* crop is equal to 14218009 Btu of energy (India Solar, 2003) and ultimately this is equal to 4165 kwh of electric energy. As per present rate of electric unit (Rs/- 3.50 per unit) about Rs 1388 is supposed to be consumed in one batch of hydro distillation. For rural industry, spending so much of money in steam distillation is expensive and, as a result the farmers go for direct using biomass as fuel, irrespective of its emission of huge amount of CO$_2$ as stated earlier.

Keep in view the quantity of gas to be utilize for hydraulic distillation of mint /*Cymbopogon* sp crop having gas capacity of 75 m$^3$ gas/day, attempt was taken to design a commercial scale biogas plant on the basis of international acceptable norms prescribed by GATE, biogas plants: Building instructions, German Appropriate technology exchange, German Agency for technical co-operation, P.O. Box 5180, 6236 Eschborni, Germany (erstwhile) 1997.

7.1.5a Selection of Biogas plant site

Generally the location of the biogas plant is supposed to be nearer to hydro distillation unit. This practice is made to decrease the amount of expenditure on hand movers, pipeline used to carry biogas to the site of utilization. Besides this it effects maintenance of biogas unit. So keeping in views the above facts for present experimental purpose, the biogas plant was constructed about 10 mt. distance from the distillation unit.

The *Mentha arvensis /Cymbopogon* sp spent residue is first kept in storage shed made up of local material like bamboo, tree limbs, bricks, tiles.

7.1.5b Estimation of Biogas requirement

Generally 1.6-kg steam is generated from one kwh of electricity (Brain and Lawrence, M., 1995) and same amount of electricity is required to produce 0.6 m$^3$ biogas (Mittal, K. M., 1996b). So, 200 kg of steam is required to run a steam distillation unit having capacity 3 tonnes per batch per day.

Therefore, 200 kg steam is generated by 75 m$^3$ gas/day (2648.25 ft$^3$)

So, 75m$^3$ gas/day is required for running a steam distillation unit having capacity 3.0 tonnes/batch.
7.1.5c Estimation of Biomass requirement

It has seen that 1 m³ (1000 kg) of slurry (40% spent residue \textit{(Mentha arvensis /Cymbopogon sp)} + 60% cattle dung} yield 0.6 m³ of gas/ day at 50 days HRT.

Therefore, $75 \text{m}^3$ Biogas needs = $75 \times 100 / 0.6$

$= 125,000 \text{ kg (125 tonnes)}.$

Daily feeding at 50 days HRT will be

Feeding rate = Total slurry (Vs) = $12,500$

\[ \frac{12,500}{50} \]

$= 2500 \text{ kg /day}$

$= 2.5 \text{ tonnes / day}$

From our experimental data it has been estimated that maximum yield of biogas was obtained by combination of 40% spent residue + 60% cattle dung (on TS basis).

So, daily spent residue slurry \textit{(Mentha arvensis /Cymbopogon sp)} requirement will be = 1000 kg (40% of total 2500 kg)

Spent residue required on TS (9%) basis = 90 kg

Water needed to make slurry (9% on TS basis) = 1000 – 90 = 910 liter
And daily cattle dung slurry requirement will be 1500 kg (60% of total slurry)

Cattle dung (fresh) required for making 9% slurry will be = 750 kg

Water needed to make slurry = 1500 – 750 =750 liter

Therefore, total spent residue (dry) required is 90 kg / day and cattle dung (fresh) is 750 kg (from 30 cattle) to make slurry of 9% (T.S. basis) by dilution with 1660 liter water.

7.1.5d Selection of Digester Capacity

It has been estimated that 2,500-kg (5,512-lb) slurry is to be feed to the digester daily, which should remain inside for 50 days for undergoing anaerobic fermentation.

Slurry with the assumed norm that 10 lb of occupies $2/7 \text{ ft}^3$ of space and 5,512 lb of slurry is feed to digester daily, volume needed for it will be

$= 2/7(\text{ft}^3) \times 1/10(\text{lb}) \times 5,512$

$= 157.49 \text{ ft}^3$

$= 4.460 \text{ m}^3$
Therefore, $1 \text{ m}^3 = 35.31 \text{ ft}^3$

For a 50 days retention period, the total digester volume needed is:

$$\text{Volume needed} = 157.49 \times 50 = 7,874.5 \text{ ft}^3 (223.01 \text{ m}^3)$$

For estimation of height & diameter there are a number of specific rules but generally ‘D’ is kept twice of ‘H’.

So, the height and diameter of the digester to match the estimated digester volume could be calculated as follows:

$$\pi D^2 \div 4 \times h = V_s$$

$V_s$ is the active slurry volume in the digester (m)

As diameter ($D$) is kept twice of $H$ (height):

$$D = 2h$$

$$\pi D^2 \div 2 \times D = V_s$$

$$D^3 = \frac{7874.5 \times 2}{3.14} = 5015.6 \text{ ft}^3$$

Hence, Diameter ($D$) = 17.1175 ft (5.2311 m)
Height ($H$) = 8.5587 ft (2.6162 m)

### 7.1.5e Estimation of GasHolder size

Based on the presumption that gas is to be used regularly and withdrawn more or less at a constant rate, the gas holder needs to have one half the volume of the estimated.

In a fixed dome-type digester,
- Daily gas production will be $2648.25 \text{ ft}^3 (73 \text{ m}^3)$
- Gas holder needs to have a capacity of $1324.13 \text{ ft}^3 (37.5 \text{ m}^3)$

### 7.1.5f For Moveable drum type digester

The diameter of the gas holder should be kept smaller by 6 inches (15-24 cm) than the digester. These for diameter ($D'$) of the gas holder will be:

$$D' = 17.1175 - 0.5 = 16.6175 \text{ ft (5.0474 m)}$$

Height of gas holder = Volume / Area

$$= \frac{1324.13 \times 4}{3.14 \times (16.6175)^2}$$
7.1.5g Estimation of Heat requirement
During winter the average temperature near digester remain $20^\circ$C ($68^\circ$F) for 3 months (Dec., Jan., Feb.). So, for obtaining higher gas yield during this period it is desired to raise the digester temperature from $20^\circ$C ($68^\circ$F) to $35^\circ$C ($95^\circ$F)

As we know that total 5,512-lb slurry is to be feed every day. If water and cattle dung are assumed to have same specific heat requisite heat to raise the temperature of input slurry from $68^\circ$F to $95^\circ$F is

$$= 5,512 \text{ lb} \times \frac{1 \text{ Btu}}{\text{lb} \times \text{F }^\circ} (95 - 68)^{\circ} \text{F}$$

$$= 1,48,824 \text{ Btu / day}$$

(1,57,009 KJ /day)

7.1.7h Estimation of Heat losses
Amount of heat losses from digester to the environment depends upon the ambient temperature. In Binyani (Rohtak) (site for bio gas plant) winter temperature during 3 months (Dec., Jan., Feb.) are as follows:

Mean ambient temperature (coldest) = $7^\circ$C ($41^\circ$F)
Mean ambient temperature close to the digester wall = $20^\circ$C ($68^\circ$F)

Area calculations are
Roof area of gas holder = $\pi D^2 / 4 = 3.14 \times (16.6175)^2 / 4$

$$= 216.7706 \text{ ft}^2 (20.163 \text{ m}^2)$$

Area of gas holder wall
$$= \pi \times 16.6175 \times 6.1084 = 318.729 \text{ ft}^2 (29.649 \text{ m}^2)$$

Area of Digester = Area of the digester bottom + area of the digester walls

$$= \text{area of annular space between digester and gas holder}$$

$$= \pi/4(17.1175)^2 + \pi \times 17.1175 \times 8.5587 + \pi/4\{(17.1175)^2 - (16.6175)^2\}$$

$$= 703.272 \text{ ft}^2 (65.4219 \text{ m}^2)$$

value of different thermal conductivity which are donated by ‘q’ are

Thermal conductivity $(q_r)$ from steel gas holder roof top and walls are given by

$$q_r = 0.16 \text{ Btu/ hr } /\text{ft}^2/\text{F }^\circ (3.27 \text{ KJ/hr/m}^2/\text{C }^\circ)$$

Thermal conductivity $(q_w)$ from digester floor, walls and annular clearance between digester and gas holder is given by
q_w = 0.12 Btu/hr/ft^2/F^° (2.45 KJ/hr/m^2/C^°)
So, heat loss from Gas holder top = 0.16 Btu x 216.7706 (95 - 41) °F
(hr. ft^2. F^°)
= 1872.8928 Btu/hr (1975.858 KJ/hr)

So,
heat loss from Gas holder wall will be = 0.16 Btu x 318.729 (95 - 68) °F
(hr. ft^2. F^°)
= 1376.9092 Btu/hr (1452.5978 KJ/hr)

Heat loss from digester bottom and digester surface area including
clearance between digester and gas holder
= 0.12 Btu x 703.272 (95 - 68) °F
(hr. ft^2. F^°)
= 2278.6012 Btu/hr (2403.8527 KJ/hr)

Thus total heat loss per day becomes.
24 (1872.8928 + 1376.9092 + 2278.6012)
= 132618.6768 Btu/day (139974.9347 KJ/day)

Total heat to be supplied = heat losses at operating temperature + Heat
needed to raise slurry to the desired operating temperature
= 148824 + 138681.6768
= 281505.6768 Btu/day.

Total efficiency of biogas plant having capacity of 75m^3 Biogas/day.
So, 0.02832 m Biogas provides = 550 Btu
75m^3 Biogas will provide = 1456567 Btu/day
So, net amount of heat produced during winter season (3 months) will be
1456567 – 281505.6768
= 1175061.323 Btu/day

Besides these 3 months, temperature loss is nearly about zero.
So net 1456567 Btu /day heat is produced that is sufficient for
running of steam distillation unit (capacity 3 tones /batch) for
8 hrs/day

7.1.5i Estimation of slurry displacement parameters
The estimation of slurry
displacement (d) can be calculated by simple equation
d = 0.16 H
d = 0.16 x 2.6162 m = 0.4185 m.
7.1.5j Computation of slurry displacement in inlet and outlet tanks

Maximum pressure exerted on gas should be equal to the pressure of the water (slurry) column above the lowest slurry level in the inlet and outlet tanks. This pressure is selected as 0.85m water gauge as a safe limit for brick/concrete domes. Thus,

\[ h + d = 0.85 \]
\[ \Rightarrow h + 0.4185 = 0.85 \]
\[ h = 0.85 - 0.4185 \]
\[ h = 0.4315 \text{ m} \]

7.1.5k Length (l) and breadth (b) of the inlet and outlet tanks

There is no specific criterion with regard to the choice of the shape of cross-section of inlet and outlet tanks, but a rectangular shape with \( l = 1.5b \) is preferred. So, breadth (b) is given by

\[ b = \left( \frac{0.2G}{1.5h} \right)^{1/2} \]

\[ G = 75 \text{ m} \]
\[ H = 0.4315 \text{ m} \]
\[ b = \left[ 0.2 \times 75 \div 1.5 \times 0.4315 \right]^{1/2} \]
\[ = \left[ 15 + 0.6472 \right]^{1/2} = (23.1749)^{1/2} \]
\[ = 4.81 \text{ m} \]
Then \( l \) will be, \( l = 1.5b \)
\[ = 1.5 \times 4.81 \]
\[ = 7.221 \text{ m} \]

7.1.5l Computation of the dome height

Volume of dome is given by \( V_d = \pi/6. \text{dh} [3(D/2)^2 + \text{dh}^2] \)
The volume of dome becomes \( V_d = G - 0.4G \)
\[ V_d = 0.6G \]

\[ 0.6G = \pi/6. \text{dh} [3(D/2)^2 + \text{dh}^2] \]

Value of \( \text{dhn} \) can be obtained by solving the above equation which is cubic. This among others can be solved by iteration. An algebraic solution also exists, which is obtained by computing series of parameters as follows:

\[ p = 0.75 \times D^2 \]
\[ = 0.75 \times (5.231)^2 \]
\[ = 20.52 \text{ m} \]
\[ q = -0.6 \times \left( \frac{6}{\pi} \right) G \]
\[ = -0.6 \times \left( \frac{6}{3.14} \right) \times 75 = -85.98 \]
\[ R = \left( \frac{p}{3} \right)^2 + \left( \frac{q}{2} \right)^2 \]
\[ = \left( \frac{20.52}{3} \right)^2 + \left( -\frac{85.98}{2} \right)^2 \]
\[ = (6.84)^2 + (42.99)^2 \]
\[ = 46.785 + 1848.140 = 1894.925 \]

\[ \sqrt{R} = \sqrt{1894.925} = 43.530 \]

\[ A = \left( \frac{-q}{2} + \sqrt{R} \right)^{1/3} \]
\[ = \left( \frac{85.98}{2} + 43.530 \right)^{1/3} \]
\[ = (42.99 + 43.530)^{1/3} \]
\[ = (86.52)^{1/3} \]
\[ = 4.42 \]

\[ B = \left( \frac{-q}{2} - \sqrt{R} \right)^{1/3} \]
\[ = \left( \frac{85.98}{2} - 43.530 \right)^{1/3} \]
\[ = (42.99 - 43.530)^{1/3} \]
\[ = (-.54)^{1/3} \]
\[ = -0.8 \]

\[ d_h = A + B \]
\[ = 4.42 - 0.8 \]
\[ = 3.622 \text{ m} \]

### 7.1.5m Estimation of the radius of dome

Dome radius is determined with the help of the following relationship.

\[ R = \left( \frac{D}{2} \right)^2 + d_h^2 \]
\[ = \frac{(5.2311)^2}{2} + (3.622)^2 \]
\[ = 2.755 \text{ m} \]

### 7.1.5n Determination of \( H' \) for curved bottom digesters

\( H' \) can be computed by the following relationship

\[ H' = 1.9 \times H \div 2.5 \]
\[ H' = 1.9 \times 2.6162 \div 2.5 = 1.988 \text{ m} \]

### 7.1.5o Size of the opening in inlet & outlet tank

Opening of inlet & outlet tanks are kept

\[ 0.5 \text{ m} \times 0.6 \text{ m} \]

Digester walls are = 9 inches thick
Inlet & outlet boxes walls are = 4.5 inches
7.1.5p Summary

\[
\begin{align*}
G &= 75.00 \text{ m}^3/\text{day} \\
Vs &= 156.103 \text{ m/day} \\
H &= 8.5587 \text{ ft (2.6162 m)} \\
D &= 17.1175 \text{ ft (5.2311 m)} \\
d &= 0.4185 \text{ m} \\
h &= 0.4315 \text{ m} \\
b &= 4.8 \text{ m} \\
l &= 7.221 \text{ m} \\
d_h &= 3.622 \text{ m} \\
r &= 2.755 \text{ m}
\end{align*}
\]

7.1.5q Conclusion

The designing of biogas plant depends on biogas requirement, manure requirement, digester capacity, gas holder size, heat requirement, heat losses and slurry withdrawn everyday from plant.

This designing based on a similar study reported in NAS publication (NAS, 1977).

7.1.6 Decision between fixed dome and movable drum Bio gas plant

On the basis of construction fixed dome type biogas plant require no steel sheet, run on a continuous batch process, provide richer fertilizer and ensures greater thermal insulation of the digester. But selection of a particular type of plant depends on several factors such as type and particle size of available waste material, skill level of local artisans and financial and technical capability of individual plant owners. So with all aspect our waste biomass is fibrous that is suitable for fixed dome type.

7.1.7 Procedure for constructing fixed dome type biogas plant

For constructing a fixed dome type biogas plant generally the following steps need be followed sequentially:

7.1.7a Collection of material

Collect all materials required including bricks, stones, cement etc.

7.1.7b Marking of digester pit

Mark the outline of the digester pit, the inlet and outlet tanks on the ground according to the above-mentioned size.

7.1.7c Excavation of the digester pit

Dig the digester pit and inlet and outlet tanks according to the marked profile. Sometimes it may happen that sides may collapse if the soil is not firm. To prevent collapsing
it is sometimes advisable to taper off the digester walls. For achieving desired insulation, soil around the digester pit should be appropriately packed.

7.1.7d Pouring the foundation slab

The floor can be either of concrete or brick work and is generally 20-40 cm thick.

7.1.7e Digester pit brickwork

For this purpose, bricks concrete block or stones can be used. Thickness of digester walls generally varies from 15 to 30 cm.

7.1.7f Construction of inlet and outlet tanks

Generally pipes of diameter 25 to 40 cm are appropriate for connecting the inlet and outlet links to the digester pit. Inlet and outlet pipes are inclined at an angle of 50 to 60 degrees.

7.1.7g Back-filling of digester pit wall

Every morning earth or sand should be filled into the space behind the section of the wall built. The previous day followed by proper ramming.

7.1.7h Building of dome

It is constructed by using full wooden shuttering.

7.1.7i Plastering of surfaces

Round off all edges and corners. Apply plastering material into layer comprising 1 portion of cement with 3 portion of sand and two parts of water. The plastering need to be carried out in 2-3 successive layer to a total 2 to 4 cm thick. If cement is not available, mortar can be used in its place which can be categorized as masonry mortar and plastering mortar.

7.1.7j Assembling of hatch cover

The hatch cover must be cast in concrete with a step all round. Water in slurry keeps the steal moist which makes it unusable after some time. As a result seal is required to be replaced after some time for breaking the fibrous material a stirrer passes through gas some lower end is pivoted into the floor of digester. It is sealed by an outer tube, which extends down below the minimum slurry level so that gas can not escape upper part of this tube is anchored and sealed in the fixed dome brick work.
7.2.1 Introduction

Organic waste is produced wherever there is human habitation the main form of organic waste are household food waste, agriculture waste, human & animal waste. In industrialized countries the amount of organic waste produced is increasing dramatically each year. Although many gardening enthusiasts ‘compost’ some of kitchen and garden waste, much of the household waste goes into landfill sites and is often the most hazardous waste. The organic waste component of landfill is broken down by microorganisms to form a liquid ‘leachate’ which contain bacteria, rotting matter and may be chemical contaminants from the landfill. This leachate can present a serious hazard if it reaches a water course or enter the water table the digesting organic matter in landfills also generates methane, which is a harmful greenhouse gas, in large quantity. Human organic waste is usually pumped to a treatment plant where it is treated then the effluents enter a watercourse, or it is deposited directly into the sea. Little efforts are made to reclaim the variable nutrients or enrage content of this waste.

In developing countries there is a different approach to dealing with organic waste. In fact, the world ‘waste’ is often an inappropriate term for organic matter, which is often put to good use. The economies of most developing countries dictates that material and resources must be used to their full potential, and this has propagated a culture of reused, repair and recycling. In many developing countries there exists a whole sector of recyclers, scavengers and collectors, whose business is to salvage ‘waste’ material and reclaim it for further use.

Where large quantities of waste are created, usually in the major cities, there are inadequate facilities for dealing with it, and much of this waste is either left to rot in the streets, or is collected and dumped on open land near the city limits. There is little environmental control in these countries to prevent such practices.

Mushroom species have been tried to be cultured on a large spectrum of plant wastes. Growth and yield of several species on such lignocellulosic substrates are reported (Nair, 1991). Reid (1989) has summarized the accessibility of herbaceous/ woody, dicot or monocot and gymnosperm waste biodegradation by mushroom species. More than 20 lignocellulosic wastes could be used as substrate for culturing more than 20 species (Poppe and Hofie, 1995). The unenriched wasted can be used: simply moistened or untreated, pasteurized, fermented or unfermented. The highest yields were achieved with
straw or corn-com based substrates for *Pleurotus*; saw dust for *shiitake, Pholiota* and *collybia*; grass chaff for *strophia* and compost for *Agaricus*. Also with sunflower peels, cotton waste, bean straw and linen waste unexpected high yields were obtained for different *Pleurotus* strain. Until now best adopted mushroom genera for fruit production on different wasted are *Agaricus, Pleurotus, Lentinus, Pholiota, Stroharia, volvariella* on fermented or pasteurized substrates, and *Auricularia, Collybia, Ganoderma, Hericium* on sterilized substrates. *Pleurotus* with its numerous enzymes and its bacteria inhabiting antibiotic pleurotine, can digest the greatest range of waste. On the other hand, *Pleurotus, Collybia* and *Volvariella* are always the fastest fructifying cultivated mushrooms showing already their first fruiting bodies 10 to 21 days after substrate-inoculation.

Waste green biomass obtains after essential oil extraction is rich in cellulose, hemicellulose and lignin. These organic chemical are basic substracts for growth and yield of mushroom. Mushrooms are highly neutraceatical having reach in protein.

Based on the aforesaid factors it has been designed to find out the possibility of using green spent residue (obtain after essential oil distillation) to produce edible mushrooms. By developing this technique, we can upgrade essential oil process unit integrated with mushroom production process.

Chemically, this essential oil bearing plants wastes based are lignocellulosic, which is difficult to degrade, or by far the most abundant renewably organic materials available for biological and chemical conversion into usable products. Lignocellulose is the building blocks of the stem, which support the plant till, attains its ultimate objective of a reproduction in the form of essential oil. It is not an organic material that can be degraded easily in the laboratory; usually one has to employ a strong acid, accompanied by high temperature. A wide range of microorganisms produces enzyme, which can degrade cellulose. Far fewer produce enzyme which can degrade the natural lignocellulosic waste because, lignin limits access to cellulose. In fact the only proven microbial means of converting unmodified lignocellulsic material is to be found in the biodegradability of various types of mushrooms (Kaneshirn, 1977). Most of the mushrooms species in general represent efficient and effective example of microbial world gifted with the unique ability of ligninolytic activity, to degrade lignin besides cellulose and hemicellulose inturn producing fruiting bodies consisting of the most of EAA, valuable vitamins, minerals and low energy carbohydrates (Crisan and sands 1978, Zakio Bano and

Bassham (1975) has estimated the net productivity of dry biomass due to photosynthesis by plants on the earth to be 155.2 billion tonnes per year. About two third of the biomass production occur on land, and remaining in the ocean. Most terrestrial plant material occur in forests (65%), with a little bit more then 15% generated in grasslands and cultivated lands. Mc Hale (1970) has calculated that about three-quarters of the approximate twenty four million tonnes of biomass generated on cultivated land and grassland is waste or residue. Recent figures of production of cultivated crop plant wastes in the world and India are presented in table 7.7

<table>
<thead>
<tr>
<th>Plant Wastes</th>
<th>World</th>
<th>India</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cereals</td>
<td>7,802,400</td>
<td>891,928</td>
</tr>
<tr>
<td>Pulse Plants</td>
<td>175,94</td>
<td>43,608</td>
</tr>
<tr>
<td>Oil Crops</td>
<td>354,185</td>
<td>35,527</td>
</tr>
<tr>
<td>Plantation Crops</td>
<td>1,370,065</td>
<td>221,580</td>
</tr>
<tr>
<td>Total</td>
<td>9,702,591</td>
<td>1,150,647</td>
</tr>
</tbody>
</table>

*Source: Derived from data by FAO Production Year Book (1994)*

**Table-7.7** Production of wastes of cultivated crop plants in the world and India (x 1000 MT)

Growth of mushrooms on lignocellulosic wastes is primarily a concept on solid state fermentation related to the absence or near absence of free liquid or free water (Hesseltine, 1992; Lonsane, et al, 1985), which corresponds to a moisture content up to 80% (Cannel and Moo-young, 1980a). The empty or void space must then be filled by gaseous phase, (Durand et al, 1983). Hence, the fungus uses undissolved material for its growth and metabolism (Mooyoung et al, 1983). The substrates are not a free liquid (Aldoo et al, 1982), where in the mushroom growth takes place on solid substrates by providing access of nutrients. The inputs and outputs during solid state fermentation of lignocellulosic waste during mushroom production are illustrated in fig. 7.2

**7.2.2 Chemistry of Lignocellulosic wastes**

These plant residues are mainly composed of cellulose, hemicellulose and lignin. Composition of wheat straw, rice straw, *mentha* spent residue and *cymbopogon* sp. spent residue are given in table 7.8. Sugar and amino acids are very low,
with major amount of about 60% holocellulose (Rajarathnam, et al., 1979). Woods vary from rice straw, in that lignin and ash content are higher; also because of more phenolics, these are very slowly degraded. Their nitrogen content, in other words, the crude protein is relatively low. Mushroom have the greatest advantage to grow over such substrates poor in nitrogen content, to produce in turn, fruiting bodies of nitrogen content higher than the growth substrate (Rajarathnam and Zakia Bano, 1981). They have tendency to concentrate the nitrogen in their growth substrate consequent to the mycelial ramification.

![Diagram](image)

* Source of C, N and minerals

SR: Spawn ramification
FN: Fructification

Fig- 7.1 Inputs and outputs during solid state fermentation (SSF) of lignocellulosic wastes by mushrooms.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Wheat straw</th>
<th>Paddy straw</th>
<th>Spent residue (Mentha arvensis)</th>
<th>Spent residue (Cymbopogon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose (%)</td>
<td>42.01</td>
<td>35.01</td>
<td>32.2</td>
<td>31.11</td>
</tr>
<tr>
<td>Hemicellulose (%)</td>
<td>22.01</td>
<td>24.01</td>
<td>20.4</td>
<td>20.1</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>7.80</td>
<td>8.55</td>
<td>16.44</td>
<td>14.22</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0.994</td>
<td>0.878</td>
<td>0.889</td>
<td>0.86</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.867</td>
<td>0.968</td>
<td>0.941</td>
<td>0.841</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.08</td>
<td>0.09</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>5.46</td>
<td>4.42</td>
<td>5.562</td>
<td>5.375</td>
</tr>
</tbody>
</table>

Table - 7.8 Chemical composition of different Agro-wastes.
7.2.3 Nutritional Value

The nutrients absorbed by the mycelium out of the decaying organic matter accumulated and gets transformed into various constituents of the mushroom fruit body. Mushrooms are thus a rich source of nutrients, particularly proteins, unsaturated fatty acids, minerals, fibres, and vitamins such as vitamin B, C and D. The nutraceutical value of different edible mushrooms are given in table-7.9. Their content of the anti-pellagra vitamin niacin is comparable to its levels found in pork or beef, which are the richest known sources of this vitamin. Mushrooms are also a good source of iron, potassium and phosphorus. They contain folic acid, which plays important role in the enrichment of the blood serum. On the other hand mushrooms are low in fat, calories, cholesterol and sodium which make them ideal food for persons with heart and kidney problems.

<table>
<thead>
<tr>
<th>Species</th>
<th>Moisture (Nitrogen-free)</th>
<th>Crude protein (N×4.38)</th>
<th>Crude fat (%)</th>
<th>Carbohydrate (nitrogen-free)</th>
<th>Crude fibre (Nitrogen-free)</th>
<th>Ash (%)</th>
<th>Energy value (K cal per 100g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Agaricus bisporus</em></td>
<td>78.3-90.5</td>
<td>23.9-34.8</td>
<td>1.7-8.0</td>
<td>51.3-62.5</td>
<td>44.0-53.5</td>
<td>8.0-10.4</td>
<td>7.7-12.0</td>
</tr>
<tr>
<td><em>Auricularia spp.</em></td>
<td>89.18</td>
<td>8.1</td>
<td>8.3</td>
<td>63.0</td>
<td>19.8</td>
<td>4.7</td>
<td>351</td>
</tr>
<tr>
<td><em>Flamulina</em></td>
<td>89.2</td>
<td>17.6</td>
<td>1.9</td>
<td>73.1</td>
<td>69.4</td>
<td>3.7</td>
<td>7.4</td>
</tr>
<tr>
<td><em>Volvariella volvacea</em></td>
<td>90.1</td>
<td>21.2</td>
<td>10.1</td>
<td>58.6</td>
<td>47.5</td>
<td>11.1</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Note: all data are presented as percentage of dry weight, except moisture (% of fresh weight) and energy value (K cal per 100g dry weight).

Source: Chang and Miles (1989); Chang and Hayes (1978).

Table – 7.9 proximate composition of cultivated species of edible mushrooms.

7.2.3a Protein Content

Edible mushrooms exhibit wide variations in their protein content. Even varietal and strainal differences in protein content have been reported. However, their value as a good source of protein is never disputed. They are considered as a potential substitute of muscle protein on account of their (i) high digestibility (Digestibility Coefficient around 89%), (ii) good amino acid content and (iii) about 1000 times higher production of mushroom protein per unit area. As mushrooms are grown on agriculture waste, hence the cost of production of mushroom protein is also lower than muscle protein. According to an estimate, 35,000 kg (dry weight) of mushroom protein can be produced from an acre of land during one year. Mushroom protein is not only
cheaper but is almost as nutritious as muscle protein. It also contains most of the essential amino acids in sufficient quantity and can prove a good supplement to those cereal diets, which lack in some essential amino acids. The amino acid compositions of different edible mushrooms are given in table 7.10.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>A. bisporus</th>
<th>L. edodes</th>
<th>P. sajor-caju</th>
<th>V. volvacea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>7.5</td>
<td>7.9</td>
<td>7.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.5</td>
<td>4.9</td>
<td>4.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Valine</td>
<td>2.5</td>
<td>3.7</td>
<td>5.3</td>
<td>5.4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2.0</td>
<td>nd</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Lysine</td>
<td>9.1</td>
<td>3.9</td>
<td>5.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>5.5</td>
<td>5.9</td>
<td>5.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.2</td>
<td>5.9</td>
<td>5.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.9</td>
<td>1.9</td>
<td>1.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.7</td>
<td>1.9</td>
<td>2.2</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Note: Data presented as grams of amino acids per 100 gram of corrected crude protein. nd = not detected
Source: Chang and Miles (1989)

Table – 7.10 Essential amino acid composition of edible mushrooms.

7.2.3b Minerals
Mushrooms are also good sources of several of minerals. However, they are particularly rich in phosphorous, potassium and iron but are low in sodium. About one third of the total iron in mushrooms is in the available form.

7.2.3c Vitamins
Mushrooms are good sources of several vitamins and excel many fruits and vegetables on this account. They are particularly rich in thiamine (B1), riboflavin (B2), niacin, biotin and ascorbic acid. Even, they are supposed to provide Vitamin A & D also, as ergosterol can be converted into Vitamin-D under ultraviolet irradiation. In *Agaricus bisporus* Vitamin K and Vitamin E have also been reported.

On the basis of all these qualities, the nutritional value of mushrooms as compared to other food-stuffs appear to be quite high as shown in table-7.11.

In addition to these, other interesting features in the nutritional qualities of mushrooms are (i) lack of starch, (ii) low fat contents, (iii) low calorific value (vi) presence of a variety of sugars and their derivatives and (v) high fibre content.
Table- 7.11 Nutritive values of mushrooms compared with various food items {Crisan and Anne Sands, 1978}

In an era, when diet-consciousness is increasing, mushrooms are becoming an important health food. Due to their nutritive qualities, they find a place in recommended diets of patients. In irregularities of carbohydrate metabolism, cardiac discomfort and Beri-beri disease, mushrooms are highly recommended due to their high contents of thiamine. Similarly, mushroom eating is helpful in Scurvy disease also.

7.2.4 Medicinal Value

Among the possible direct medicinal values of mushrooms the most important ones are their anticancer, hypolipidemic, hypcholesteremic and anti-hypertension effects. Agaricus bisporus, Auricularia poytricha, Lentinula edodes etc have been shown to lower down the blood cholesterol. Maximum activity of this kind was noticed with Lentinula edodes. When 5% dry powder of this mushroom was fed to rats for ten weeks, a reduction of 24% in the average plasma cholesterol level was observed. Similarly, antiviral and antibiotic principles have been reported in several mushroom species. Calvatia gigantea and Lentinula edodes also produce high molecular weight substances, which inhibit both influenza and polio-viruses.
They induce the formation of ‘interferon’, a compound that helps to fight virus infections. Calvacin, potential anti-tumour agent has been isolated from the giant puffball, *Calvatia gigantea*, which can be used against cancer. A polysaccharide, Lentinan, isolated from *Lentinula edodes* was also found to inhibit the growth of tumors in mice. Antitumor activity has been found in the Maitake mushroom (*Grifola frondosa*). Water extract of this mushroom has recently been reported to kill the HIV virus causing AIDS in human and was as effective against this disease as the widely used drug AZT. Maitake has beneficial effects against blood pressure and diabetes also. Reishi mushroom (*Ganoderma lucidum*) is perhaps the only mushroom today which is being used for preparing pills for marketing in several countries including India, in view of its rich content of immuno-regulating compounds. In fact, Reishi mushroom is called as the “Mushroom of Immortality” in Japan and as “Longevity Mushroom” in Korea.

7.2.5 Materials and Methods

Different steps are involved for determining the Efficacy of mint and palmarosa crops spent residue and their combinations for different kind mushroom production. The following steps are involved:

7.2.5a Maintenance of culture

The culture of *Agaricus bisporus* (Photo- 7.3) (Lange) sing, *Pleurotus sajor caju* (Photo- 7.4), *Calocybe indica* (Photo-7.5) and *Volvariella* sp. (Photo- 7.6) were used in the present investigation, were procured from the department of plant pathology, CCS Haryana Agricultural University, Hissar. The culture was multiplied and maintained on potato-dextrose agar (PDA) medium. It was transferred on PDA slants at periodic intervals of 20 days and stored at 5° C for further studies. Potato dextrose agar medium (PDA) had the following ingredients:

- Potato: 250gm
- Dextrose: 20gm
- Agar-Agar: 20gm

Distilled water to make 1000ml volume.

7.2.5b Cleaning and sterilization

All the glassware were cleaned with chromic acid mixture (100g potassium dichromate + 400ml hot water + 600ml concentrated sulphuric acid) and subsequently washed thoroughly under tap water. The glassware were sterilized in an oven at 180 ± 5°C for 90 min. The medium was sterilized in an autoclave at 15 lb. per sq. inch fresher for 20 min.
Photo- 7.3 Cultivation of *Agricus bisporus*

Photo- 7.4 Cultivation of *Pleurotus* sp.
Photo- 7.5 Cultivation of Calocybe indica

Photo- 7.6 Cultivation of Volvariella sp.
7.2.5c Inoculation
For multiplication and transfer of the fungus, culture slants of medium prepared in test tubes and quarter-sized flat bottles were used. The incubation was done under aseptic condition using laminar flow.

7.2.5d Spawn Preparation
For preparing spawn, the fungus mycelium was propagated on wheat grains (var. WH-147). The wheat grains were boiled for 30 min. using water 1.5 times the weight of grains and were allowed to soak water for another 15 min. without heating. The excess water was drained off and the grains were allowed to cool at room temperature. Calcium Carbonate (2% w/w) was mixed in the cooled boiled grains. The grains were then filled into half liters empty glucose bottles at the rate of 400gm per bottle. Bottles were plugged with non-absorbent cotton, sterilized and allowed to cool down over night.

7.2.5e Mother-spawn Preparation
To prepare mother spawn the sterilized grain bottles were inoculated with stock culture of *Agaricus bisporus* (Lange) sing, *Pleurotus sajor caju*, *Calocybe indica* and *Volvariella* sp. already grown in flat bottles. Putting two growth discs of 5mm diameter per bottles under aseptic conditions did the inoculation. The inoculated bottles were incubated at 25 ± 2°C

7.2.5f Sterilization
Polypropylene bags/bottles containing different substrates were sterilized at 20lb pressure per sq. inch for 2 hours.

7.2.5g Inoculation with commercial spawn
Polypropylene bags were inoculated with commercial spawn by transferring about 10 gm of spawn per bag aseptically using Laminar-flow. Inoculated bags were incubated at 25 ± 2°C.

7.2.5h Growth of fungus on substrates
Periodical observations were made to record the fungal growth and development of different substrates during spawn preparation.
7.2.6 Mushroom Cultivation

7.2.6a Preparation of compost

The compost was prepared by long method of composting using ingredients shown in table 7.12. Composting yard/place was cleaned and disinfected with 2 percent formalin. Wheat straw, the main ingredient of compost, was spread on the floor up to a height of 12 inches. Straw was wetted thoroughly by spraying water over it and kept as such for 48 hours so that every portion of the straw absorbed enough water. Two third quantity of calcium ammonium nitrate and urea each and full dose of super phosphate and muriate of potash were mixed in half the quantity of wheat bran and the mixture was then covered with wet gunny bags for 16 hours. The wet straw was spread on a floor and the above prepared mixture was thoroughly mixed in it and stacked (60” x 60”). The temperature of the stag reached 75°C in three days. The first turning to the compost was given on the sixth day to bring out inner portion out and vice versa and was left for four hours to cool down. 24 hr. prior to their mixing in the above compost, the remaining one third quantity of CAN and urea and half of the wheat bran were mixed separately and added to the above cooled compost. The whole contents were thoroughly mixed and stacked again. On the tenth day, the dry patches observed on the stack were watered and second turning was given and the stack was spread on the floor so that it cools down. After cooling down of straw, molasses and Furadan 3G were mixed and applied on straw and then again stacked. On the 13th day, similar turning as above was given and stacked. Like wise, turning and stacking was performed on the 16th, 19th and 22nd day as well. On the 25th day, 7th turning was given to the compost and BHC was added and thoroughly mixed in it. On 28th day (i.e. 8th turning), the compost was turned and checked for the presence of ammonia and moisture. Compost for sporophore production of *pleurotus sajor caju* was same as given in table 7.12 except paddy straw in place of wheat straw.

7.2.6b Supplementation at spawning

In the experiment, where compost was prepared with or without the use of gypsum and calcium carbonate, thorough mixing the grain spawn with compost did spawning.

7.2.6c Casing

To explore the possibility of using various locally available substrates, casing material in different combinations were prepared. Efficacy of
different combinations of casing material was compared with the substrate used alone. The casing materials prepared by thorough mixing were chemically treated with Formalin solution (3%). To disinfect the materials the solution of formalin in water was calculated at the rate of 40 L per m³ of the casing material. The treated materials were covered with polythene sheet for 48 hours. Subsequently the materials were turned on alternative day upto 7 days to remove the excessive fumes of formalin. The materials prepared were used for casing the spawned compost upto a depth of 1.5 inch. This was done after 20 days of spawning.

Three replications per treatment we were taken. The mushroom yield against each was observed and calculated per 10 kg of compost

<table>
<thead>
<tr>
<th>Compost</th>
<th>Spent residue (kg)</th>
<th>Wheat straw (kg)</th>
<th>CAN (Kg)</th>
<th>Urea (Kg)</th>
<th>Muriate of potash (Kg)</th>
<th>Super phosphate (kg)</th>
<th>Wheat bran (Kg)</th>
<th>Furadan (3G) (Kg)</th>
<th>HHC (5%) (kg)</th>
<th>Molasses (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat straw</td>
<td>-</td>
<td>1</td>
<td>0.030</td>
<td>0.012</td>
<td>0.010</td>
<td>0.010</td>
<td>0.100</td>
<td>0.00050</td>
<td>0.00083</td>
<td>0.0166</td>
</tr>
<tr>
<td>Mint leaves</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mint stem</td>
<td>1</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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</tr>
<tr>
<td>Mint whale plant</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mint leaves + wheat straw (1:1)</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mint stem + wheat straw (1:1)</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Mint whale plant + wheat straw (1:1)</td>
<td>0.5</td>
<td>0.5</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cymbopogon leaves</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cymbopogon leaves + wheat straw (1:1)</td>
<td>0.5</td>
<td>0.5</td>
<td>-</td>
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</tr>
</tbody>
</table>

Table- 7.12 Composition of different compost used for growth and development of *Agricus bispora*, *pleurotus sajor caju*, *Calocybe indica* & *Volvariella* sp.

246
Preparation of casing media

<table>
<thead>
<tr>
<th>Components</th>
<th>Proportions (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spent compost + Farm Yard manure + Soil</td>
<td>2:1:1</td>
</tr>
<tr>
<td>Spent Compost + Rice Burnt Husk + Soil</td>
<td>1:1:1</td>
</tr>
<tr>
<td>Rice Burnt Husk + Farm Yard Manure + Soil</td>
<td>2:1:1</td>
</tr>
<tr>
<td>Farm Yard Manure + Soil</td>
<td>1:1</td>
</tr>
<tr>
<td>Rice Burnt Husk + Soil</td>
<td>1:1</td>
</tr>
<tr>
<td>Rice Burnt Husk</td>
<td>-</td>
</tr>
<tr>
<td>Farm Yard Manure</td>
<td>-</td>
</tr>
<tr>
<td>Soil</td>
<td>-</td>
</tr>
</tbody>
</table>

7.2.6d Cropping Conditions

The spawned bags were placed in the cropping room over the racks. During the spawn running till pinhead initiation stage the temperature of cropping room was maintained at 23 ± 2°C. The relative humidity in the cropping room was maintained at 85 ± 5%. During spawn running the windows and doors of cropping room were kept closed to increase the concentration of carbon dioxide as it was thought to be beneficial for spawn running. Just after the pinhead initiation the room temperature was reduced to 16 ± 2°C. At this stage fresh air was allowed daily for two hours in the room during morning and evening by opening the windows and doors. The surface of the casing materials was kept moistened by spraying water with a fine sprayer twice a day. To check the saprophytic and other pathogenic infestations during cropping sprays with Bavistin and Dithane Z-78 (two sprays of each) at a concentration of 0.05 and 0.15 per cent, respectively were given. For checking insect infestation two sprays with Nuvan 76 EC (1 ml/lit) were given.

7.2.6e Picking of Mushrooms

The mushroom fruiting bodies developed were harvested by gentle twisting before opening of their caps when they attained a diameter of about 3 cm and causing minimum disturbance to the small developing mushroom. The number of harvested mushrooms for each treatment was recorded. The weight of fresh mushrooms was also recorded. The average of replicated data was taken on the basis of per kg substrate. After initiation of pinning the harvesting of mushrooms continued upto the 75th day.

The degradation of composts after 75 days was analyzed by the different methods as earlier stated in this chapter.

7.2.7 Result & Discussion

Table 7.13 shows the yield of mushrooms grown on different substrates. Agrious bisporus did not show good growth when it was

247
grown on mint leaves, mint stem or in combination. Hardly 30-35% in *Agricus bispora* was noticed as compare to control. By adding wheat straw to mint crop-spent residue the growth of *Agricus* was noticed to be about 60-70%. Using *Cymbopogon* leaves alone did not show encouraging results on *Agricus bispora* production. However, when the same was substituted with 50% wheat straw the production raise upto 70%. These results suggest that mint or *Cymbopogon* crop in combination with straw can be used as a low cost substrate for the production of *A. bispora*.

*Pleurotus sajor caju* showed good response when grown on spent residue obtained from steam distillation of mint or *Cymbopogon* crop. About 13% increase in mushroom productivity by using such spent residue over value of control.

By using spent residue plus wheat straw the production of *P. sajor caju* was increased about 25%. However, straw in combination with mint stem showed only 13% increase in *P. sajor caju* production. Use of mixture of whole plant & straw (1:1) as substrate showed about 24% increase in production of *P. sajor caju*.

*Cymbopogon* spent residue was also proven to be good substrate for the growth of *P. Sajor caju*. About 17% increase in *P. Sajor caju* was noticed when *Cymbopogon* leaves were alone used as substrate by addition of straw (1:1). The increase production amount of *P. Sajor caju* was raised to 35%.

*Calcybe indica* gave good response when grown on spent residue both of mint as well as *Cymbopogon* crop. The maximum increase in production of *Calcybe indica* was seen when mixture of mint leaves and wheat straw was used as substrate for growth.

*Cymbopogon* spent residue in combination with wheat straw also gave encouraging results of *Calcybe indica* production. By mixing of *cymbopogoan* with wheat straw, the production of *Calcybe indica* was increased upto 40%.

*Volvariella* sp did not show much increase in production by using spent residue of *Cymbopogon* crop. However, the value of production of control and spent residue grown *Volvariella* sp was noticed to be about same.

Mushroom cultivation have carried out by taking wide variety of Agro waste (Thomas G.V. et al. 2003). A lot of literature is available on production of variety of mushroom by taking green waste like banana leaves, bean straw, corn cobs, cotton straw, grass chaff, sugar cane (Chand Rashekar, 2003), bagerse, shredded paper, saw dust, rice straw, wheat straw, etc (Rajarathanam S. 1997).

Generally the highly lignified tissue takes more time for bio-degradation (Smith, et al, 1987). So the regular time period of treatment is not adequate for bio-degradation of lignin and as a result
cellulose and hemicellulose of biomass take time to its highly resisted crystalline structure and also its lignin surrounding (Cowing, 1975, T590, 1978, Fan et al 1980a; 1980b). This is the reason that high lignin content mint stem and Cymbopogon leaves did not suit to Agricus bisporus for luxury growth. The reason for co-response of mint leaves for Agricus growth was not investigated.

Generally the process of sporophore production appearance of pinhead and its final growth and development depend on the potential of lignolytic enzyme and cellulose enzyme activity of type of mushroom grown on a substrate (Boyle et al, 1992). In this connection P. Sajor caju was examined for its response to different green waste and the result was given in table 7.14. Mushroom (P. Sajor caju) grown on paddy straw took maximum time for pinhead appearance followed by mint stem, mint leaves. Combination of paddy straw with mint or Cymbopogon spent residue did not change the time of appearance of pinhead.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Substrate &amp; combination</th>
<th>Yield of mushroom species/ kg day spent residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Agricus bisporus</td>
</tr>
<tr>
<td>1</td>
<td>Wheat straw</td>
<td>2.80 ± 0.021</td>
</tr>
<tr>
<td>2</td>
<td>Mint leaves</td>
<td>0.97 ± 0.017</td>
</tr>
<tr>
<td>3</td>
<td>Mint stem</td>
<td>0.80 ± 0.016</td>
</tr>
<tr>
<td>4</td>
<td>Mint whale plant</td>
<td>0.95 ± 0.015</td>
</tr>
<tr>
<td>5</td>
<td>Mint leaves + wheat straw (1:1)</td>
<td>1.91 ± 0.016</td>
</tr>
<tr>
<td>6</td>
<td>Mint stem + wheat straw (1:1)</td>
<td>1.60 ± 0.017</td>
</tr>
<tr>
<td>7</td>
<td>Mint whale plant + wheat straw (1:1)</td>
<td>1.86 ± 0.018</td>
</tr>
<tr>
<td>8</td>
<td>Cymbopogon leaves</td>
<td>1.21 ± 0.016</td>
</tr>
<tr>
<td>9</td>
<td>Cymbopogon leaves + wheat straw (1:1)</td>
<td>2.02 ± 0.017</td>
</tr>
</tbody>
</table>

Table -7.13 Yield of different Mushroom species on various lignocellulosic substrates & their combination with wheat straw
On the basis of weight and morphology of sporophore mixture of mint spent residue & paddy straw residue was proven to be a better substrate as compare to paddy straw alone.

Table 7.15, shows degradation of green spent residue of mint & Cymbopogon crop by P. Sajor caju. Combination of mint spent residue and paddy straw or Cymbopogon leaves and paddy straw shows good mycellium growth as compared to spent residue alone. The bio-degradation of cellulose, hemicellulose and lignin was noticed to be high in such substrate used for P. Sajor caju growth. During such activity the availability of N, Ca, Mg to P. Sajor caju was noticed to be maximum as compared to control.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Days of pinhead appearance</th>
<th>Number of sporophores</th>
<th>Weight of sporophores (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paddy straw</td>
<td>22 ± 0.6</td>
<td>10.0 ± 0.3</td>
<td>140 ± 0.3</td>
</tr>
<tr>
<td>Mint leaves</td>
<td>18 ± 0.5</td>
<td>10.0 ± 0.3</td>
<td>142 ± 0.2</td>
</tr>
<tr>
<td>Mint stem</td>
<td>19 ± 0.6</td>
<td>10.0 ± 0.4</td>
<td>140 ± 0.3</td>
</tr>
<tr>
<td>Mint whole plant</td>
<td>18 ± 0.6</td>
<td>10.0 ± 0.4</td>
<td>142 ± 0.4</td>
</tr>
<tr>
<td>Mint leaves + Paddy straw (1:1)</td>
<td>18 ± 0.6</td>
<td>13.0 ± 0.4</td>
<td>152 ± 0.2</td>
</tr>
<tr>
<td>Mint stem + wheat straw (1:1)</td>
<td>19 ± 0.6</td>
<td>12.0 ± 0.5</td>
<td>149 ± 0.3</td>
</tr>
<tr>
<td>Mint whole plant + Paddy straw (1:1)</td>
<td>18 ± 0.6</td>
<td>12.0 ± 0.5</td>
<td>150 ± 0.2</td>
</tr>
<tr>
<td>Cymbopogon leaves</td>
<td>19 ± 0.6</td>
<td>10.0 ± 0.4</td>
<td>143 ± 0.3</td>
</tr>
<tr>
<td>Cymbopogon leaves + Paddy straw (1:1)</td>
<td>18 ± 0.7</td>
<td>13.0 ± 0.5</td>
<td>154 ± 0.5</td>
</tr>
</tbody>
</table>

Table-7.14 Sporophore production of pleuroteus sajor caju on mint & Cymbopogon waste & their combination with paddy straw.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Major %</th>
<th>Minor %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose</td>
<td>H-cellulose</td>
</tr>
<tr>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Paddy straw</td>
<td>35.0±0.2</td>
<td>27.0±0.02</td>
</tr>
<tr>
<td>Mint leaves</td>
<td>46.1±0.04</td>
<td>26.0±0.05</td>
</tr>
<tr>
<td>Mint stem</td>
<td>30.2±0.5</td>
<td>24.2±0.15</td>
</tr>
<tr>
<td>Mint whole plant</td>
<td>32.2±0.02</td>
<td>23.0±0.04</td>
</tr>
<tr>
<td>Mint leaves + Paddy straw (1:1)</td>
<td>35.5±0.12</td>
<td>23.0±0.02</td>
</tr>
<tr>
<td>Mint stem + wheat straw (1:1)</td>
<td>33.5±0.03</td>
<td>23.0±0.02</td>
</tr>
<tr>
<td>Mint whole plant + Paddy straw (1:1)</td>
<td>34.0±0.3</td>
<td>23.0±0.05</td>
</tr>
<tr>
<td>Cymbopogon leaves</td>
<td>35±0.2</td>
<td>24.0±0.13</td>
</tr>
<tr>
<td>Cymbopogon leaves + Paddy straw (1:1)</td>
<td>35.0±0.4</td>
<td>22.0±0.02</td>
</tr>
</tbody>
</table>

Table 7.15 Bio degradation of green spent residue of mint & *Cymbopogon* by *P. sajor caju*.