CHAPTER 5

RESULTS

&

DISCUSSION
The microorganisms' VIABILITY depends on the used energy nature, the amount of absorbed energy, the microorganisms' susceptibility and the irradiation condition. For the same microbial culture dilution, microwave cavity type, microwave power, culture condition, microorganisms concentration (MOC), for differences in time period of exposure, shows remarkable decrease in viable count. To examine the non thermal effect of microwaves a series of experiment was performed, in which rate of inactivation of viable microorganisms was compared with duration of microwave irradiation. By measuring the optical density of sample we can determine change in cell mass and cell number, for this we have used spectrophotometer, which measures amount of light passing through suspension of microbe and the amount of light passing absorbed or scarrered by microscope is proportional to its cell density.

Fig 6. given below shows the variation of the number of microorganisms in CFU/ml as a function of the sample absorbance measured at 600 nm. The results show the linear dependence of the absorbance on the number of microorganisms in CFU/ml. By using this relation we can calculate the number of the microorganisms/ml (C) from the measured value of its absorbance (A). The relation can easily express the linear dependence:

\[ C = 9.7 \times 10^9 A \]
Effect of 2450 MHz microwave radiation on *E. coli*

![Graph showing the OD (nm) over time for E. coli under different conditions.](image)

**Figure 7**

Effect of 2450 MHz microwave radiation on *Bacillus subtilis*

![Graph showing the OD (nm) over time for B. subtilis under different conditions.](image)

**Figure 8**
5.1 viability of irradiated cell

It is clear from figure 7 and 8 that the exposure periods 4 and 8 hrs decreased the absorbance and in accordance with equation (1), indicates a decrease in the cells number and consequently an inhibition case for the bacteria. For this reason we used the exposure period of 4 h (volume A) and 8 hr (volume B) as an inhibition case where the number of cells was $10^8$ and became $10^7$ cells/ml.

It is clear from the figure 7 that there is a decrease in the growth rate of the E. coli cells exposed to 4 hr, and further decrease in growth rate of E. coli cells exposed to 8 hours relative to its unexposed control ones.

Fig. 8 explains the growth rate of Bacillus Subtilis, volumes C exposed for 4 hours and volume D exposed for 8 hours. It is clear from the figure that there was a slight decrease in the growth rate of volume C, cell density in both untreated and microwave treated slightly decrease in similar pattern. Volume D shows further decrease in growth rate.

And when figure 7 and figure 8 were compared, we can clearly make out that volume C and volume D shows decrease in growth to much lesser extent compared to volume A and volume B. It is because that cell wall of Bacillus subtilis which is a gram positive bacteria is more resistant to stress condition, the cell wall is thicker, amorphous, single layered, with 80% of cell wall composed of several layers of peptidoglycans distributed uniformly in 3 dimensional network, all making it stronger than the multilayered, thin cell wall of gram negative bacteria.
FIGURE 9
First Petridish is control one showing growth of E. coli without any microwave treatment. The Petridish is showing quite dense growth.

Second Petridish is prepared from culture that has been exposed to microwave radiation for 4 hour. The Petridish is showing lesser growth of E. coli cells compared to untreated one.

Third Petridish is prepared from culture that has been exposed to microwave radiation for 8 hour. The Petridish is showing least growth of E. coli cells compared to above two plates.
First Petridish is control one showing growth of Bacillus subtilis without any microwave treatment. The Petridish is showing quite dense growth.

Second Petridish is prepared from Bacillus culture that has been exposed to microwave radiation for 8 hour. The Petridish is showing lesser growth Bacillus subtilis cells compared to above Petridish.
Effect of 2450 MHz microwave radiation on 
E. coli when treated with SDS

![Graph of E. coli data](image1)

Figure 11

Effect of 2450 MHz Microwave Radiation on Bacillus 
Subtilis when treated with SDS

![Graph of Bacillus data](image2)

Figure 12
5.2 Irradiated cell viability in presence of SDS

Fig.11 shows that how the presence of SDS influence the growth rate of *E.Coli* relative to their control. The irradiated cells at 4 hr exposure period shows enormously large decrease in growth rate relative to their control in presence of SDS. but it did not decrease in absence of SDS. In the case of untreated cell suspension, no significant reduction in cell density was observed during 4hr of incubation, regardless of presence of SDS. these results support the conclusion that most of cells inactivated by microwave radiation remain unlysed in cell suspension in the absence of SDS. Also it shows that microwave injured *E.coli* cells would be sensitive to SDS and that untreated cells are some how resistant.

Fig.12 shows that how the presence of SDS influence the growth rate of *Bacillus subtilis* relative to their control. Though we cannot see measurable amount of differences in the growth rate of bacillus exposed for 4 hours with respect to there relative control but 8 hr exposure shows some differences with respect to their control. so here in case of *Bacillus subtilis*, both untreated and microwave treated cells were unexpectedly resistant to SDS. This may be due to fact that the cells of *B.subtilis*, a gram positive bacteria, were not lysed even in presence of SDS because of their thick and rigid cell wall structure.
Figure 13:
Showing effect of microwave on SDS treated E.coli culture.
First Petridish at the top is prepared from only SDS treated culture, as we move anticlockwise, the second Petridish is prepared from 4hr irradiated culture, we can see reduction in viable cells the third plate is showing vast reduction in cells after 8 hr microwave exposure.

Figure 14:
Petridishes showing effect of SDS on microwave treated Bacillus subtilis culture and their respective unexposed control.
The plate at bottom is the control one, only SDS treated, as we move clockwise from here the second petridish is showing little reduction in viable cells after the culture have been 4 hr irradiated, third petridish is showing quite more reduction after 8 hr irradiated.
Figure 15

Effect of 2450 MHz Microwave radiation on E.coli at pH 5

Effect of 2450 MHz Microwave radiation on E.coli at pH 6
Figure 16

Effect of 2450 MHz Microwave radiation on E. coli at pH 7

Time (Hr)

0 0.5 1 1.5 2 2.5
O.D (nm)

c 4hr 8hr

Figure 17

Effect of 2450 MHz Microwave radiation on E. coli at pH 8

Time (Hr)

0 0.5 1 1.5 2 2.5
O.D (nm)

c 4hr 8hr
5.3 Effect of pH on irradiated sample of *E.coli*

Fig.15, Fig.16, Fig.17, Fig.18 and fig19, shows how differences in pH change at pH 5, pH 6, pH 7, pH 8 and pH 9, influence the growth rate of irradiated *E.coli* bacterial culture and their respective control. *E.coli* bacteria is showing maximum growth rate at pH 6 and pH 7. We can also observe that there is remarkable decrease in growth rate of 8 hr irradiated bacteria at pH 5 and pH 9.
Figure 20: Petridishes showing effect of pH on microwave treated E.coli culture and their respective unexposed control. The petridish at bottom is showing most dense growth at pH 7. The upper petridish is also showing good growth.

Figure 21: Petridish at pH 8 is showing good growth, while culture at pH 5 and pH 9 is showing less dense growth.
Effect of 2450 MHz Microwave radiation on Bacillus subtilis at pH 5

Figure 22:

Effect of 2450 MHz Microwave radiation on Bacillus subtilis at pH 6

Figure 22:
Figure 23:

Effect of 2450 MHz Microwave radiation on Bacillus subtilis at pH 7

Figure 24:

Effect of 2450 MHz Microwave radiation on Bacillus subtilis at pH 8
5.4 EFFECT OF PH ON IRRADIATED SAMPLE OF *Bacillus subtilis*

Fig.22, Fig.23, Fig.24, Fig.25 and Fig.26, shows how differences in pH change, at pH 5, pH 6, pH 7, pH 8 and pH 9, influence the growth rate of irradiated *Bacillus subtilis* bacterial culture and their respective control. Bacterial culture of *Bacillus subtilis* is showing maximum growth rate at pH 7 - pH 8.

At pH 5 and pH 6, there is vast decrease in growth rate of bacteria irradiated for 8 hour, rest other pH condition does not show much effect on growth rate of irradiated bacterial culture of *Bacillus subtilis* and their respective control.
Figure 27:
Petri dishes showing how, irradiated Bacillus subtilis culture at different pH grew on agar medium. Petri dishes at pH 7 and pH 8 is showing maximum growth. Petri dish at pH 6 is showing lower density.

Figure 28:
Both petri dishes of irradiated culture of Bacillus subtilis at pH 5 and pH 9 is showing very low density of viable cells of Bacillus subtilis. There have been quite lot of inhibition in its growth after microwave irradiation.
<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Mode of action</th>
<th>Diameter of inhibition zone in cm for <em>E. coli</em></th>
<th>Diameter of inhibition zone in cm for <em>Bacillus subtilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (8 hr)</td>
<td>Exposed (8 hr)</td>
</tr>
<tr>
<td>Nalidixic acid (30μg/ml)</td>
<td>Inhibition of bacterial protein synthesis and act on ribosome</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Tetracycline (30μg/ml)</td>
<td>Inhibition of protein synthesis by combining with 30s ribosomal subunit.</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Amoxicillin (30μg/ml)</td>
<td>Inhibition of cell wall synthesis of bacteria</td>
<td>.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Kanamycin (30μg/ml)</td>
<td>Inhibition of protein synthesis by combining with 30s ribosomal subunit.</td>
<td>1</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Table 4: The antibiotic test for exposed and unexposed bacterial strain:-
Figure 29:
First petridish is prepared from culture of microwave treated Bacillus subtilis; it is clear from the photograph that, zone of inhibition have increased compared to the second petridish of control culture, if we check it clockwise from top, there is increase in diameter of inhibition zone for Nalidixic acid, Tetracycline, Kanamycin shows maximum increase in inhibition zone, while Amoxycillin is showing zero zone of inhibition after treatment also.

Figure 30:
First petridish is prepared from untreated E.coli culture; it is clear from the photograph that, zone of inhibition of second petridish of microwave treated culture have increased, if we check it clockwise from top, there is increase in diameter of inhibition zone for Nalidixic acid, Tetracycline, Kanamycin, while Amoxycillin is showing minute increase in zone of inhibition after treatment also.
Bradford protein assay

**KNOWN**

<table>
<thead>
<tr>
<th>BSA (µg/ml)</th>
<th>O.D (595 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0732</td>
</tr>
<tr>
<td>5</td>
<td>0.093</td>
</tr>
<tr>
<td>10</td>
<td>0.1007</td>
</tr>
<tr>
<td>20</td>
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<tr>
<td>40</td>
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<td>60</td>
<td>0.1887</td>
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<td>80</td>
<td>0.2183</td>
</tr>
<tr>
<td>100</td>
<td>0.2411</td>
</tr>
<tr>
<td>120</td>
<td>0.2603</td>
</tr>
<tr>
<td>140</td>
<td>0.2776</td>
</tr>
</tbody>
</table>

Table 5

**figure 31:**

**TIME OF EXPOSURE**

<table>
<thead>
<tr>
<th>TIME OF EXPOSURE (HR)</th>
<th>E.coli</th>
<th>CONCENTRATION (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O.D INT(I)</td>
<td>O.D EXT (II)</td>
</tr>
<tr>
<td>4</td>
<td>.1028</td>
<td>.1005</td>
</tr>
<tr>
<td>8</td>
<td>.1193</td>
<td>.1032</td>
</tr>
</tbody>
</table>

O.D INT-optical density for intrinsic protein
O.D EXT-optical density for extrinsic protein

Table 6: protein concentration determined in *E.coli* with the help of Bradford Protein Assay
Table 7: Protein concentration determined in *Bacillus subtilis* with the help of Bradford Protein Assay.

### 5.6 Leakage of protein from cells due to microwave irradiation

We have determined concentration of the protein that had been released, due to microwave injury of cell with the help of Bradford method of protein assay. Data shows that as the time period of irradiation increases, the amount of protein released from cell suspension have also increased. Though there have not been much difference in amount of intracellular and extra cellular protein released from both the strains, still it is clear from the data that the amount of protein released from *Bacillus subtilis* is much lower than that in *E.coli*. 

<table>
<thead>
<tr>
<th>TIME OF EXPOSURE (HR)</th>
<th>Bacillus subtilis</th>
<th>CONCENTRATION (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O.D INT(III)</td>
<td>O.D EXT(IV)</td>
</tr>
<tr>
<td>4</td>
<td>.098</td>
<td>.087</td>
</tr>
<tr>
<td>8</td>
<td>.1023</td>
<td>.091</td>
</tr>
</tbody>
</table>
