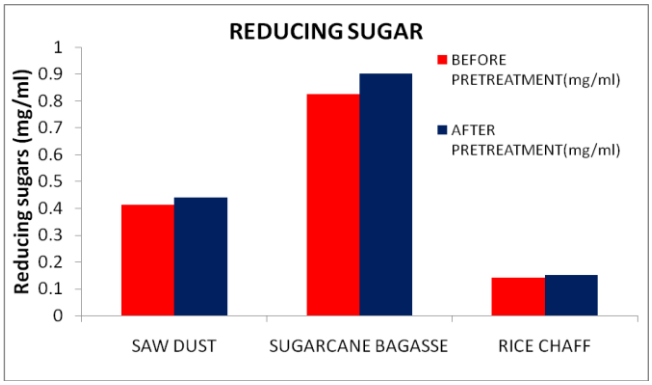

Figure 3: Estimation of reducing sugar in different substrate

Table 1: Estimation of reducing sugar content

Substrate	*Before pre-treatment(mg/ml)*	*After pre-treatment(mg/ml)*
Saw dust	0.412	0.44
Sugarcane bagasse	0.8241	0.9
Rice chaff	0.1413	0.15

3.3 Enzyme assay of Lignocellulosic biomass

1g of each pre treated lignocellulosic biomass was taken and added as a carbon source in 100ml of MSM media. The microbes were inoculated with each respective biomass at a constant pH of 5.6 for fungi and 7.0 for bacteria. Every 48 hr 10ml of cultural broth was taken and was checked for enzyme activity by CMC assay. It was found that *Aspergillus niger* showed the highest enzyme activity of 600.52IU/ml in sugarcane bagasse. *Trichoderma viride* showed the highest enzyme activity of 480.42 IU/ml in rice chaff where as *Bacillus subtilis* showed the highest enzyme activity of 390.34IU/ml in sugarcane bagasse. These biomass were selected as substrates for the production of bioethanol.

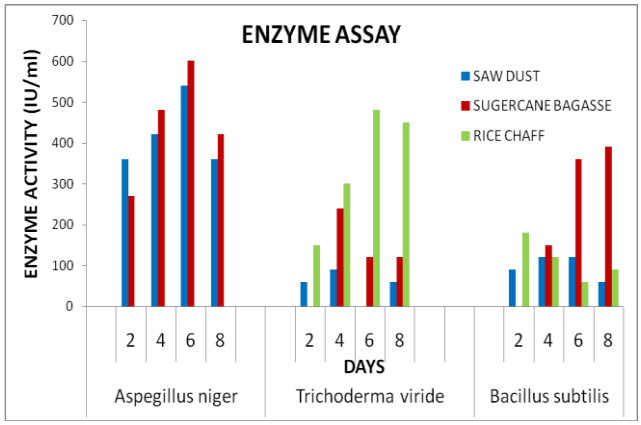

Figure 4: Enzyme activity of Microbes on different substrate

3.4 Fermentation

The microbes that showed the highest activity with respect to the given substrate were chosen. *Aspergillus niger* showed the highest enzyme activity on sugarcane bagasse. *Trichoderma viride* showed the highest enzyme activity on rice chaff. *Bacillus subtilis* showed the highest activity on sugarcane bagasse. 10g substrate was taken as a carbon source in MSM and the fermentation media was made up to 1liter.For fermentation by bacteria the pH was set to 7.0 and for both the fungi the pH was set to 5.6. After 5 days *Saccharomyces cerevisiae* was inoculated in the fermentation broth and after 10 days the fermentation broth was taken out for the process of distillation. Another set of batch was prepared and the above process was repeated with *Zymomonas mobilis* (MTCC 2428). The batch was then inoculated with *Zymomonas mobilis* (MTCC 2428) and after 10 days fermented broth was taken out for distillation. Simultaneous Saccharification and Fermentation process (SSF) was performed.

3.5 Distillation

The distillate obtained from the fermentated broth was collected and was measured. The highest distillate obtained was from the substrate sugarcane bagasse that was simultaneously saccharified and fermented by *Aspergillus niger* and *Saccharomyces cerevisiae* which was found to be 35ml. With same substrate *Bacillus subtilis* and *Saccharomyces cerevisiae* distillate was found to be 18ml. With *Aspergillus niger* and *Zymomonas mobilis(MTCC 2428)* the distillate was found to be 15ml. Rice chaff that was simultaneously saccharified and fermented by *Trichoderma viride* and *Saccharomyces cerevisea* the distillate obtained was 25ml.

Table 2: Amount of distillate obtained from the biomass and type of microorganism used

Substrate	**Microorganism**	**Distillate(ml)**
Sugarcane bagasse	*Aspergillus niger* and *Saccharomyces cerevisiae*	35
Sugarcane bagasse	*Bacillus subtilis* and *Saccharomyces cerevisiae*	18
Rice chaff	*Trichoderma viride and Saccharomyces cerevisiae*	25
Sugarcane bagasse	*Aspergillus niger and Zymomonas mobilis MTCC 2428*	15

3.6 Analysis of distillate by HPLC

After the distillates were obtained they were filtered and sonicated. The samples prepared were injected into the HPLC machine and the chromatogram was observed.

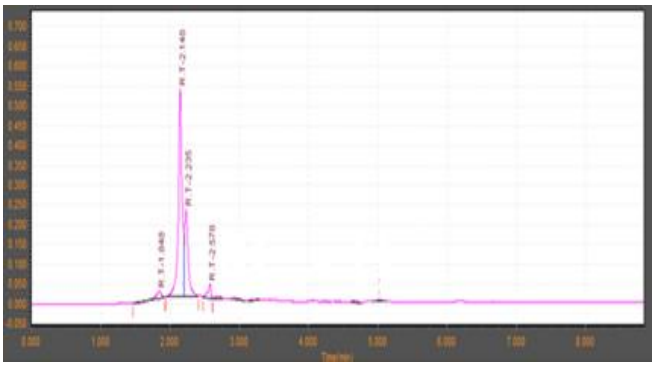

Figure 5: Chromatogram showing peak of 5% ethanol run in HPLC as standard

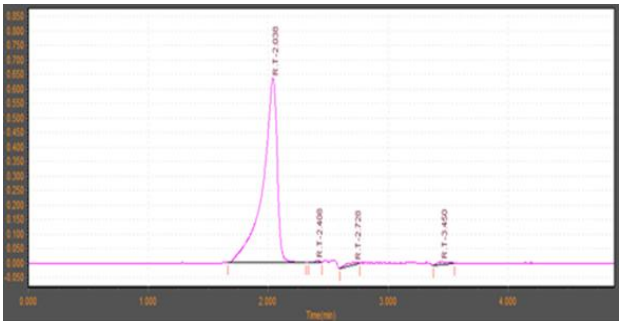

Figure 6: Chromatogram showing distillate sample obtained from sugarcane bagasse which was simultaneous saccharified and fermented by *Bacillus subtilis* and *Saccharomyces cerevisiae.*

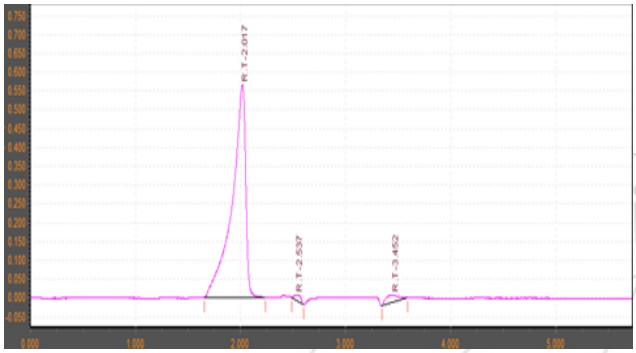

Figure 7: Chromatogram showing distillate sample obtained from sugarcane bagasse which was simultaneous saccharified and fermented by *Aspergillus niger* and *Saccharomyces cerevisiae.*

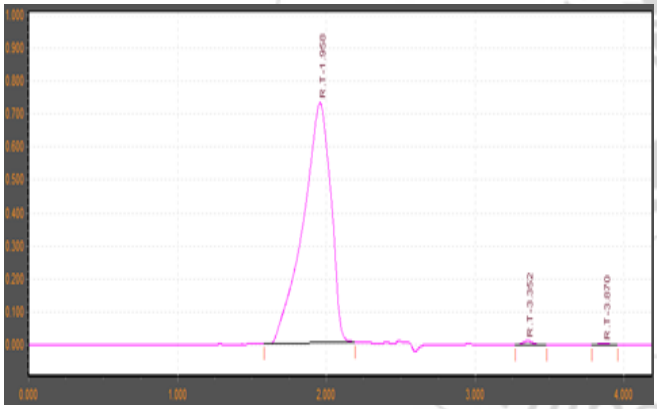

Figure 8: Chromatogram showing distillate obtained from Rice chaff which was simultaneous saccharified and fermented by *Trichoderma viride* and *Saccharomyces cerevisiae.*

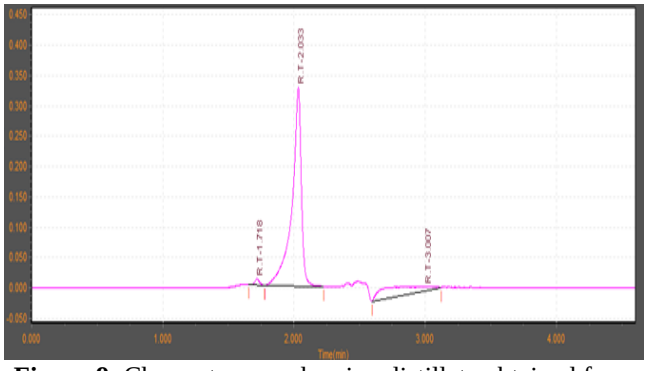

Figure 9: Chromatogram showing distillate obtained from sugarcane bagasse which was simultaneous saccharified and fermented by *Aspergillus niger* and *Zymomonas mobilis(* MTCC 2428).

4. Discussion

The present work carried out with the aim to produce bioethanol from lignocellulosic biomass by simultaneous saccharification and fermentation. Cellulose degrading microbes were isolated and identified. From the above study it was found out that for the production of bio ethanol from lignocellulosic material, firstly the cellulase enzyme activity of each microorganism was checked with each substrate. Sugarcane bagasse and rice chaff were chosen on which the highest cellulase activity of microorganism were recorded. In a study conducted it was found that the substrates such as Banana peel and sugarcane waste had more capability to produce ethanol as compared to waste paper by using cellulase enzyme [11]. Simultaneous Saccharification and Fermentation process was performed. After the saccharification process was over the fermentation microbes such as *Saccharomyces cerevisiae* and *Zymomonas mobilis* (MTCC 2428) were incorporated for the conversion of soluble sugars into ethanol. After the Fermentation process the broth was taken was distillation. The distillate obtained was measured and they were analysed for ethanol content. The reference was made by 5% ethanol and it was runned in HPLC. In the same way the distillate were also runned in HPLC. The peak of the reference and the sample were compared based on their retention time. In a similar study conducted for the analysis of the contents of ethanol were determined using Gas Chromatography (GC), propenol was used as standard [12]. In the comparison of these two fermenting microbes it was found that *Saccharomyces cerevisiae* was able to ferment the soluble sugars easily but *Zymomonas mobilis (MTCC 2428)* showed results only with sugarcane bagasse that was saccharified by *Aspergillus niger*.

5. Conclusion

From the present study conducted it was found that *Aspergillus niger* and *Bacillus subtilis* showed the highest cellulase activity on sugarcane bagasse indicating it as a good substrate for the production of cellulase enzyme and also a very low cost, readily available lignocellulosic biomass for the production of bioethanol.

6. Future Scope

The isolated microorganism can be further studied and used to optimize the fermentation process for the production of Bioethanol.

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