CHAPTER 6

SUMMARY

Studies on microbial interaction on biomass in biogas production were conducted with the aim of utilizing weed biomass and sugarcane press-mud as renewable energy sources as alternative to cow-dung to meet the energy demand. An attempt also has been made to understand the mechanism of microbial activity in the degradation of biomass leading to the methane gas production.

The different weeds selected for the present study were subjected to various treatments:

Treatment 1: A mixture of cow-dung, biomass of weed and water in a ratio of 5.0:1.0:6.5

Treatment 2: A mixture of cow-dung, biomass of weed, sugarcane press-mud and water in a ratio of 5.0:1.0:1.0:7.5

Treatment 3: A mixture of cow-dung, biomass of weed, sugarcane press-mud and water in a ratio of 2.5:2.0:2.5:7.5

Treatment 4: Formulation of treatment 3 + pure liquid culture of press-mud bacteria (*Bacillus subtilis*, *Citrobacter koseri* and *Proteus rettgeri*, 50 ml each)
Treatment 5: Formulation of treatment 3 + pure liquid culture of press-mud fungi (Aspergillus flavus, A. niger, Chaetomium oleavacearum, Fusarium oxysporum and Periconia prolifica, 50 ml each)

Of the selected weeds, Dodonaea viscosa, Eichhornia crassipes, Jatropha gossypifolia, Parthenium hysterophours and Prosopis spicigera have high biogas potential. Calotropis gigantea, Cassia auriculata and Datura metel have moderate biogas potential. Antigonon leptopus, Croton bonplandianum, Ipomaea carnea and Martynia annua have low biogas potential in treatments 1, 2 and 3.

The addition of sugarcane press-mud in treatment 2 accelerated the production of biogas. The biogas acceleration ranged from 10.53 to 20.90 per cent at 30°C and from 9.11 to 19.03 per cent at 35°C over treatment 1 (without sugarcane press-mud). The optimum temperature for maximum production of biogas was found to be 35°C.

It was observed that the cellulose content from 17.98 per cent to 24.10 per cent and hemicellulose content from 10.03 per cent to 14.40 per cent produced more biogas, provided the cellulolytic bacterial count was high.

It is obvious from the present study that for higher biogas production, it is essential that carbon and nitrogen ratio should range between 25:1 and 31:1 and the volatile
fatty acids content between 8.61 and 9.10 mg per litre. Phosphorus and potassium content of the substrate do not seem to have any significant influence on biogas production.

Under different types of treatment, the maximum percentage 71.17 of methane was yielded by *D. viscosea* in treatment 4, 65.00 per cent of methane by *E. crassipes* in treatment 2, 62.67 per cent of methane by *P. spicigera* in treatment 5, 62.33 per cent of methane by *J. gossypifolia* in treatment 3 and 53.67 per cent of methane by *P. spicigera* in treatment 1.

Of the different treatments, treatment 3 could be the best formulation, in which the cow-dung proportion was reduced to 50 per cent and the biogas output was maintained more or less stable.

The presence of bacteria such as *Eubacterium tortuosum* (coliform-fermentative), *Clostridium butyricum* (cellulolytic)—*Selenomonas ruminantium* (lipolytic) and *Bacteriodes ruminicola* (proteolytic), ensures hydrolytic, acetogenic and homoacetogenic stages of methanogenesis.

The presence of *Methanobacterium ruminatum*, *M. soehngenii* and *Methanosarcina barkeri* ensures the last phase of methanogenesis by specifically consuming the substrate like acetate, butyrate, formate and hydrogen and...
carbon dioxide to produce methane. Further it was confirmed that the recently put forward four-stage mechanism of methanogenesis is also applicable to lab-scale fermentors as in large-scale biogas plants. It was observed that these bacterial counts had been quite significant in relation with enhanced biogas production.

The bacteria isolated from sugarcane press-mud such as *Bacillus subtilis*, *Citrobacter koseri* and *Proteus rettgeri* have also contributed significantly to biodegradation of substrate along with the bacteria found in the fermentor, leading to accelerated biogas production in treatments 2 and 4 and stabilization of biogas output in treatment 3.

The fungi isolated from sugarcane press-mud (such as *Aspergillus flavus*, *A. niger*, *Chaetomium olevacearum*, *Fusarium oxysporum* and *Periconia prolifica*) and from fermentor (*A. fumigatus*, *Cladosporium herbarum*, *F. solani*, *Humicola fuscoatra*, *Memnoniella echinata*, *Paecilomyces carneus* and *Trichothecium roseum*) played an effective role in biodegradation of the substrate generating CO₂, which was utilized as one-carbon carrier and reduced to methane through methanogenesis.

The bacteria such as *B. subtilis*, *C. koseri* and *P. rettgeri* and the fungi such as *C. olevacearum,*
F. oxysporum and P. proliferica isolated from sugarcane press-mud, and F. solani, H. fuscoatra, M. echinata, P. carneus and T. roseum isolated from the fermentor were identified as potent microbes on the basis of their higher degradation capacities.

It is concluded:

1. Under favourable conditions the weed biomass such as D. viscosa, E. crassipes, J. gossypifolia, P. hystero- phours and P. spicigera can generate more biogas with higher percentage of methane;

2. The addition of sugarcane press-mud to the above weeds produced more or less stable biogas at the cost of 50 per cent reduction in cow-dung. Therefore, the above said weeds and sugarcane press-mud are found to be the best alternative for renewable energy source;

3. These weeds and sugarcane press-mud are the best suited raw-materials for interaction of microbes especially substrate-specific methanogenic bacteria to produce biogas with higher percentage of methane.