CHAPTER 5

DISCUSSION* AND CONCLUSION

The present studies document the roles of important bacteria concerning in the production of biogas from cellulose or cellulosic wastes. More understanding in the characters and activities of those cellulolytic bacteria and methanogens may lead to improve the degradation of cellulose and gas yields in the cellulosic waste biogas digesters. Many bacterial types were found in the conventional type of digestive tanks, i.e., strict anaerobes and facultative anaerobes, gram-negative and gram-positive bacteria. Not only Escherichia coli, Salmonella spp., Pseudomonas spp., Bacillus spp., Staphylococcus spp., Streptococcus spp., but also other bacterial groups might be included. In was found that Shigella spp. was excluded. Moreover, the total counts of the digestive bacteria in an anaerobic condition were not much different and higher than in an aeobic condition. This evident suggested that a number of the strict anaerobes might be included in the digestive tank.

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*The Literature Survey contains discussion of the characters and activities of cellulolytic bacteria and methanogens in the anaerobic digester. These aspects will not again be extensively covered here.

Based on the cellulosic substrates, isolation and selections of cellulolytic bacteria and methanogens under an anaerobic condition were initiated and originated in Thailand. From ruminal fluid, three strains of cellulolytic bacteria were isolated, characterized and selected as the tested organisms. All were gram-negative, small curved rod, filamentous, 1 x 2-6 μ size, and yellow-pigment forming bacteria. They were cellulolytic bacteria resembling any of succinivibrio, butyrivibrio or selenomonas groups. Volatile fatty acids, H_2 and CO_2 were produced from the fermentation of cellulose. Two acids, succinic and acetic, were detected, finally, the results shows that acetic acid is an important product in the fermentation processes of cellulose and cellulosic wastes. In addition, different degrees of cellulolytic activities were elucidates especially, from different kinds of cellulosic wastes.

From digestive fluid, five strains of methanogens were isolated, characterized and selected as the tested organisms. All were gram negative, coccal formed, 1-2 μ in size, and yellow-pigment forming bacteria. Under fluorescence at 420 nm suggested they were methanogens resembling Methanococcus. However, Balch (15) reported that H_2 and formate were the substrates for growth and CH_4 production in the pure culture of Methanococcus spp. From these studies, H_2 was the main substrate for CH_4 production, and acetate might be another substrate for growth and CH_4 production. Therefore, more details of these tested methanogens should be further investigated.

The results demonstrates a two-organism fermentation of cellulose to CH_4 and CO_2. The two bacteria are representatives of the two general bacterial types proposed by Bryant (53), and their involvement in the cellulose fermention were summerized in Figure 5.1. In Thailand, this is the first demonstration of a co-culture of two bacteria capable of degrading cellulose to CH_4 and CO_2 in less than 2 weeks. As mention before in the Literature Survey, H_2 plays a critical role in the overall process of the fermentation. These studies confirmed that evidence and also showed that H_2 was used by all tested methanogeus for methanogenesis. Thus the use of H_2 by these bacteria would result in channelling of the electrons in the tested cellulolytic bacteria fermentation to H_2 production Without ethanol determination in the present studies, acetate seemed to become the main product of the saccharide fermentation (23, 121, 135). When H_2, CO_2 and acetate were present, the H_2-CO_2 were utilized preferentially, and only when most had been converted to CH_4, acetated was readily used by methanogens. The results were similar to others (22, 43, 55, 122, 123) : H_2 disappeared and then acetate might be metabolized. Succinate, detecting only in monoculture, was used by the methanogenic strains in all systems of co-culture.

Acetate was the main end product in the co-culture systems of the tested cellulolytic strain CU1 and methanogen strain Sc4 from the fermentation of different kind of cellulosic wastes. It is

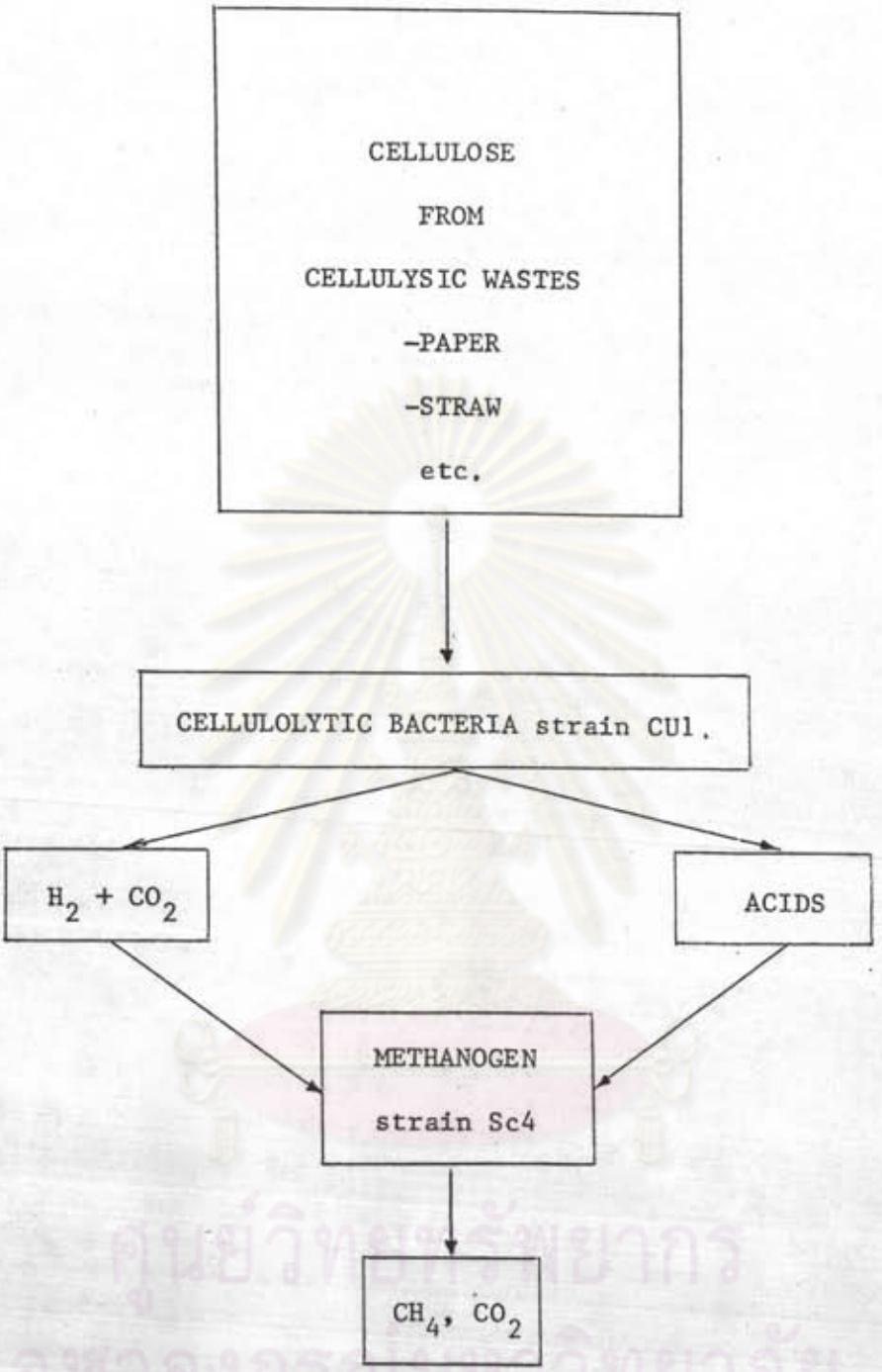

Figure 5.1 Scheme showing the fermentation of cellulosic wastes by cellulolytic bacteria CU1 and methanogen Sc4.

noticeable that only in the pineapple wastes mono-culture of CU1, acetate, butyrate, and succinate were formed, and the latter two acids could not be detected in the co-culture and in any mono- or co-culture of all systems of other tested substrates. It may be concluded that H_2 and acetate were primarily utilized for CH_4 production and growth by methanogens. Butyrate and succinate were not accumulated in any of the co-culture systems, those might be possibly utilized and/or not formed by the cellulolytic bacteria in the presence of methanogens. Another possibity might be depended on the sensitivity of the determination method of volatile fatty acid (2 µg of acid content) in these studies. The percentage of cellulose contents seems to be important for the yields of biogas production, i.e., in waste paper, high yield of gas has produced from high cellulose content; and also the efficiency of the fermentation process is considerably important. Compared with the former investigations, the amount of methane production from the fermentations of cellulose and cellulosic waste (paper) are presented in Table 5.1, and almost equal to other systems of the co-culture, but less than the tri-culture systems.

The results presented here emphasized the interdependence of the microbial processes involved in the cellulosic waste or cellulose to methane fermentation. In the present work, the ability of the selected bacteria to utilize either pure cellulose or the cellulose component of plant fibrous material has been demonstrated. Co-culture with the selected methanogens resulted in the conversion of cellulose to methane and carbon dioxide and at the same time there

Table 5.1 The amount of methane production compared with the former investigation

CH_4 (mol/mol hexose	$\%CH_4$ (in Biogas)	Substrate	Organisms	Reference
0.51	51.5	glucose	S.ruminantium + M.ruminantium	Chen (1977)
0.56	33.65	cellulose	C.thermocellum + M.thumoautotrophicum	Weimer 1977
0.58	39.82	cellulose	Rumen Anaerobic Fungus + Rumen Methanogen	Bauchop 1981
0.64	28.50	cellulose	A cellulolyticus + M.barkeri	Lanbe 1981
2.08	50.66	cellulose	A cellulolyticus M.barker + Desulfovibrio sp.	Laube 1981
2.00	50.00	cellulose	Anaerobic Fungus M. brevibacter R41 + M. barkeri	Mountfort (1982)
0.52	62.6	cellulose	Cellulolytic bacteria CU3 + Methanogen Sc5	
0.45	67.7	cellulose	Cellulolytic bacteria CU1 + Methanogen Sc4	
CH_4 (mol/mol lactate)				
0.39	48.1	lactate	S.ruminantium + M. ruminantium	Chen (1977)
CH_4 (lit/kg paper)				
47.49	68.7	waste paper	Cellulolytic bacteria CU1 + Methanogen Sc4	

was an increase in the rate and extent of cellulose degradation compared with the multiple strains in the fermentation process. The combination of the cellulolytic bacteria and methanogens may offer remarkable advantages in fermentative systems for the conversion of plant residue to methane.