CHAPTER 4

RESULTS

4.1 Some Bacterial organisms in Digestion Tank

Total bacterial counts in aerobic and anaerobic environments were 9.7×10^7 and 10.2×10^7 cells/ml, respectively. Bacterial counts for gram-negative bacteria, salmonellae group, bacillus group, streptococci and staphylococci were 3.3×10^7, 1.1×10^5, 1.1×10^7, 2.0×10^7 and 4.7×10^4 cells per ml, respectively, or about 6.4×10^7 cells/ml.

4.2 Isolation and Selection of Pure Cultures

4.2.1 Cellulolytic Bacteria

Three isolates from 113 pure cultures of suggestive cellulolytic bacteria were selected and named CU1, CU3 and CU4. They were characterized as :

CU1 : Colonial characteristics grown on rumen fluid cellulose agar--3mm in diameter, yellow to brown, flat, rugged edge, surrounded with 3-mm clear zone (See Figure 4.1).

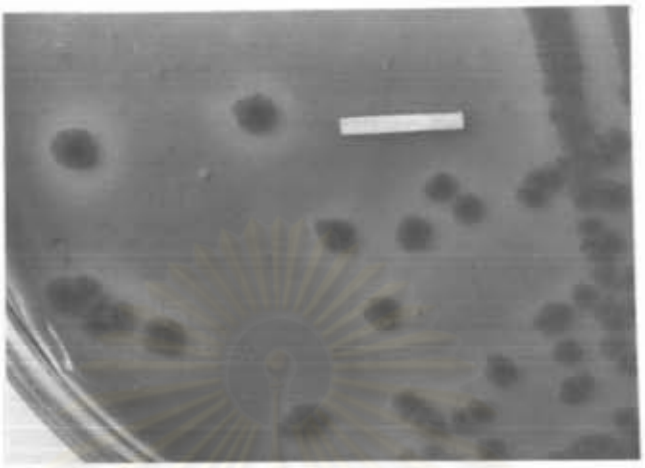

Figure 4.1 Colonial characteristic of cellulolytic bacteria CU1 (x 1.6), on rumen fluid cellulose agar, incubate 4 weeks at 37°C in 5 % CO_2 10 % H_2 N_2 balance atmosphere.

Figure 4.2 Morphological characteristic of cellulolytic bacteria CU1 (x330) cultivated in cellulose broth 4 weeks at 37°C in 5 % CO_2 10 % H_2 N_2 balance atmosphere.

Morphological characteristics--rod-shape, gram-negative bacilli, 1 x 3-6μ (See Figure 4.2).

CU3 : Colonial characteristics--2-5 mm in diameter, yellow, clear, flat, rugged edge, surrounded with 3-mm clear zone (See Figure 4.3).

Morphological characteristics--rod-shape, gram-negative bacilli, 1 x 2-5 μ (See Figure 4.4)

CU4 : Colonial characteristics--5 mm in diameter, pale yellow, flat, rugged edge, and surrounded with 3-mm clear zone, (See Figure 4.5)

Morphological characteristics--rod-shape, gram-negative bacilli, 1 x 2-6 μ (See Figure 4.6).

Pre-testes of cellulolytic activities of those three selected strains were performed. The media contained 0.1 or 0.2 % α-cellulose fibre were used to test acid formation, and the results are shown in Table 4.1. It was found that number of produced acid was depended on the concentration of cellulose in the medium, i.e., in low, only one acid or acetic acid but in high, two acids--acetic and succinic acids, were produced. Moreover, the amount of acetic acid in the high cellulose medium was about 2 times higher than the lower one. The capacity to degrade cellulose in CU1 and CU3 seems to be equal, but Figure 4.7 shows the rates of cellulose utilization by CU1 is better than the others. The pH of each monoculture was

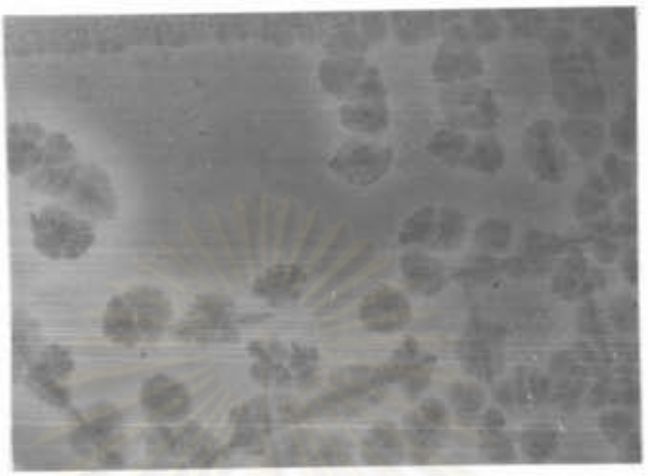

Figure 4.3 Colonial characteristic of cellulolytic bacteria CU3 (x 1.6), on rumen fluid cellulose agar 4 weeks at 37°C in 5 % CO_2 10 % H_2 N_2 balance atmosphere.

Figure 4.4 Morphological characteristic of cellulolytic CU3 (x 330), cultivated in cellulose broth 4 weeks at 37°C in 5 % CO_2 10 % H_2 N_2 balance atmosphere.

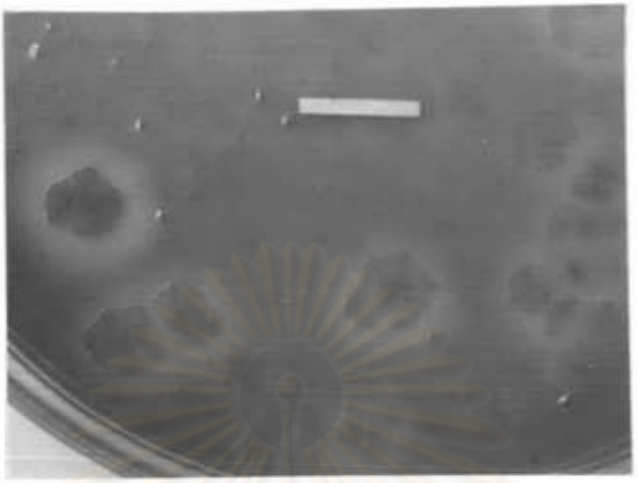

Figure 4.5 Colonial characteristic of cellulolytic bacteria CU4 (x 1.6), on rumen fluid cellulose agar, incubated 4 weeks at 37°C in 5 % CO_2 10 % H_2 N_2 balance atmosphere.

Figure 4.6 Morphological characteristic of cellulolytic bacteria CU4 (x 330), cultivated in cellulose broth 4 weeks at 37°C in 5 % CO_2 10 % H_2 N_2 balance atmosphere.

Table 4.1 Acid production from α - cellulose by three selected strains of cellulytic bacteria.

Organisms	Initial No. of Organisom (cells x 10^7)	α-cellulose (mg)	Production (μ mol) of	
			Acetic acid	Succinic acid
CU 1	3.5	10	5.98(± 0.46)	ND
	3.5	20	11.85(± 2.15)	1.66(±0.25)
CU 3	3.9	10	6.06(± 0.73)	ND
	3.9	20	10.95(± 0.65)	1.50(± 0.18)
CU 4	3.8	10	5.50(± 1.36)	ND
	3.8	20	10.82(± 1.66)	1.70(± 0.16)

Alls were means of 5 replicate

ND = cannot be detected

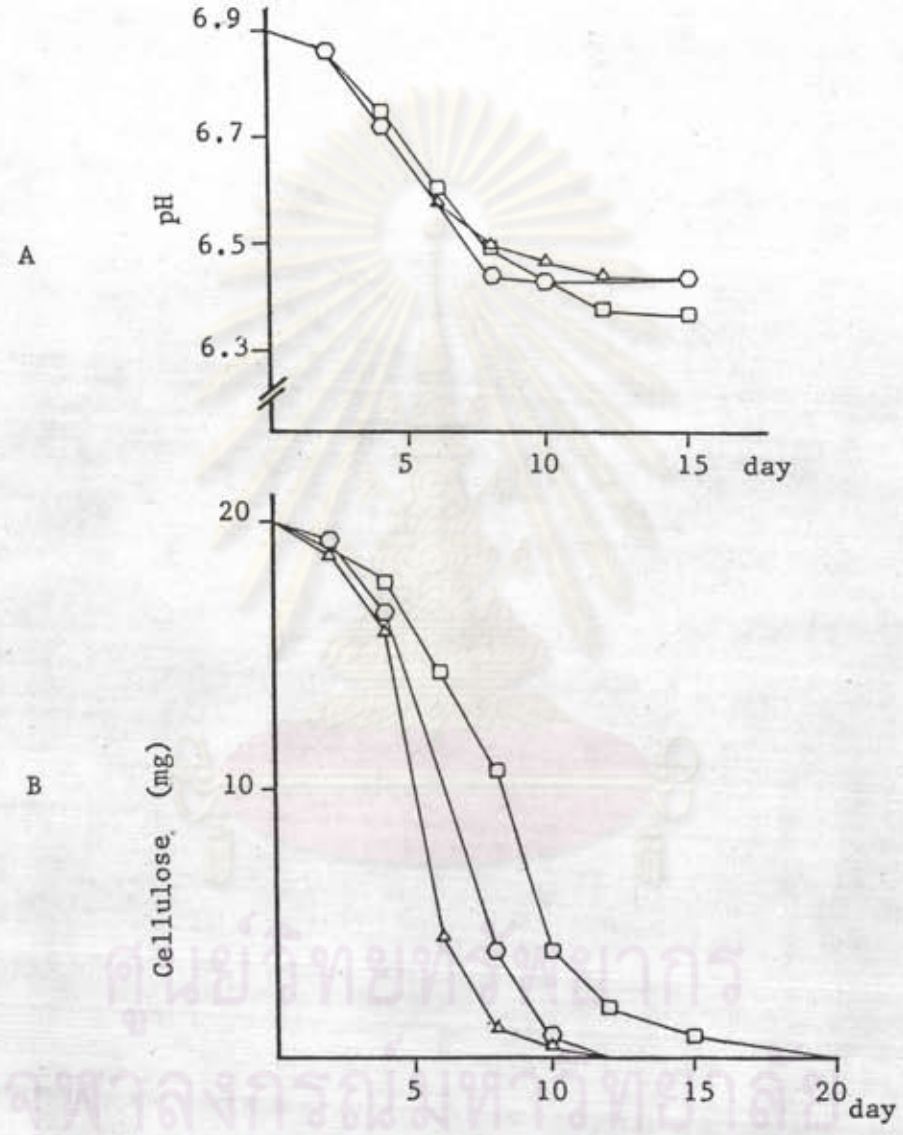

Figure 4.7 Remained cellulose contents and pH reduction in cellulose fermentation by three selected strains of cellulolytic bacteria : CU 1 (△), CU 3 (○), and CU 4 (□).

gradually drop until day 8. Anyway, there was no difference in the rate of pH reduction among those strains. Gaseous products of cellulose fermentation by the tested strains were H_2 and CO_2 (Table 4.2). Figure 4.8 shows the gas production rates of those. The rates of hydrogen gas production were constant for about 5, 8 and 10 days in monocultures of CU1, CU3 and CU4 respectively. Difference in the time course and lesser in the amounts, the rates of carbon dioxide production of those were similar to H_2 From these results indicated the selected cellulolytic bacteria strain CU1 might be suitable to use as the tested organism in other experiments.

4.2.2 Methanogens

Five isolates from 51 pure cultures of suggestive methanogenic bacteria were selected and they were named Sc1, Sc2, Sc3, Sc4 and Sc5. All isolates were tested for methanogenic activities. They were characterized as :

Sc1 : Colonial characteristics grown on Balch medium I agar--1 mm in diameter, smooth, round, convex, shining, pale yellow-green (102.5 μmol CH_4). See Figure 4.9.

Morphological characteristics—coccal form, gram-negative cocci, 1-2 μ (See Figure 4.10)

Table 4.2 H_2 CO_2 Production from cellulose fermentation of three selected strain of cellulolytic bacteria

Organisms	Productions (μ mol) of			H_2 : CO_2
	H_2	CO_2	CH_4	
CU 1	1032.5	501.1	0	2.05
CU 3	1135.5	487.2	0	2.33
CU 4	877.4	455.8	0	1.92
Mixed Culture	0	111,3	83.1	

Inoculated - 3×10^7 cell into 20 mg

α cellulose fibre, incubated at 37°C for 9 days

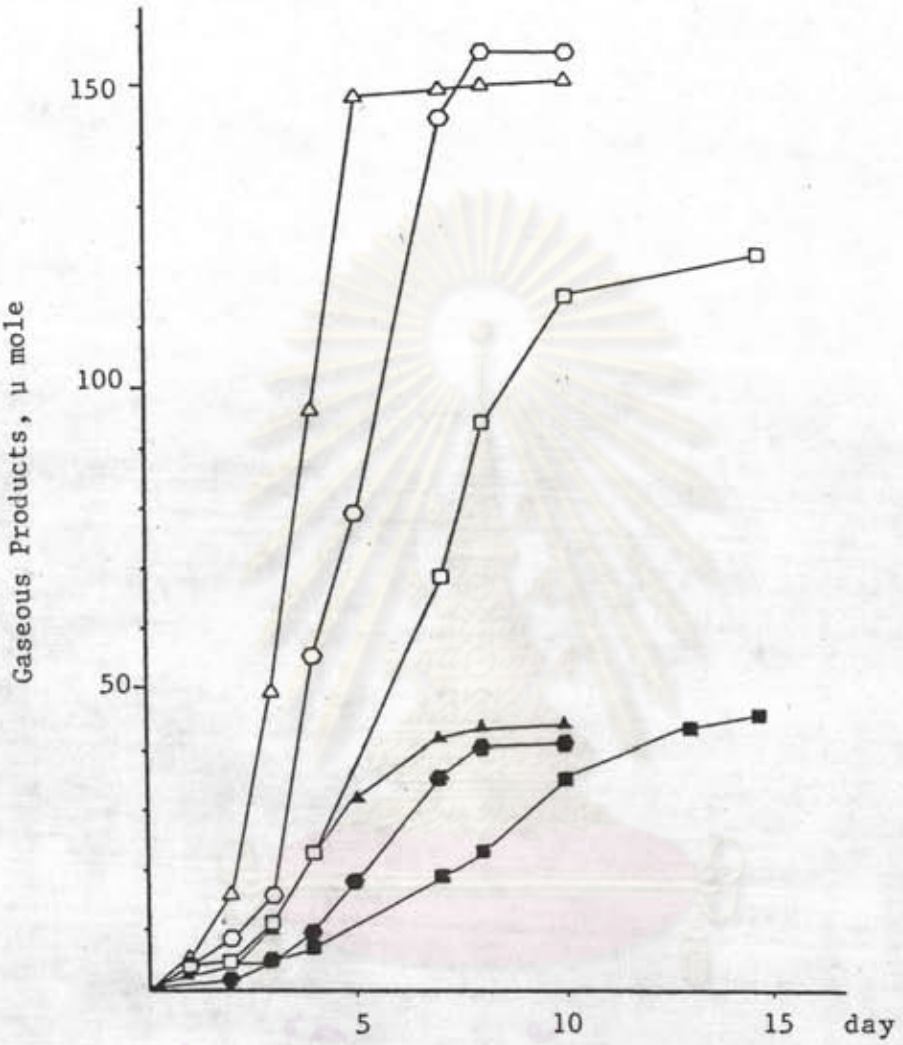

Figure 4.8 Gaseous products of cellulose fermentation by three selected strains of cellulolytic bacteria :

CU 1 (△ = H_2, ▲ = CO_2),

CU 3 (○ = H_2, ● = CO_2) and

CU 4 (□ = H_2, ■ = CO_2)

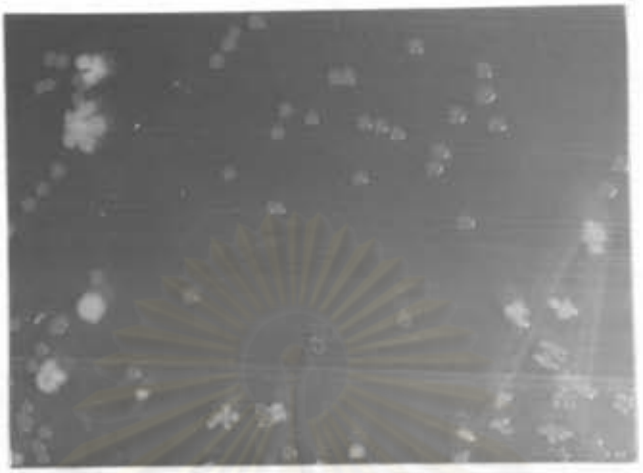

Figure 4.9 Colonial characteristic of methanogen Scl (x 1.6) on balch medium 1 agar, incubated 4 weeks at 37°C in 80 % H_2 20 % CO_2 pressurized 2 atmosphere.

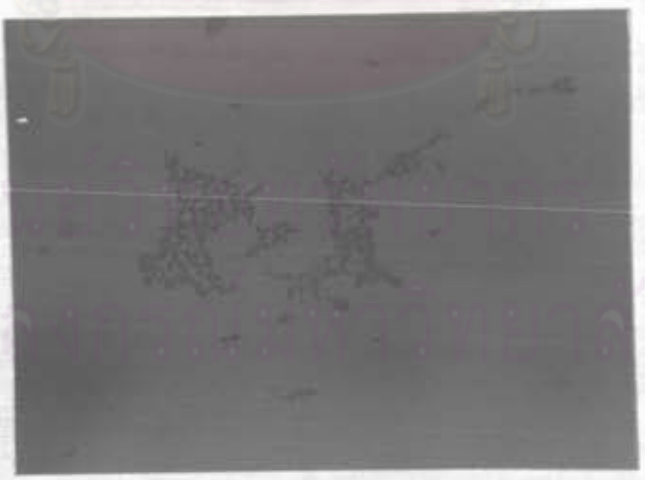

Figure 4.10 Morphological characteristic of methanogen Scl (x 330), cultivated in balch medium 1 broth, incubated 4 weeks at 37°C in 80 % H_2 20 % CO_2 pressurized 2 atmosphere.

Sc2 : Colonial characteristics--1mm in diameter, shining, smooth, round, convex, yellow (113.4 μmol CH_4). See Figure 4.11.

Morphological characteristics—coccal form, gram-negative cocci, 1-2 μ (See Figure 4.12)

Sc3 : Colonial characteristics-- 1-2 mm, shining, round, smooth, convex, yellow-green (111.5 μmol CH_4). See Figure 4.13.

Morphological characteristics--coccal form, gram-negative cocci, 1-2 μ (See Figure 4.14).

Sc4 : Colonial characteristics--1-2 mm, shining, round, smooth, cenvex, pale yellow-green (151.2 μmol CH_4). See Figure 4.15.

Morphological characteristics—coccal form, gram-negative cocci, 1 μ (See Figure 4.16).

Sc5 : Colonial characteristics--2 mm, shining, round, smooth, convex, yellow green (152.3 μmol CH_4). See Figure 4.17 .

Morphological characteristics--coccal form, gram-negative cocci, 1 μ(See Figure 4.18).

H_2-CO_2 utilizing capacity is a common character of all methanogens. To compare this, five tested methanogens were initially cultivated in different amounts of H_2-CO_2, and the results are shown in Tables 4.3 and 4.4. It was found that the more in H_2-CO_2 concentration,

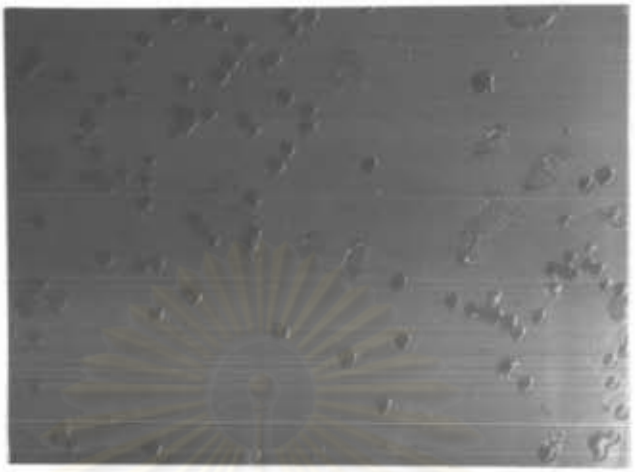

Figure 4.11 Colonial characteristic of methanogen Sc2 (x1.6) on balch medium 1 agar, incubated 4 weeks at 37°C in 80% H_2 20% CO_2 pressurized 2 atmosphere.

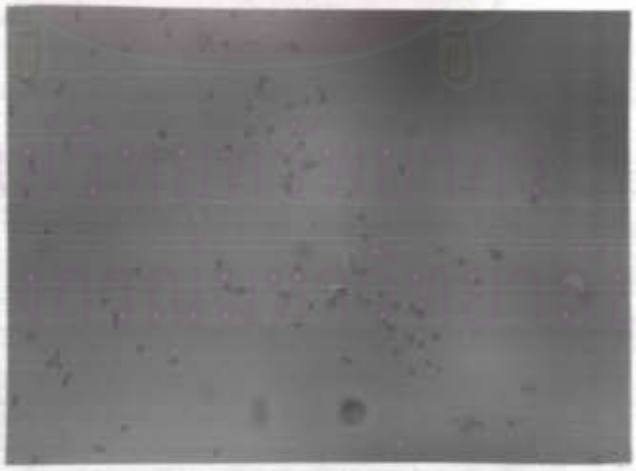

Figure 4.12 Morphological characteistic of methanogen Sc2 (x 330), cultivated in balch medium 1 broth, incubated 4 weeks at 37°C in 80 % H_2 20% CO_2 pressurized 2 atmosphere.

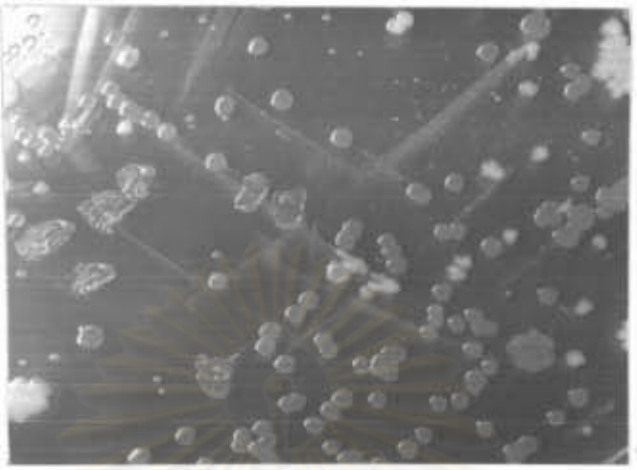

Figure 4.13 Colonial characteristic of methanogen Sc 3 (x 16) on balch medium 1 agar, incubated 4 weeks at 37°C in 80% H_2 20% CO_2, pressurized 2 atmosphere .

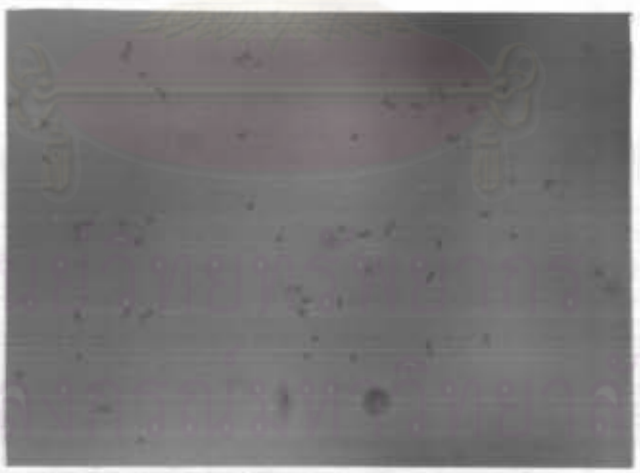

Figure 4.14 Morphological characteristic of methanogen Sc3 (x 330), cultivated in balch medium 1 broth, incubated 4 weeks at 37°C in 80% H_2 20% CO_2 pressurized 2 atmosphere .

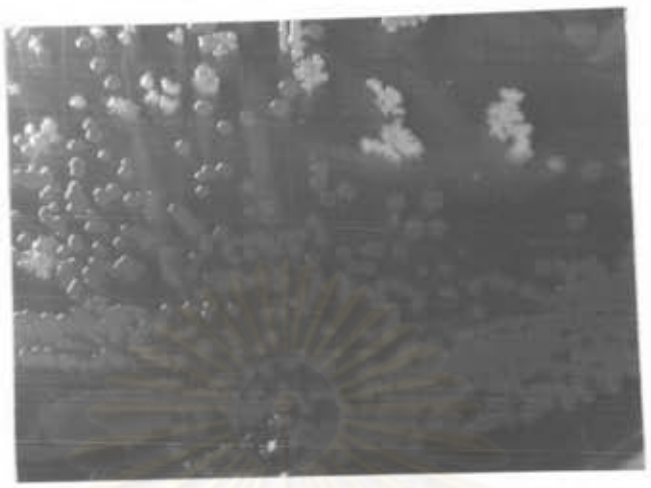

Figure 4.15 Colorial characteristic of methanogen Sc4 (x 1.6) on balch medium 1 agar incubated 4 weeks at 37'c in 80 % H_2 20% CO_2 pressurized 2 atmosphere .

Figure 4.16 Morphological characteristic of methanogen Sc4 (x 330), cultivated in balch medium 1 broth, incubated 4 weeks at 37'c in 80% H_2 20% CO_2 pressurized 2 atmosphere .

Figure 4.17 Colonial characteristic of methanogen Sc5 (x1,6) on balch medium 1 agar, incubated 4 weeks at 37°C in 80% H_2 20% CO_2 pressurized 2 atmosphere.

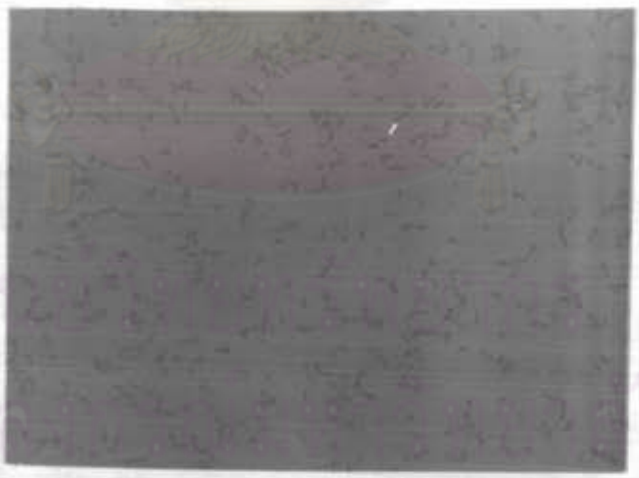

Figure 4.18 Morphological characteristic of methanogen Sc5 (x330), cultivated in balch medium 1 broth, incubated 4 4 weeks at 37°C in 80% H_2 20% CO_2 pressurized 2 atmosphere.

Table 4.3 H_2-CO_2 utilizing capacity of the tested methanogen Sc1-Sc5 in low amount of H_2-CO_2 condition

Bacteria	H_2 and CO_2 loss (µl)		CH_4 Production (µl)	Ratio CH_4/H_2 cons
	H_2	CO_2		
Sc 1	590	77.4	1100.7	1.86
Sc 2	590	77.5	977.8	1.66
Sc 3	590	77.5	1134.0	1.92
Sc 4	590	58.4	1138.2	1.93
Sc 5	590	45.9	1480.2	2.50

Alls cultivated in 10 ml balch medium 1 broth with 10% + $H_2$5% CO_2 N_2 balance gas mixture in headspace, incubate 5 days at 37°C

Initial stage	H_2	590.0	µl
	CO_2	118.5	µl
	CH_4	0	µl

Table 4.4 H_2-CO_2 utilizing capacity of the tested methanogen Sc1-Sc5 in high amount of H_2-CO_2 condition.

Bacteria	H_2 and CO_2 loss (µl)		CH_4 Production (µl)	Ratio CH_4/H_2 cons
	H_2	CO_2		
Sc 1	1694.0	1763.5	2296.7	1.35
Sc 2	1601.6	1775.0	2539.5	1.58
Sc 3	1828.2	1748.4	2498.5	1.36
Sc 4	2306.8	1433.1	3001.5	1.30
Sc 5	2183.8	1778.8	3415.9	1.56

Alls cultivated in 10 ml balch medium 1 broth with 80% H_2 20 % CO_2 in head space, incubated 5 days at 37°c

Initial stage	H_2	3953.8	µl
	CO_2	1869.2	µl
	CH_4	0	µl

the more in CH_4 formation. In H_2 excess, the selected strains, Sc4 and Sc5, utilized large amounts of H_2 to form CH_4 gas. Comparing between the two systems, the ratio of H_2 loss and CH_4 production in low H_2 condition was 1 to 2,but in excess condition, only 1 to 1.3, and also both Sc4andSc5 produced high amounts of CH_4 in both systems.

4.3 Fermentation of Cellulose by Co-culture

In fifteen co-culture sets, the amounts of remained cellulose are summerized in Table 4.5, detailed results of certain sets are presented in Figures 4.19-4.24 in Appendix F. Depended on the cellulose digestion capacity of the tested organisms, CU1, CU3 and CU4, cellulose was not detectable on day 12, day 12-15 and day 20, respectively. The fermentation of α-cellulose by co-culture resulted in the formation of at least 3 to 4 products, acetic acid or acetate, succinic acid or succinate, carbon dioxide and methane. The amounts of acids are shown in Table 4.6. Only five sets, succinate could not be detected. The percentages of acid loss were widely ranged, from 0.1 — 39 % and 0-100 % for acetate and succinate, respectively. The pH of the medium decreased from 6.9 at the beginning of the incubation to 6.3 at the end of fermentation. Hydrogen was not detected during fermentation of all co-culture sets. Productions of CO_2 and CH_4 in co-culture systems of cellulolytic

Table 4.5 Remained cellulose in the fermentation of cellulose (20 mg) by monocullures of cellulolytic bacteria CU1, CU3 and CU4 and by co-culture with methonogens Sc1, Sc2, Sc3, Sc4 and Sc5

Organism	Remained cellulose	
	day 6	day 12
CU 1 system	4.5	0
CU 1 + Sc1	2.6	0
+ Sc 2	4.1	0
+ Sc 3	3.7	0
+ Sc 4	2.5	0
+ Sc 5	3.9	0

Organism	Remained cellulose	
	day 6	day 12
CU 3 system	10.4	0
CU 3 + Sc 1	10.7	0
+ Sc 2	8.8	0
+ Sc 3	6.4	0.1
+ Sc 4	8.3	0.6
+ Sc 5	7.1	0.5

Organism	Remaned cellulose	
	day 6	day 12
CU 4 system	14.2	2.0
CU 3 + Sc 1	13.5	1.7
+ Sc 2	14.5	0.6
+ Sc 3	13.9	2.0
+ Sc 4	12.5	0.7
+ Sc 5	13.3	0.8

Table 4.6 Acid production in the fermentation of cellulose (20 mg) by monoculture of cellulolytics bacteria, Cu 1, CU 3 and CU 4 and by co-culture with methanogens, Sc1, Sc2, Sc3, Sc4 and Sc 5, for 15 day incubation.

Organism	Initial No of Organism (cells) x 10^7	Production (μ mole) of			
		Acetic acid	% loss	Succinic acid	% loss
CU 1 System	3.5	11.85 (+ 2.15)	0.0	1.66 (+ 0.25)	0.0
CU 1 + Sc1	3.5 + 3.7	7.65 (± 1.14)	35.4	ND	100
+ Sc2	3.5 + 2.1	7.25 (± 1.06)	38.8	0.75 (± 0.09)	54.8
+ Sc 3	3.5 + 2.3	8.46 (± 1.35)	28.6	1.36 (± 0.13)	18.1
+ Sc 4	3.5 + 1.4	8.21 (±0.90)	30.7	0.90 (± 0.16)	45.8
+ Sc 5	3.5 + 2.5	9.63 (+ 1.03)	18.7	ND	100
CU 3 System	3.9	10.95 (± 0.65)	0.0	1.50 (± 0.18)	0.0
CU 3 + Sc 1	3.9 + 3.7	9.30 (± 1.20)	15.0	1.56 (± 0.23)	0.0
+ Sc 2	3.9 + 2.1	8.20 (± 0.82)	25.1	1.05 (± 0.13)	30.0
+ Sc 3	3.9 + 2.3	7.73 (± 1.06)	29.4	1.06 (± 0.16)	29.3
+ Sc 4	3.9 + 1.8	8.10 (± 1.09)	26.0	1.07 (± 0.17)	28.6
+ Sc 5	3.9 + 2.5	10.25 (± 1.84)	6.4	1.13 (± 0.12)	24.6
CU 4 System	3.8	10.82 (± 1.66)	0.0	1.07 (± 0.16)	0.0
CU 4 + Sc 1	3.8 + 3.7	8.68 (± 1.30)	19.7	ND	100
+ Sc 2	3.8 + 2.1	10.81 (± 1.23)	0.1	ND	100
+ Sc 3	3.8 + 2.3	7.70 (± 0.81)	28.8	ND	100
+ SC 4	3.8 + 1.8	10.76 (± 1.32)	0.5	0.83 (± 0.08)	22.4
+ Sc 5	3.8 + 2.5	8.33 (± 1.60)	23.4	0.72 (± 0.11)	32.7
Mixed	23.1	ND		ND	
Sc 1, 2, 3, 4, 5	3.7, 2.1, 2.3, 1.8, 2.5	ND		ND	

ND = could not be detected

bacteria strain CU 1, CU 3 and CU 4 are summerized in Tables 4.7-4.9, respectively, detailed result of certain sets are presented in Figures 4.25-4.30 in Appendix F. After 10-day incubation, total amounts of biogas production from co-culture sets were widely varied i.e, 1.8-4.3 mmol per gram of cellulose and composed of 58-75 % methane. Moreover, in the individual systems of cellulolytic bacteria, CU 1, CU 3 and CU 4, the maximal percentages of CH_4 composition were 68, 64 and 75, respectively. Degradation of cellulose in the mixed culture could not be observed. Comparison to co-culture fermentation, any of the cellulose fermentation products could not be detected. Table 4.10 compares the fermentation products and the periods of cellulose disappearence in six co-culture sets. The shortest period of absolute disappearence of cellulose high degree in acid utilizing, high level of total amount of biogas production and also high percentage of CH_4 composing, the co-culture system of CU 1-Sc4 was chosen for the next experiment.

4.4 Fermentation of Cellulosic Wastes by Co-culture

Similar to cellulose fermention, the end products from the fermemtation of cellulosic wastes, i.e., pineapple peel (25 % α - cellulose), paper (71 % α - cellulose), straw (45 % α - cellulose), bermuda grass (24 % α - cellulose) and water hyacinth (19 % α - cellulose) by mono culture and co-culure are volatile fatty acids, hydrogen, carbon dioxide, methane and others. Table 4.11 compares

Table 4.7 Gas Production from the fermentation of cellulose (20 mg) by the selected strain of cellulolytic bacteria, CU 1 alone and individually plus methanogen strain Sc1, Sc2, Sc3, Sc4, and Sc5, for 20-day incubation

Organism	H_2 Production µmole (%)		CO_2 Production µ mole (%)		CH_4 Production µ mole (%)		CH_4 : CO_2	
	5 day	10 day	5 day	10 day	5 day	10 day	5 day	10 day
Cellulolytic bacteria CU 1	146.5 (82.1)	150.1 (77.3)	32.0 (17.9)	44.0 (22.7)	ND*	ND		
Cellulolytic bacteria CU 1+Methanogen Sc1	2.0 (3.2)	ND	25.0 (39.6)	30.0 (38.0)	36.2 (57.2)	49.0 (62.0)	1.44	1.63
Cellulolytic bacteria CU 1+Methanogen Sc 2	ND	ND	26.0 (40.5)	30.0 (37.2)	38.2 (59.5)	50.7 (62.8)	1.46	1.66
Cellulolytic bacteria CU 1+Methanogen Sc 3	ND	ND	24.2 (39.2)	27.1 (36.1)	37.5 (60.8)	48.0 (63.9)	1.54	1.77
Cellulolytic bacteria CU 1+Methanogen Sc 4	5.0 (9.0)	ND	20.2 (36.5)	22.0 (32.3)	30.2 (54.5)	46.2 (67.7)	1.50	2.09
Cellulolytic bacteria CU 1+Methanogen Sc 5	ND	ND	29.1 (43.4)	33.0 (41.3)	38.0 (56.6)	47.0 (58.7)	1.30	1.42
Mixed Culture	ND	ND	ND	ND	ND	ND		
Control	ND	ND	ND	ND	ND	ND		

* ND = Any product could not be detected.

Table 4.8 Gas production from fermentation of 20 mg α cellulose fiber

Organism	H_2 Production μ mole (%)		CO_2 Production μ mole (%)		CH_4 Production μ mole (%)		CH_4 : CO_2	
	5 day	10dday	5 day	10 day	5 day	10 day	5 day	10 day
Cellulolytic bacteria CU 3	77.8 (81.6)	152.5 (79.2)	17.5 (18.4)	40.0 (20.8)	ND	ND		
Cellulolytic bacteria CU 3+Methanogen Sc 1	2.0 (5.6)	ND	10.5 (29.6)	30.3 (36.7)	23.0 (64.8)	52.0 (63.3)	2.30	1.70
Cellulolytic bacteria CU 3+Methanogen Sc 2	ND	ND	17.5 (43.2)	35.0 (41.2)	23.0 (56.8)	50.0 (58.8)	1.35	1.40
Cellulolytic bacteria CU3+Methanogen Sc 3	2,0 (12.9)	ND	25.0 (37.6)	34.8 (41.5)	36.0 (57.1)	49.0 (58.5)	1.44	1.44
Cellulolytic bacteria CU 3+Methanogen Sc 4	6.0 (12.9)	ND	17.5 (37.6)	26.8 (35.8)	23.0 (49.5)	48.0 (64.2)	1.35	1.8
Cellulolytic bacteria CU 3+Methanogen Sc 5	ND	ND	20.0 (38.1)	32.1 (37.4)	32.5 (61.9)	53.8 (62.6)	1.60	1.60
Mixed culture	ND	ND	ND	ND	ND	ND		
Control	ND	ND	ND	ND	ND	ND		

Note Infermintation of 20 mg α cellulose fiber by pure culture of methanogen Sc1-Sc5 no gas production could be detected

*Percent calenlated from total volume of biogas production

Table 4.9 Gas production from fermentation of 20 mg α celluloge fibre

Organism	H_2 Production μ mole (%)			CO_2 Production μ mole (%)			CH_4 Production μ mole (%)			$CH_4 : CO_2$		
	5 day	10 day	15 day	5 day	10 day	15 day	5 day	10 day	15 day	5 day	10 day	15 day
Cellulolytic bacteria CU 4	38.1 (82.6)	115.0 (76.7)	121.0 (72.9)	8.0 (17.4)	35.0 (23.3)	45.0 (27.1)	ND	ND	ND			
Cellulolytic bacteria CU 4+Methanogen Sc 1	ND	ND	ND	8.0 (42.6)	18.8 (34.9)	19.0 (25.5)	10.8 (57.4)	35.0 (65.1)	55.5 (74.5)	1.25	1.94	2.89
Cellulolytic bacteria CU 4+Methanogen Sc 2	ND	ND	ND	5.2 (36.6)	9.1 (24.8)	16.6 (24.9)	9.0 (63.4)	27.5 (75.2)	50.1 (75.1)	1.8	3.0	3.1
Cellulolytic bacteria CU 4+Methanogen Sc 3	ND	ND	ND	8.0 (43.2)	12.5 (31.2)	2 5 (32.0)	10.5 (56.8)	27.5 (68.8)	53.0 (68.0)	1.2	2.2	2.12
Cellulolytic bacteria CU 4+Methanogen Sc 4	ND	ND	ND	8.0 (50.0)	19.0 (41.3)	21.0 (31.3)	8.0 (50.0)	27.0 (58.7)	46.0 (68.7)	1.0	1.4	2.19
Cellulolytic bacteria CU 4+Methanogen Sc 5	2.0 (7,4)	ND	ND	10.8 (40.4)	18.9 (33.2)	28.0 (32.5)	13.9 (52.2)	38.0 (66.8)	58.0 (67.5)	1.3	2.1	2.0
Mixed culture	ND	ND	ND	ND	ND	ND	ND	ND	ND			
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND			

Note In fermentation of 20 mg -cellulose fibre by pure culture of methanogen Sc 1-Sc5 no Gas production could be detected.

*Percent calculated from total volume of biogas production

Table 4.10 Cellulose fermentation products by six co-culture sets, some combinations of cellulolytic bacteria strains (CU1, CU3 and CU4) and methanogens (Sc1, Sc2, Sc4 and Sc5)

Co-culture Set	100% cellulose loss (days)	Production (μ mol) of		Gas production at day 10 (μ mol)				CH_4/cellulose lose μ mol/mg
		Acetic acid	Succinic acid	total	H_2	CH_2 (%CH_4)	CH_4 : CO_2	
CU 1	10	11.85 (± 2.15)	1.66 (± 0.25)	194.1	150.1	ND (0)	0	0
CU 1 + Sc1	12	7.65 (± 1.14)	ND	79.0	ND	49.0(62.0)	1.6	2.5
CU 1 + Sc 4	12	8.21 (± 0.90)	0.90 (± 0.16)	68.2	ND	46.2(67.7)	2.1	2.4
CU 1 + Sc 5	12	9.63 (± 1.03)	ND	80.0	ND	47.0(58.7)	1.4	2.4
CU 3	12	10.95 (± 0.65)	1.50 (± 0.18)	192.5	152.5	ND (0)	0	0
CU 3 + Sc4	20	6.10 (± 1.09)	1.07 (± 0.17)	74.8	ND	48.0(64.2)	1.8	2.5
CU 4	20	10.82 (± 1.66)	1.07 (± 0.16)	150.0	115	ND (0)	0	0
CU 4 + Sc 2	20	10.81 (± 1.23)	ND	36.6	ND	27.5(75.2)	3.0	1.8
CU 4 + Sc 4	20	11.96 (± 1.32)	0.83 (± 0.08)	46.0	ND	27.0(58.7)	1.4	1.6
Mixed culture		Fermentation could not be detected						

ND = could not be detected

Table 4.11 Acid production Gas production and remained cellulose in fermentation of cellulosic wastes (1g) by cellulolytic bacteria CU1, CU1 + methanogen Sc 4 and mixed-culture, incubated 25 day at 37°c

Substrates	Actual							Alkali treated						
	Remained Cellulose mg (%)	Acid production (μ mol)	pH		Gas production (μ mol)			Remained cellulose mg (%)	Acid production (μ mol)	pH		Gas production (μmol)		
			day 0	day 25	H_2	CO_2	CH_4			day 0	day 15	H_2	CO_2	CH_4
Pineapple peel														
- CU 1	242 (94.5)	acetic 532.0, butyric 54.0, succinic 107.2	6.99	6.75	2180	700		184 (80.0)	acetic 397.8	6.90	6.48	ND	48	
- CU 1 + Sc 4	228 (89.0)	acetic 435.2	6.99	6.62		194	484	192 (83.5)	acetic 262.0	6.90	6.46		102	198
- Mixed culture	252 (98.4)	-	6.99	6.79		168	146	222 (96.5)	acetic 470.8, propionic 182.4, butyric 456	6.90	6.78		244	10
Paper														
- CU 1	0 (0)	acetic 892.5	7.00	6.45	6125	1100		79 (11.7)	acetic 782.5	7.00	6.39	5625	1325	
- CU 1 + Sc 4	0 (0)	acetic 644.0	7.00	6.37		965	2120	0 (0)	acetic 743.0	7.00	6.43		1050	2610
- Mixed culture	595 (82.0)	ND	7.00	6.95		ND	130	560 (82.3)	ND	7.00	6.89		150	75
Straw														
- CU 1	296.6 (76.7)	acetic 423.3	7.00	6.75	3900	837		8 (2.5)	acetic 1054.6	7.00	6.36	3403	557	
- CU 1 + Sc 4	200 (51.7)	acetic 441.6	7.00	6.51		636	1433	0 (0)	acetic 757.6	7.00	6.41		617	1267
- Mixed culture	283.3 (73.3)	ND	7.00	6.96		240	633	293 (89.8)	ND	7.00	6.49		177	70
Bermuda grass														
- CU 1	98 (35.7)	acetic 583.6	6.99	6.45	3200	922		212 (84.8)	acetic 116.0	6.91	6.69	ND	ND	
- CU 1 + Sc 4	42 (15.3)	acetic 501.6	6.99	6.46		660	1600	196 (78.4)	acetic 147.8	6.91	6.66		60	ND
- Mixed culture	230 (63.9)	ND	6.99	6.88		154	ND	238 (95.2)	acetic 672.4, propionic 180.4	6.91	6.84		392	ND
Water hyacinth														
- CU 1	90 (41.6)	acetic 233.6	7.00	6.48	1000	304		178 (89.0)	acetic 147.0	6.85	6.52	ND	46	
- CU 1 + Sc 4	122 (56.4)	acetic 249.2	7.00	6.59		202	260	142 (71.0)	acetic 127.4	6.85	6.44		68	60
- Mixed culture	182 (84.2)	ND	7.00	6.91		164	42	186 (93.0)	ND	6.85	6.69		120	ND

the fermentation products and remained cellulose contents from those wastes under the actual and alkali pre-treated conditions. The degree of cellulose degradation in those wastes was widely varied. After 25 day incubation, the cellulose (about 12 mg) was disappeared from the actual paper by the mono-culture of CU 1, and from paper (both actual and alkali-treated) and alkali-treated straw by the co-culture of CU 1 and Sc 4. Less than 25 % of α - cellulose contents of those wastes were degraded by mono-and/or co-cultures (See Table 4.12). In these studies, the most resistance to cellulose degradation was pineapple waste and the best substrate was paper waste. Compared with the mono-culture more cellulose was degraded in the co-culture except the two sets used actual water hyacinth and alkali-treated pineapple waste as substrates. The pH of the medium in both mono- and co-cultures was gradually decreased depending the degradation of cellulose.

The acid productions in those systems were varied qualitatively and/or quantitatively. It was found that acetate, the major acid, could be detected after 25-day incubation in all systems except the actual pineapple waste in monoculture system (Table 4.13).

The amounts of carbondioxide in co-cultures were generally lower than in the monoculture (Table 4.14). Hydrogen could be detected only in the monoculture of cellulolytic bacteria but not in co-culture. It was found that the amounts of hydrogen production and methane production were related, i.d., high in the first one

Table 4.12 Degree of cellulose degradation in 5 cellulosic wastes under actual and alkoli treated condition, incubated 25 day.

Substrate	% Cellulose Degradation	
	CU 1	CU1 + Sc4
1. Actual paper	100	100
2. Treated straw	97.5	100
3. Treated paper	88.3	100
4. Actual bermuda grass	64.3	84.7
5. Actual water hyacinth	58.4	43.6
6. Actual straw	23.3	48.3
7. Treated pineapple peel	20.0	16,5
8. Treated bermuda grass	15.2	21.6
9. Treated water hyacinth	11.0	29.0
10. Actual pineapple peel	5.5	11.0

Table 4.13 Acid production (μmol/g substrate) in cellulosic waste fermentation by monoculture CU 1 and co-culture CU1+Sc4, in incubated 25 day, 37°C .

Substrate	Mono-culture CU1 Acetate	Co-culture CU1+Sc4 Acetate
1. Treated straw	1054.6	757.6
2. Actual paper	897.5	644.0
3. Treated paper	782.5	743.0
4. Actual bermuda grass	583.6	510.6
5. Actual pineapple peal	532.0 (Butyrate 54.0, Succinate 107.2)	435.2
6. Actual straw	423.3	441.6
7. Treated pineapple peel	397.8	262.0
8. Actual water hyacinth	233.6	249.2
9. Treated water hyacinth	147.0	127.4
10. Treated bermuda grass	116.0	147.8

Table 4.14 Gas production from the fermentation of cellulosic waste (μmol/g) by monoculture CU 1 and co-culture CU 1 + Sc4

Substrate	CU 1		CU 1 + Sc 4				
			The Amount of gas (μmol/g)			$\%CH_4$	$CH_4 : CO_2$ Ratio
	H_2	CO_2	total	CO_2	CH_4		
Actual paper	6125	1100	3085	965	2120	68.7	2.20
Treated paper	5625	1325	3660	1050	2610	71.3	2.48
Actual straw	3900	837	2069	636	1433	69.2	2.25
Treated straw	3403	557	1879	617	1262	67.1	2.04
Actual bermuda grass	3200	922	2260	660	1600	70.7	2.42
Actual piapple peel	2180	700	678	194	484	71.3	2.49
Actual water hyacinth	1006	304	462	202	260	56.3	1.29
Treated pineapple peel	ND*	48	260	102	158	60.7	1.55
Treated water hyacinth	ND	46	128	68	60	46.8	0.88
Treated bermuda grass	ND	ND	60	60	ND	0	0

ND = could not be detected

Table 4.15 Gas production in the fermentation of cellulosic wastes by the cellulolytic bacteria CU1 + methanogen Sc4, incubated at 37°C for 25 days.

Substrates	Gas production (μmol/g)				
	Dry Weight		Cellulose loss		CH_4 : CO_2
	Total Gas*	CH_4	Total Gas	CH_4	Ratio
α-cellulose fiber**	3,410	2,400	3,410	2,400	2.13
Treated paper	3,660	2,610	5,382	3,838	2.48
Actual paper	3,085	2,120	4,251	2,922	2.19
Actual bermuda grass	2,260	1,780	9,720	6,882	2.42
Actual straw	2,069	1,430	11,076	7,625	2.25
Treated straw	1,884	1,270	5,775	3,888	2.05
Actual pineapple peel	678	480	24,068	16,519	2.49
Actual water hyacinth	462	260	4,899	2,757	1.28
Treated pineapple peel	300	170	7,895	5,312	1.94
Treated water hyacinth	128	60	2,207	1,500	0.88
Treated bermuda grass	60	0	1,111	0	0

*Total gas = the net amount of CH_4 and CO_2

**incubated 37°C for 10 days, and cellulose could not be detected

caused high in the second one. Table 4.15 compares the gaseous products products of the fermentation of pure cellulose and cellulosic wastes. In pure cellulose system, the amounts of total gas and methane per gram of dry weight and per gram of utilized cellulose was equal. In the treated paper system, both amounts of total gas and CH_4 per gram of dry weight and per gram of cellulose loss were higher than the pure cellulose system. It meaned that certain amount of gaseous products might be partly produced from other components of the waste paper. The results showed that the amounts of total gas and CH_4 per gram of cellulose loss were higher than per gram of dry weight in all systems of cellulosic wastes. The ratio of CH_4 to CO_2 was varied in range from 0-2.49 in case of the cellulosic wastes but in the pure cellulose system was 2.13. However, the percentages of methane production from those four top systems, i.e., treated paper, actual paper, actual bermuda grass and treated straw, were rather high, 67-71. From five kinds of the tested substrates, the results indicated that the waste paper might be the best substrate for biogas production from cellulosic wastes.