

生質能源技術研討會

中高溫厭氧微生物活性評估

鄭幸雄

國立成功大學環境工程系名譽教授

2015.7.17



前言

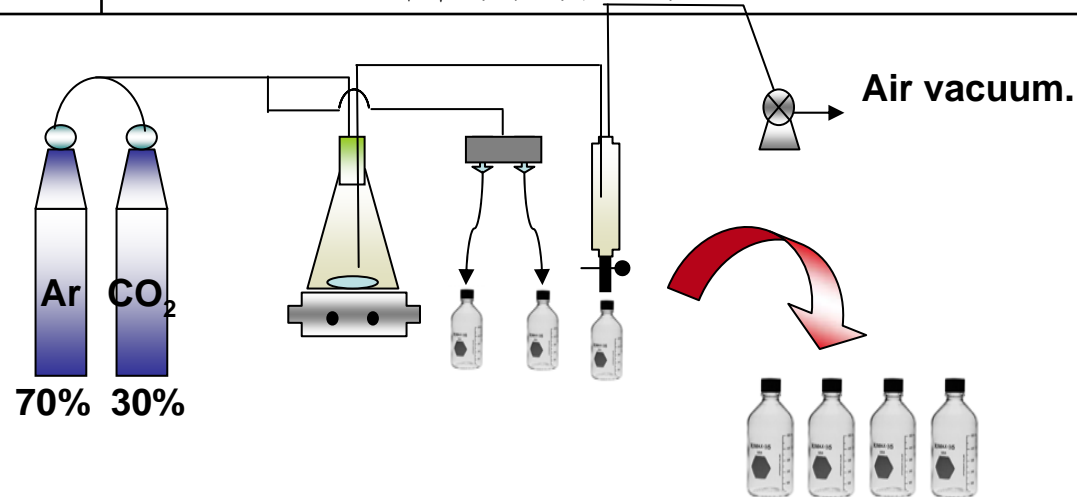
在台灣，一般生活污水隨著人口密集的增加和生活品質的提昇，也逐年迅速的增加。台灣衛生下水道工程興建尚未完備。近年都市污水處理廠採取厭氧生物處理污泥的技術剛起步不久，對於厭氧生物的特性了解尚有不足，有機性污泥含量及比例，被不同馴養程度之厭氧菌群逐次水解酸化及至甲烷化，隨厭氧污泥消化槽啟動運轉至長期培養而提升厭氧菌活性。本研究引用批分式生甲烷產能實驗評估中溫及高溫厭氧菌種分群及半固態生質污泥及乙酸基質之單位重厭氧菌產生甲烷量，以食微比結果代表厭氧菌活性。因此加強對厭氧生物活性進行定性定量分析是直接的，可應用於厭氧污泥消化槽之功能評估，藉以調控其食微比之操作參數，以提昇甲烷產氣速率。



實驗方法與設計

本研究主要採用W.F.Owen等(1979)之生化甲烷產能試驗(Biochemical Methane Potential, BMP)方法，來評估甲烷菌的生化潛能。

代號	植種源與基質源
HAc1	Seeding : 實驗室馴養八里污泥
	Substrate : 醋酸鈉
HAc2	Seeding : 八里消化模型槽(20L)污泥
	Substrate : 醋酸鈉
CS1	Seeding : 實驗室馴養八里污泥
	Substrate : 八里都市污水的初沉污泥



Anaerobic transport of seeding & substrates Lab of Professor Sheng-Shung Cheng



表 三批次厭氧污泥分解乙酸及初級污泥之生化甲烷產能試驗條件

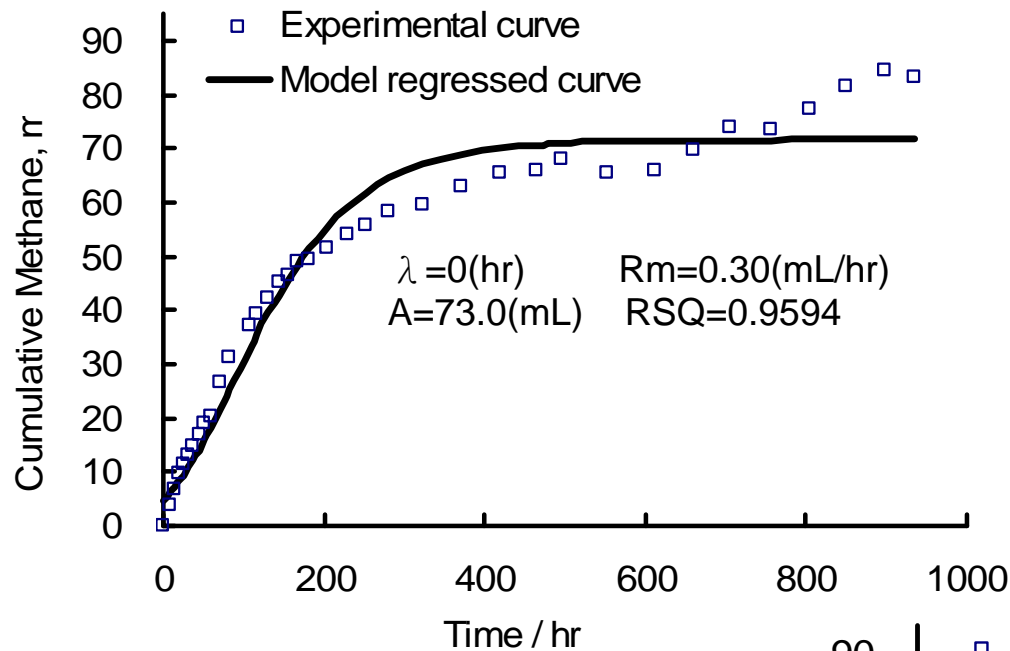
HAc1										35°C										
食微比 S_0/X_0	0	0.05	0.1	0.15	0.2	0.25	0.375	0.5												
基質濃度 S_0 CODt(mg/L)	0	200	400	600	800	1000	1500	2000												
微生物濃度 X_0 MLVSS(mg/L)	4000	4000	4000	4000	4000	4000	4000	4000												
HAc2										35°C										
食微比 S_0/X_0	0	0.02	0.04	0.07	0.1	0.12	0.16	0.2												
基質濃度 S_0 CODt(mg/L)	0	200	400	700	1000	1200	1600	2000												
微生物濃度 X_0 MLVSS(mg/L)	10,000	10,000	10,000	10,000	10,000	10,000	10,000	10,000												
CS1										35°C										
食微比 S_0/X_0	0	0.05	0.1	0.15	0.2	0.25	0.375	0.5	-											
基質濃度 S_0 CODt(mg/L)	0	200	400	600	800	1000	1500	2000	4000											
微生物濃度 X_0 MLVSS(mg/L)	4000	4000	4000	4000	4000	4000	4000	4000	4000	0										

3 Biogas Production Curve model fitting Michael is Menten Model



厭氧甲烷產氣速率模式化計算法

Biogas production curve model fitting



$$y = A \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{A}(\lambda - t) + 1\right]\right\}$$

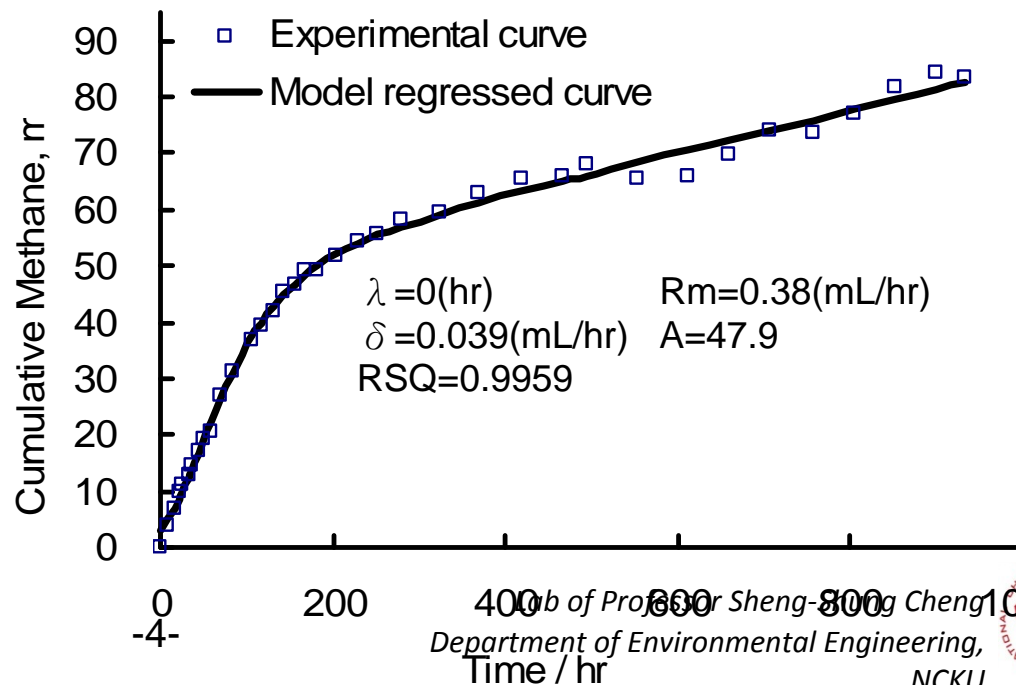
λ : 遲滯期 (hr)
 R_m : 最大產氣速率 (mL/hr)
 A : 平衡產氣量 (mL)

由 R_m 換算而得的比產甲烷速率 = 9.69 (mL/gVSS-day)

$$y = (\delta \cdot t + A) \cdot \exp\left\{-\exp\left[\frac{R_m \cdot e}{(\delta \cdot t + A)}(\lambda - t) + 1\right]\right\}$$

λ : 遲滯期 (hr)
 R_m : 最大產氣速率 (mL/hr)
 δ : 曲線後段直線斜率 (mL/hr)
 A : 曲線後段直線與 y 軸截距 (mL)

由 R_m 換算而得的比產甲烷速率 = 12.27 (mL/gVSS-day)



厭氧甲烷產氫速率動力特性模式表示法

Michaelis-Menten Model for Kinetic Study

$$V = V_0 + \frac{V_{max} \cdot S}{S + K_m} \text{-----Eq2.}$$

- V** : Specific methane production rate (mL/gVSS/day)
- V₀** : Substrate blank specific methane production rate (mL/gVSS/day)
- V_{max}** : Maximum specific methane production rate (mL/gVSS/day)
- S** : Substrate concentration (mg COD/L)
- K_m** : Michaelis-Menten constant (mg COD/L)

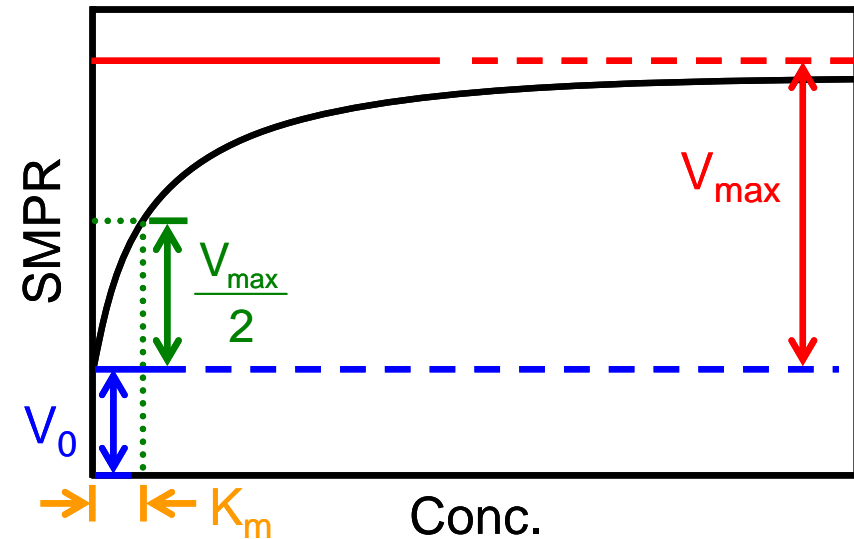


Fig.11 Michaelis-Menten model curve.



三、結果與討論

3.1 中溫厭氧菌群分解乙酸基質之甲烷產氣動力特性

The Cumulative Gas of HAc1

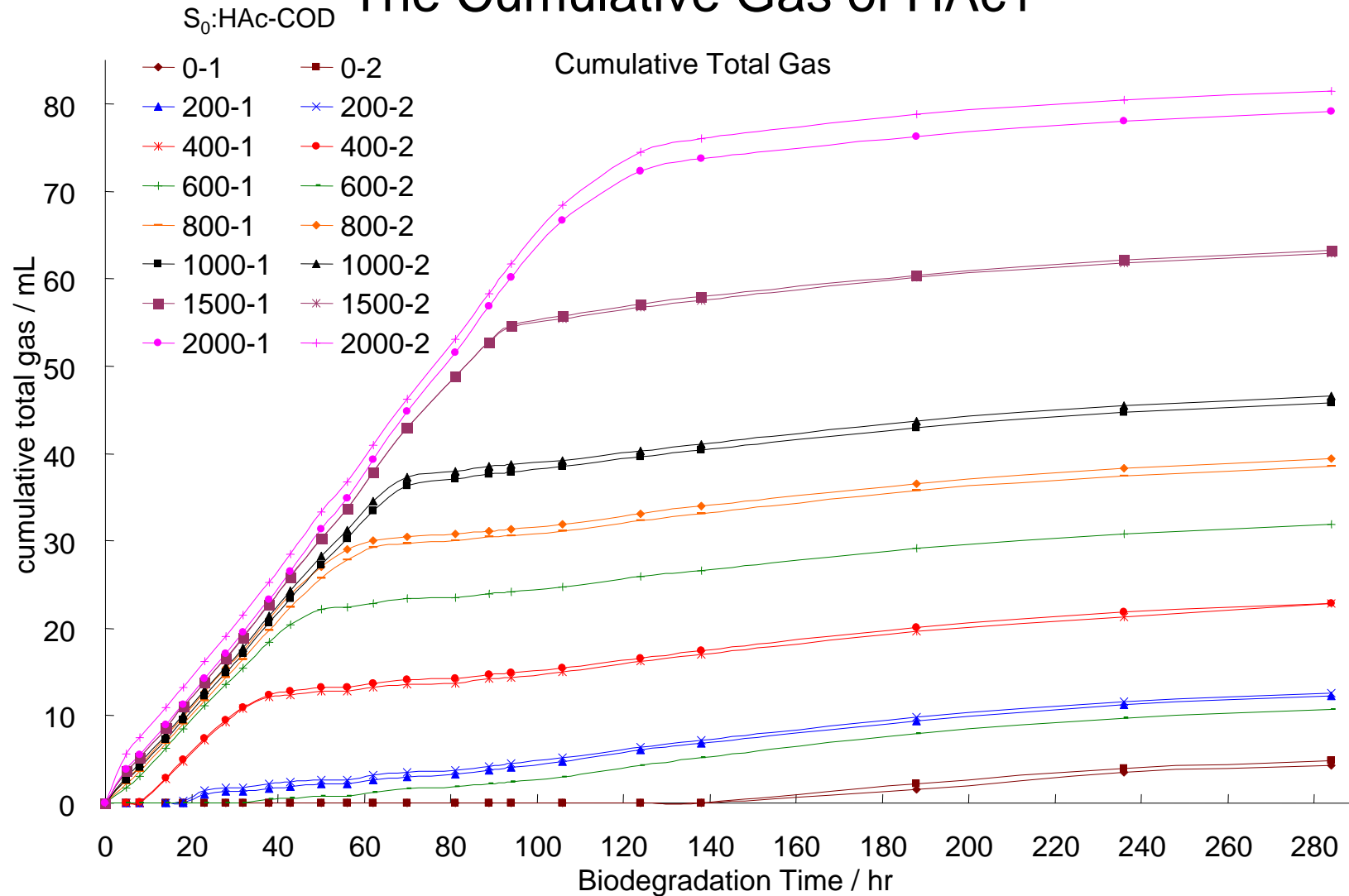


Figure Cumulative biogas production(mL) vs Batch HAc biodegradation time(hr)



實驗室馴養厭氧污泥分解乙酸基質之前後水質分析項目(醋酸基質第一批)		35℃								
基質濃度		FB	200	400	600	800	1000	1500	2000	
Initial	MLSS(mg/L)	5720	5550	5810	5910	5840	6100	5950	6080	
	MLVSS(mg/L)	3970	3900	4120	4310	4200	4460	4390	4390	
	VSS/SS	0.69	0.70	0.71	0.73	0.72	0.73	0.74	0.72	
	CODt(mg/L)	6182	5683	6298	6221	6912	7728	7920	8064	
	CODs(mg/L)	171	330	407	484	706	960	1594	2131	
	NH ₄ ⁺ -N(mg/L)	168	-	-	-	-	-	-	-	157
	TKN(mg/L)	477	-	-	-	-	-	-	-	591
Final (After 284hrs)	MLSS(mg/L)	6550	5180	5340	5280	5190	5390	5410	6550	
	MLVSS(mg/L)	4770	3970	3940	3920	3930	4160	4320	5120	
	VSS/SS	0.73	0.77	0.74	0.74	0.76	0.77	0.80	0.78	
	CODt(mg/L)	4918	4702	4643	4564	4761	4722	4427	4210	
	CODs(mg/L)	314	270	277	276	258	282	265	304	
	COD _t (%)	20.4	17.3	26.3	26.6	31.1	38.9	44.1	47.8	
	NH ₄ ⁺ -N(mg/L)	178	-	-	-	-	-	-	-	161
TKN(mg/L)	431	-	-	-	-	-	-	-	467	

Total COD biodegradation and Cell growth within 12 days



三、結果與討論

3.1 中溫厭氧菌群分解乙酸基質之甲烷產氣動力特性

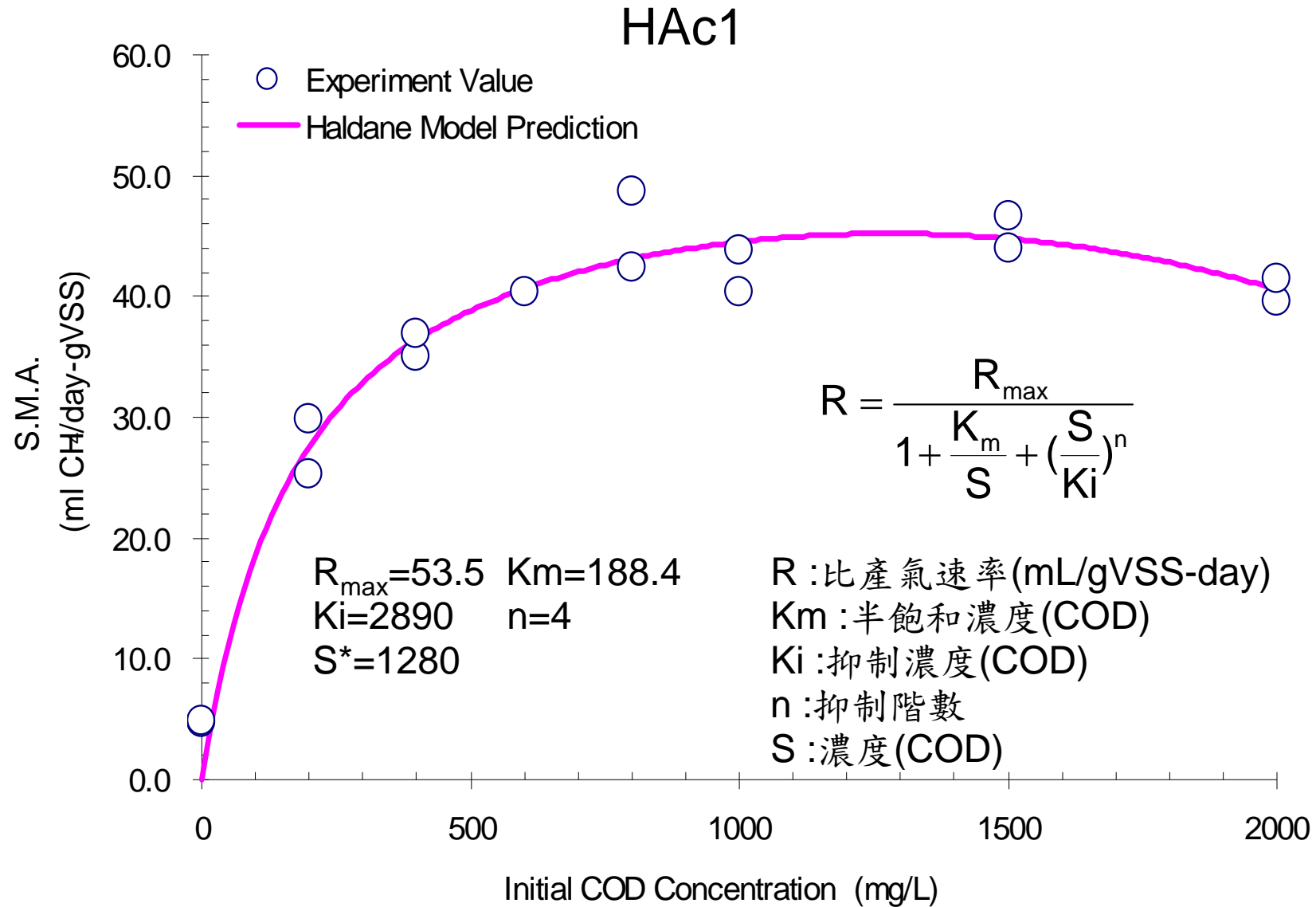


Figure Initial HAc-COD-biogas conversion rate vs HAc-COD concentration *Lab of Professor Sheng-Shung Cheng*



The Cumulative Gas of HAc2

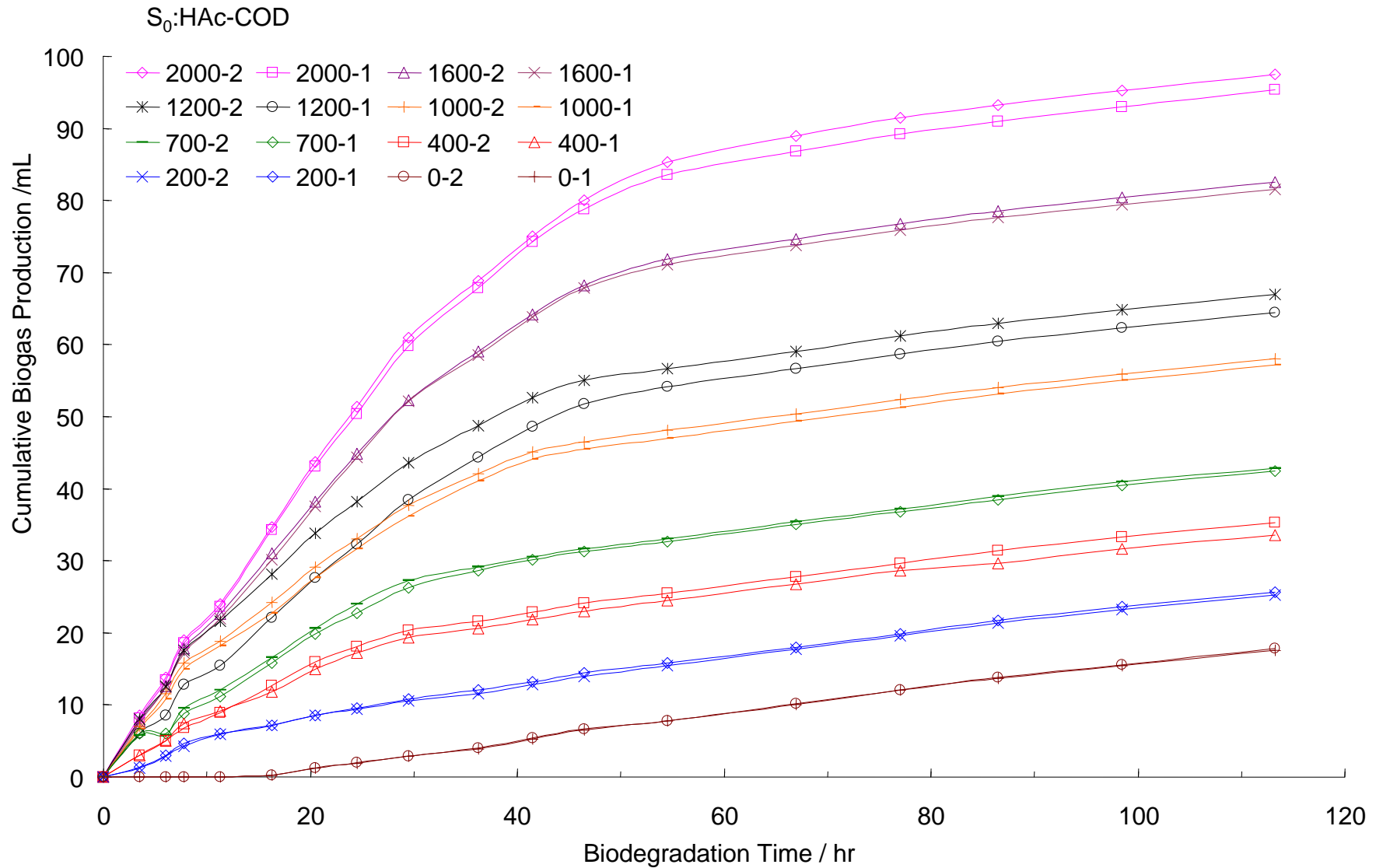


Figure Cumulative biogas production(mL) vs Batch HAc biodegradation time(hr)



模型槽馴養厭氧污泥分解乙酸基質之前後水質分析項目(醋酸基質第二批)		35°C							
基質濃度		FB	200	400	700	1000	1200	1600	2000
Initial	MLSS(mg/L)	20640	22295	15130	20410	20323	20828	16470	21305
	MLVSS(mg/L)	8447	9095	6175	8328	8293	8550	6755	8675
	VSS/SS	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
	CODt(mg/L)	17593	16290	16507	16507	17050	17810	18027	18462
	CODs(mg/L)	266	343	605	837	1099	1691	1412	2259
	NH ₄ ⁺ -N(mg/L)	256	-	-	-	-	-	-	262
	TKN(mg/L)	867	-	-	-	-	-	-	852
Final (After 113hrs)	MLSS(mg/L)	20785	21395	20905	20885	21328	20868	20143	21100
	MLVSS(mg/L)	8420	8575	8355	8413	8615	8440	8243	8500
	VSS/SS	0.41	0.40	0.40	0.40	0.40	0.40	0.41	0.40
	CODt(mg/L)	15376	15474	15076	14977	15477	14979	17069	16263
	CODs(mg/L)	256	335	357	369	408	460	421	721
	COD _t (%)	12.6	5.0	8.7	9.3	9.2	15.9	5.3	11.9
	NH ₄ ⁺ -N(mg/L)	253	-	-	-	-	-	-	233
TKN(mg/L)	857	-	-	-	-	-	-	885	

The initial HAc biodegradation without cell growth within 5 days



HAc2

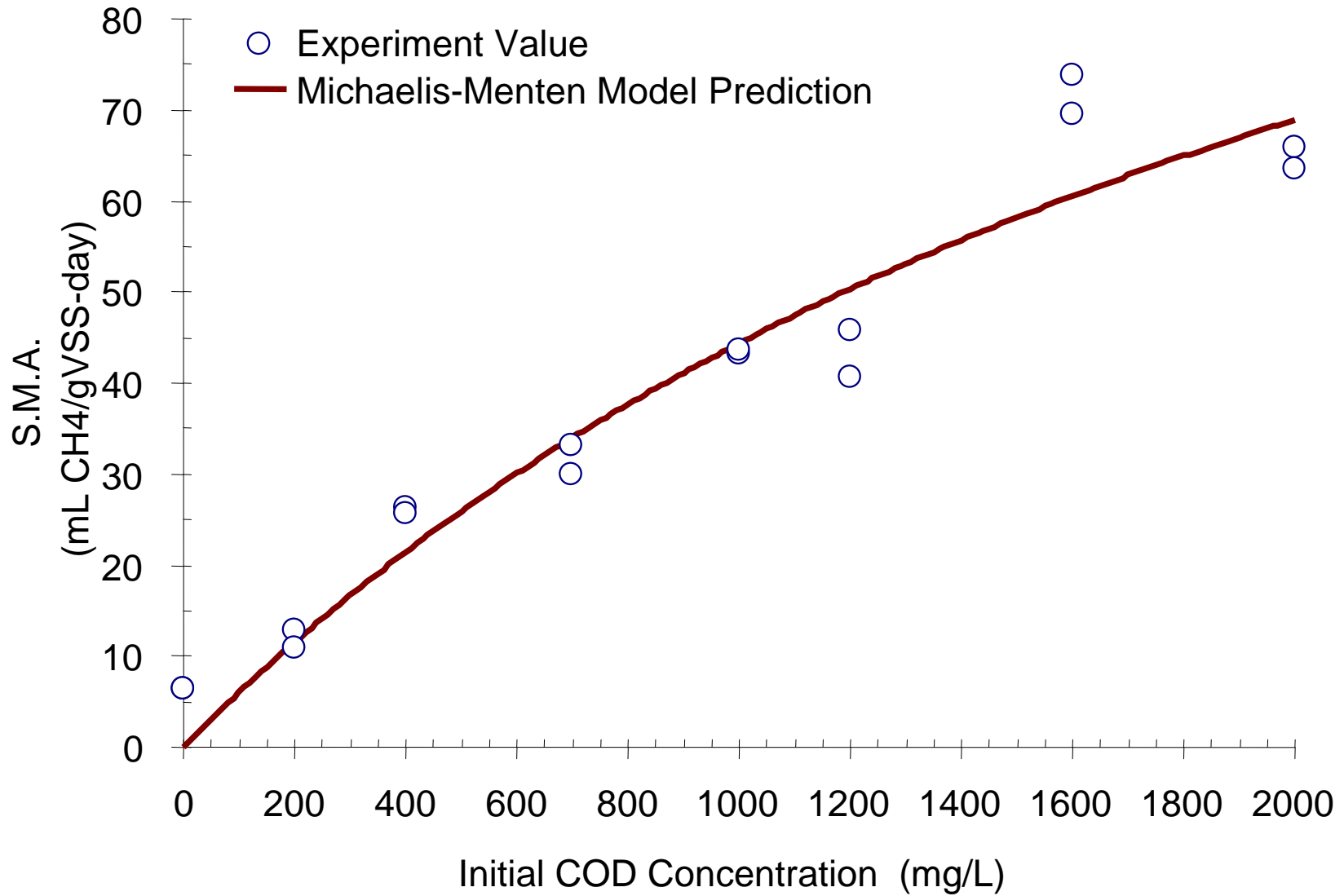


Figure Specific initial HAc-COD-biogas conversion rate vs HAc-COD concentration

Lab of Professor Sheng-Shung Cheng

Department of Environmental Engineering,

NCKU



小結

- 由醋酸基質的BMP test中確認植種污泥內有利用醋酸來產Methane的甲烷化菌，且在高食微比下醋酸會對厭氧菌產生抑制。
- 由動力模式的迴歸曲線來看，連續流所培養的污泥其最大比產甲烷速率達 $154.3 \text{ mLCH}_4/\text{gVSS-day}$ ，約為批次培養 $53.5 \text{ mLCH}_4/\text{gVSS-day}$ 的3倍。因此利用乙酸的甲烷菌以連續流所培養的污泥活性較佳。
- 就半飽和濃度 K_m 來看，批次培養的為 188.4 mg/L ，換算其食微比為 $0.0471 \text{ gCODr/gVSS-day}$ ；而連續流培養的 K_m 值為 2479 mg/L ，換算成食微比為 $0.25 \text{ gCODr/gVSS-day}$ 。故批次培養的污泥對乙酸的親和性比連續流培養的還好，可能是批次培養的污泥長期處在乙酸的親合馴養環境的緣故。



3-2 中溫厭氧菌群分解初沉污泥之甲烷產氣動力特性

The Cumulative Gas of CS1

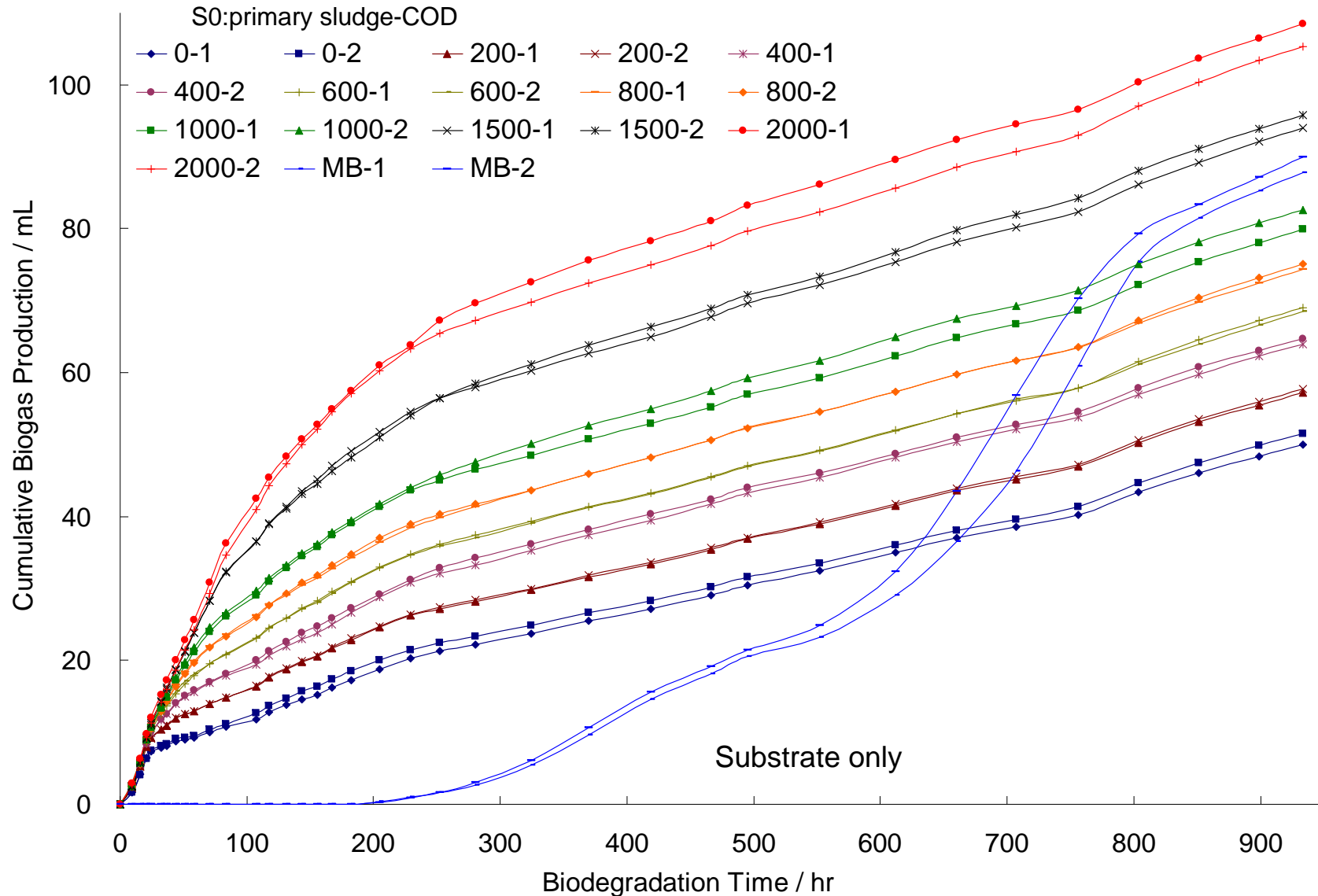


Figure Cumulative biogas production (mL) vs Batch sludge digestion time (hr)



馴養厭氧菌種消化分解污水廠初沉污泥之BMP test前後水質分析項目										35°C
	基質濃度	FB	200	400	600	800	1000	1500	2000	MB
Initial	MLSS(mg/L)	9300	8700	9800	9700	8750	10220	9450	11366	3460
	MLVSS(mg/L)	6250	6550	6550	7000	6700	6480	6700	7433	1600
	VSS/SS	0.67	0.75	0.67	0.72	0.77	0.63	0.71	0.65	0.46
	COD _t (mg/L)	7475	7082	7475	8853	8459	11213	9639	9443	3266
	COD _s (mg/L)	254	257	272	280	299	382	437	527	289
	NH ₄ ⁺ -N(mg/L)	209	219	212	203	208	215	237	238	107
	TKN(mg/L)	732	-	-	-	699	751	773	803	203
	pH	7.64	7.62	7.61	7.63	7.58	7.58	7.59	7.48	6.77
Final (After 934hrs)	ORP(mV)	-408	-422	-430	-425	-424	-421	-421	-419	-360
	MLSS(mg/L)	6930	8100	8520	8683	9567	9683	11967	10833	3200
	MLVSS(mg/L)	5430	6350	5990	6733	6750	7100	7850	7483	1700
	VSS/SS	0.78	0.78	0.70	0.78	0.71	0.73	0.66	0.69	0.53
	COD _t (mg/L)	6977	7032	6996	6977	7074	7364	7425	7482	1861
	COD _s (mg/L)	396	385	365	379	389	370	418	401	242
	COD _{t_r} (%)	6.67	0.71	6.41	21.2	16.4	34.33	22.98	20.76	43
	NH ₄ ⁺ -N(mg/L)	220	-	-	-	-	-	-	212	57
	TKN(mg/L)	694	-	-	-	-	-	-	737	190

Anaerobic sludge digestion with limited MLVSS bio conversion ratio after 39 days



CS1

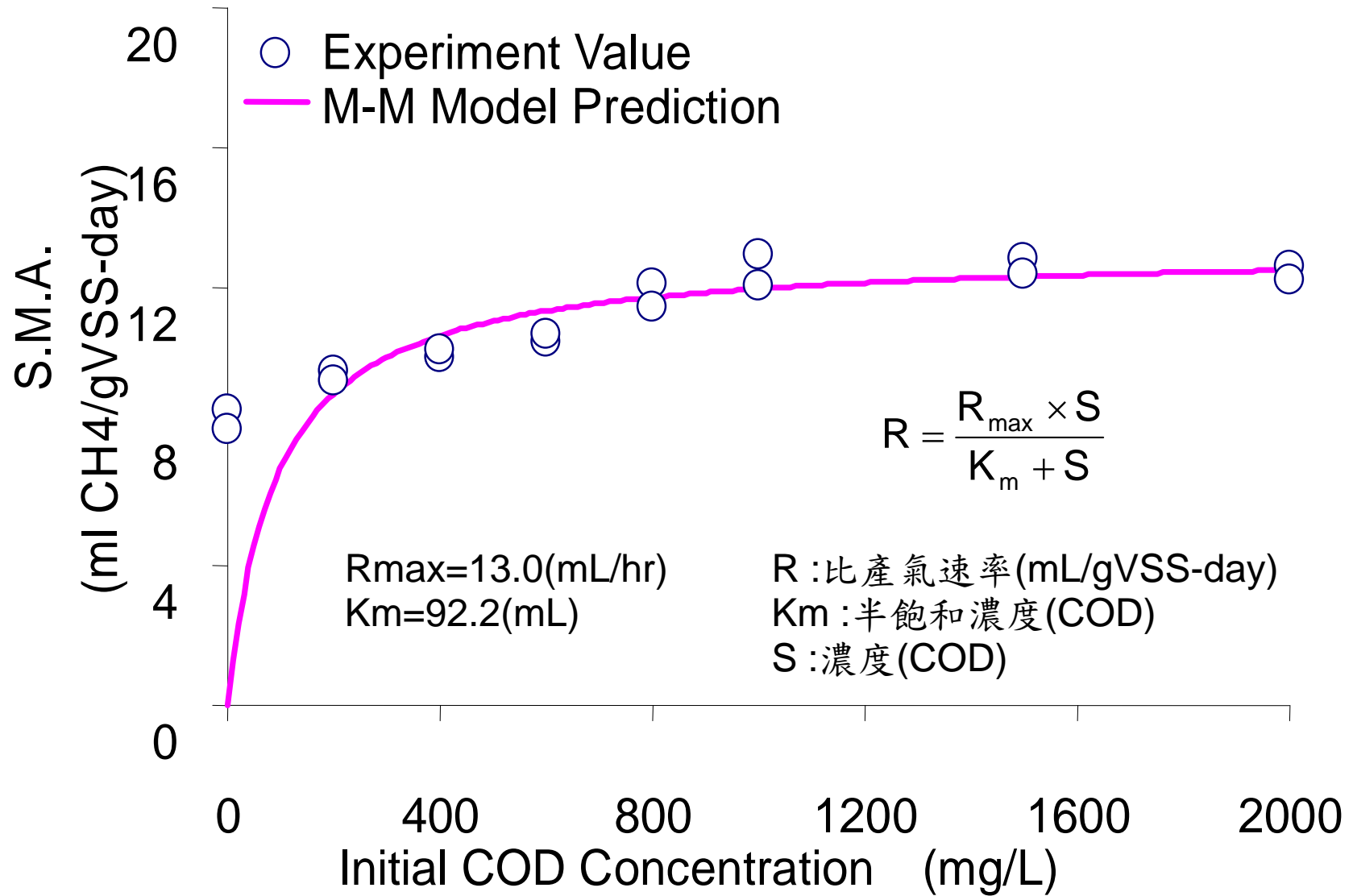


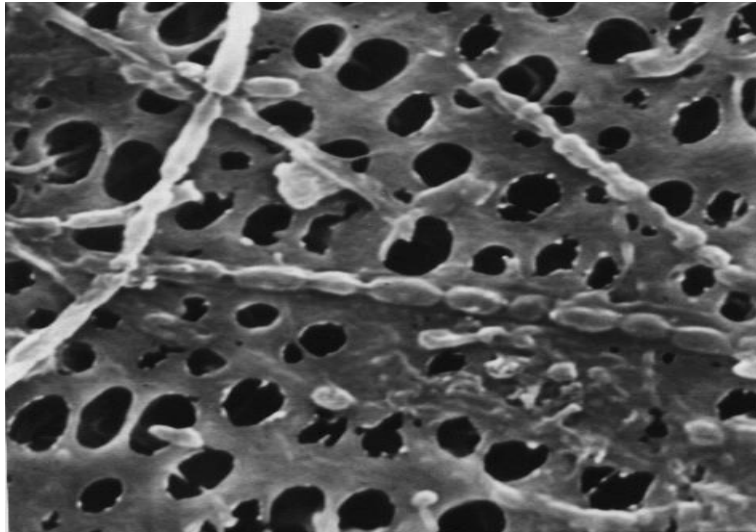
Figure Specific sludge-COD-biogas conversion rate vs primary sludge concentration



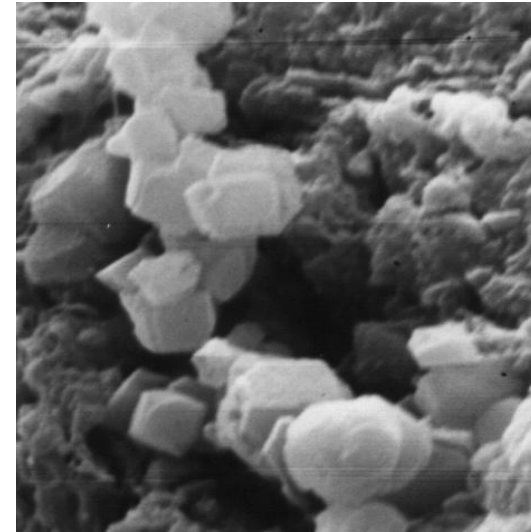
結語

- 都市污水的初沉污泥中所含的原始菌種，經35°C厭氧培養約10天後可使其厭氧菌群活性增加。
- 以初沉污泥作為基質的BMP test中(40天)，不管微生物的來源為何，到最後都會以某穩定速率產氣，其斜率在0.96mL/day~1.44mL/day間，換算成比產氣速率皆在1.84mL/gVSS-day附近；因此可以判定此處的產氣速率主要是由固體污泥水解酸化成溶解性COD供甲烷菌利用的水解速率來決定。
- 由上面的實驗可知，大型厭氧污泥消化槽內的甲烷菌利用半固體污泥主要取決於固體污泥的水解效率，故如何增加水解酸化程度將是消化槽操作及功能提升的主要因素
- 本污水處理廠之初沉污泥水解速率慢，需長期馴養厭氧菌群及較長消化日數，才能有效產生甲烷。而溶解性乙酸甲烷化速率快而完成於5天內。

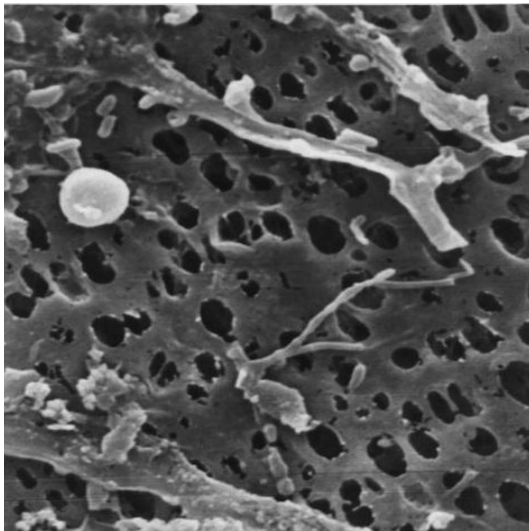




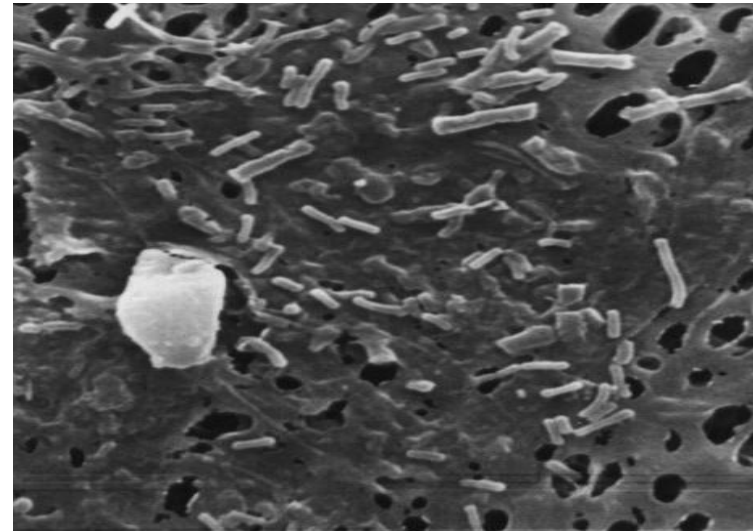
CS1的FB實驗組：許多串聯在一起的短桿菌。
代表植種厭氧菌群 倍率X7000



CS1的MB實驗組：有類似八聯球菌的菌群
在其中。-初沉污泥即有菌群 倍率X 7000



CS1的2000實驗組：除了短桿菌以外，也
有球菌在其中。代表植種與污泥混合菌群
倍率X6000



CS1的FB實驗組：有許多短桿菌(約 $2\mu\text{m}$)散
落在膠羽上。代表植種菌類之一

-17-

倍率X5000

Lab of Professor Sheng-Shung Cheng
Department of Environmental Engineering,

NCKU



3-3 中溫厭氧菌群分解乙酸基質之甲烷產氣動力特性

Methane Production of AP-HAc-BMP Batch Test

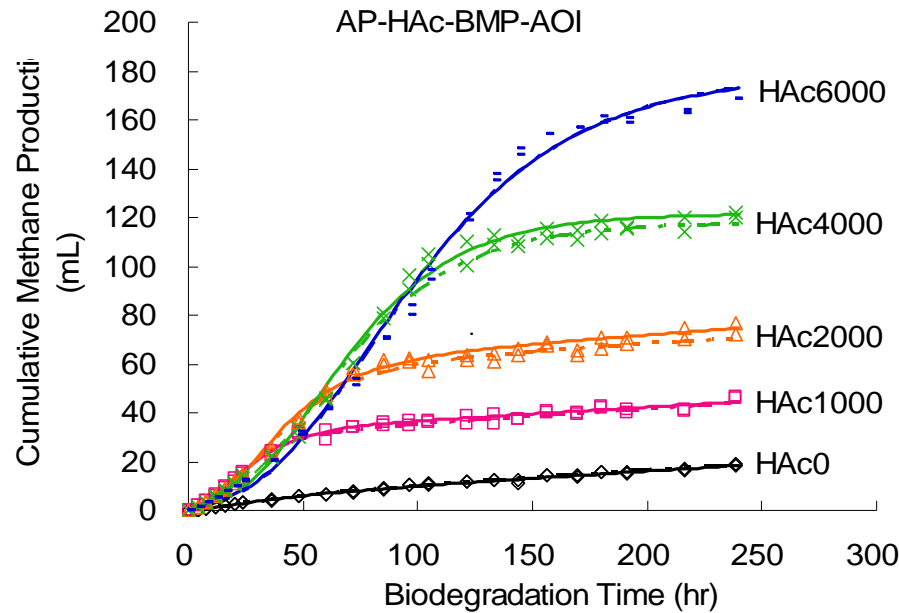


Fig.3 Cumulative methane production curve of different HAc concentrations degraded by AP sludge digester anaerobes.

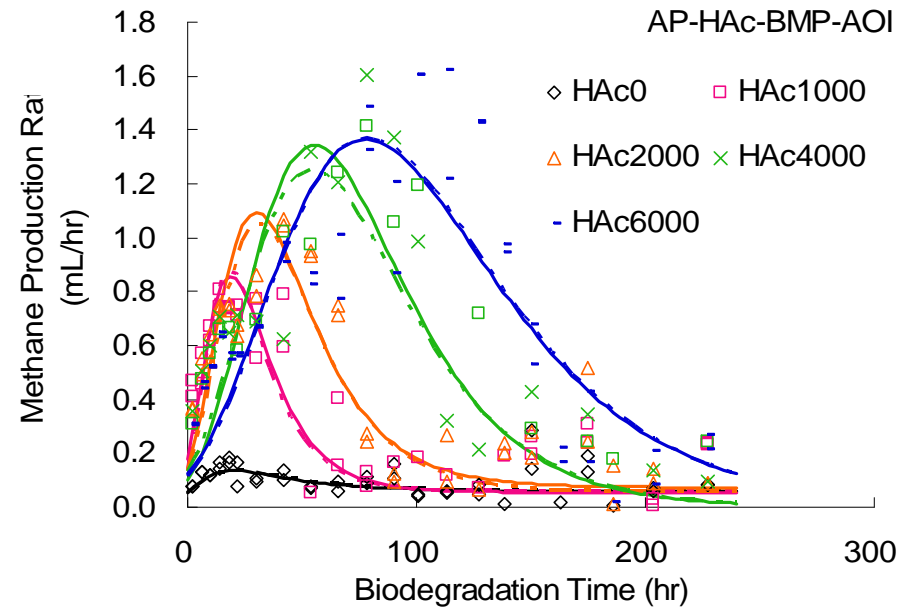


Fig.4 Methane production rate curve of different HAc concentrations degraded by AP sludge digester anaerobes.

Table1. BMP experimental design of AP sludge digester anaerobes degrading acetic acid substrate.

Item	HAc0	HAc1000	HAc2000	HAc4000	HAc6000
Substrate - S_0 mg COD/L	0	976	1,915	3,890	6,011
Seeding - X_0 VSS (mg/L)	12,530 \pm 1,182				
S_0/X_0	0.00	0.08	0.15	0.31	0.48



3-4 中溫厭氧菌群分解廢棄活性污泥之甲烷產氣動力特性

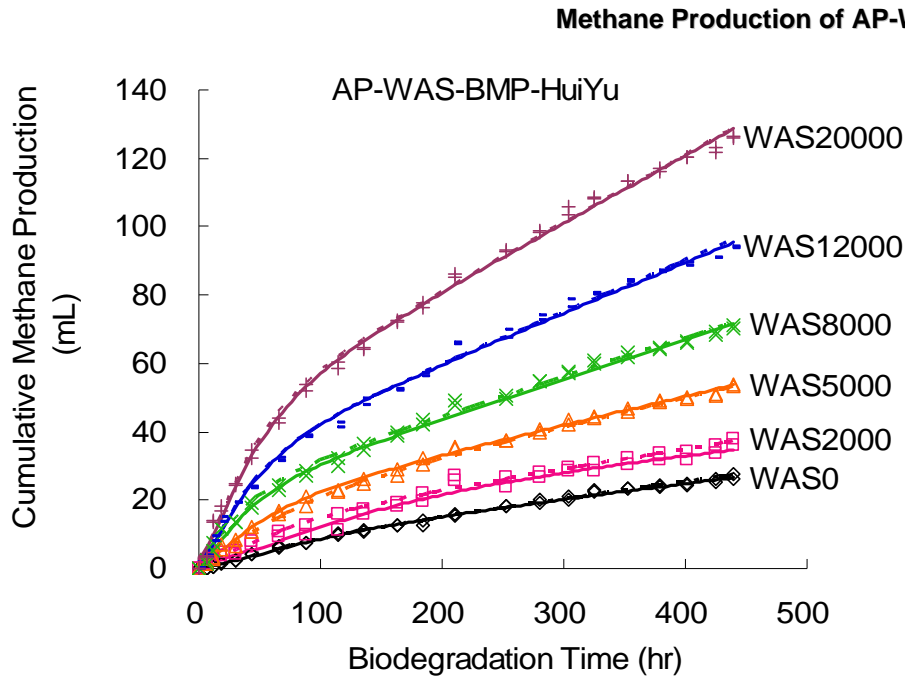


Fig.7 Cumulative methane production curve of different WAS concentrations degraded by AP sewage sludge acclimated anaerobes.

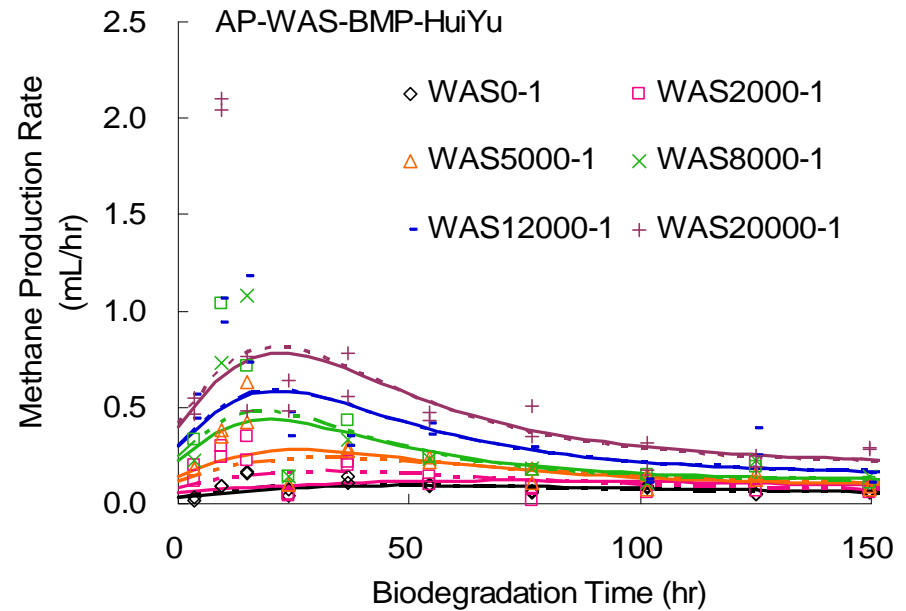


Fig.8 Methane production rate curve of different WAS concentrations degraded by AP sewage sludge acclimated anaerobes.

Table3. BMP experimental design of **CL primary sludge acclimated anaerobes degrading primary sludge substrate**

Item	WAS0	WAS2000	WAS5000	WAS8000	WAS12000	WAS20000
Substrate - S_0 mg COD/L	0	2,820	6,109	12,218	20,677	31,570
Seeding - X_0 VSS (mg/L)	14,550					
S_0/X_0	0.00	0.19	0.42	0.84	1.42	2.17



Michaelis-Menten Model Fitting Curves

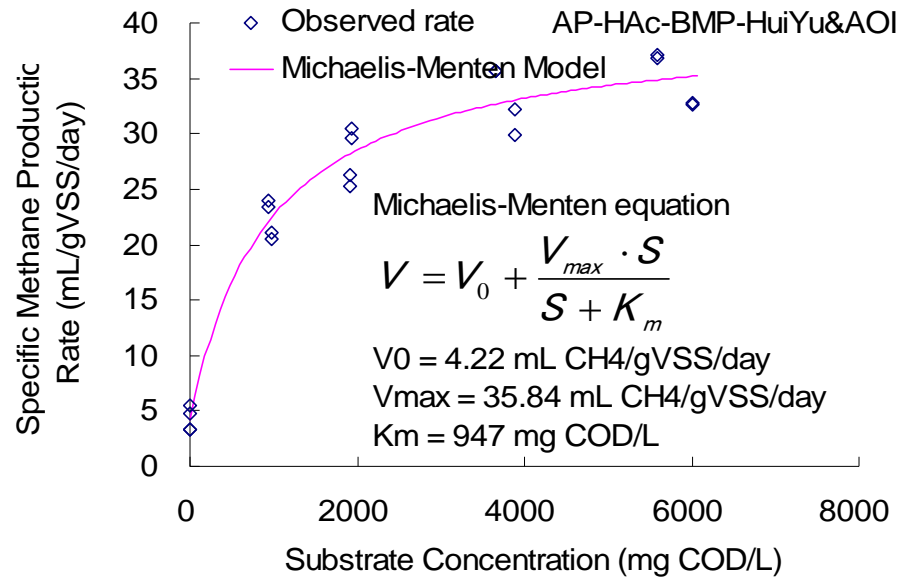


Fig. Kinetic model of specific methane production rate versus different substrate concentrations of HAc degraded by AP sewage sludge acclimated anaerobes.

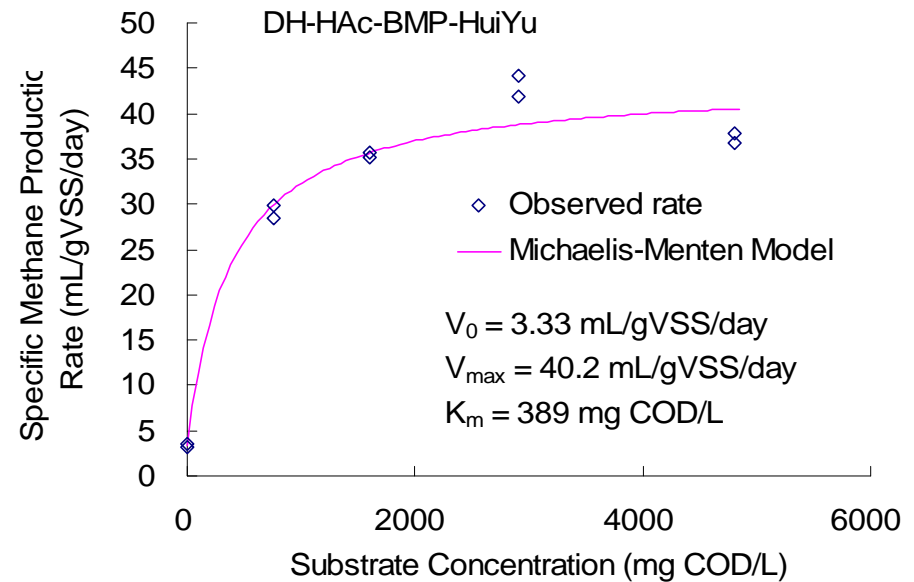


Fig. Kinetic model of specific methane production rate versus different substrate concentrations of HAc degraded by DH sewage sludge acclimated anaerobes.



Michaelis-Menten Model Fitting Curves (Continuous)

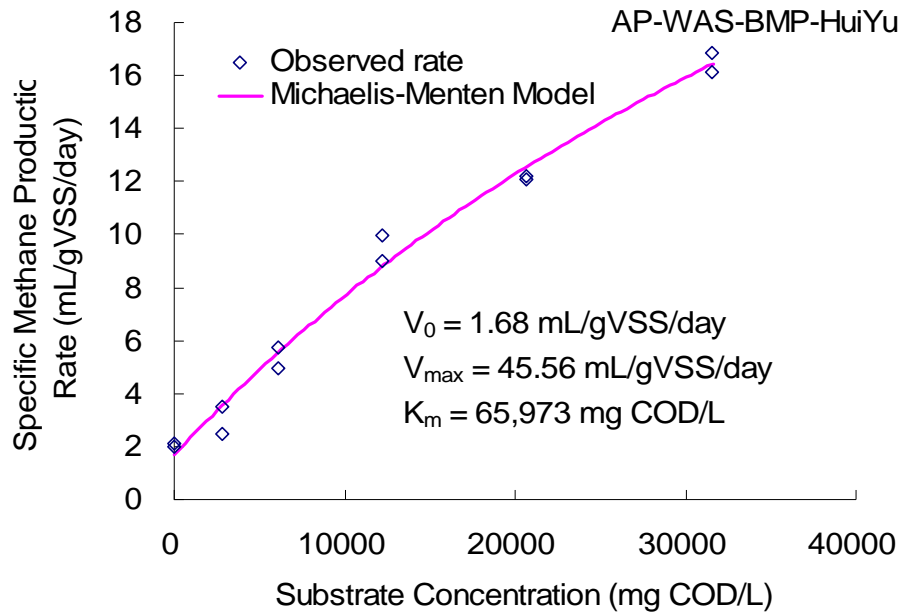


Fig. Kinetic model of specific methane production rate versus different substrate concentrations of WAS degraded by AP sewage sludge acclimated anaerobes.

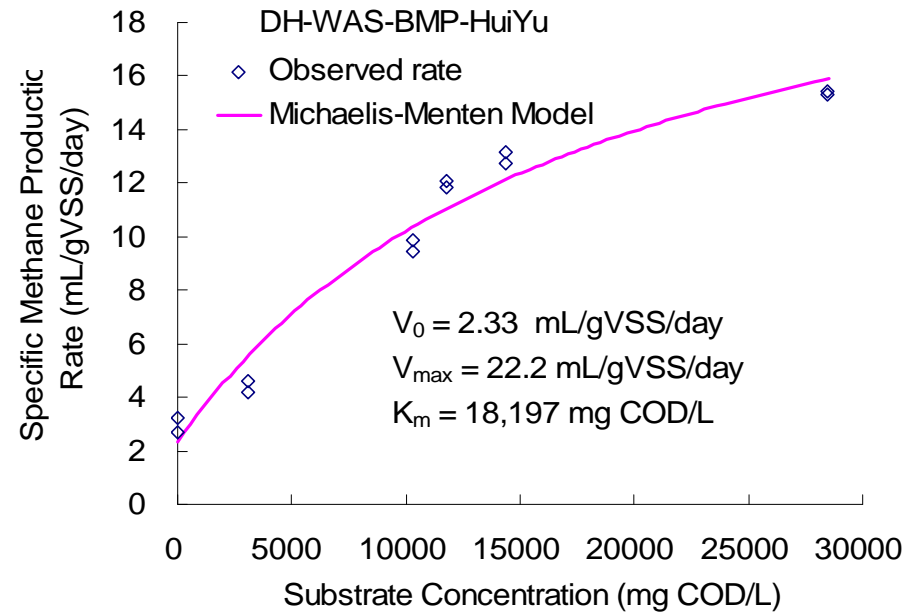


Fig. Kinetic model of specific methane production rate versus different substrate concentrations of WAS degraded by DH sewage sludge acclimated anaerobes.



3-5 中溫厭氧菌群分解乙酸基質及廢棄活性污泥之甲烷產能比較

Michaelis-Menten Parameters of Each Batch Test

Table 7. The relationship of Michaelis-Menten parameters of each batch test.

Batch test	V_0 mL/gVSS/day	V_{max} mL/gVSS/day	K_m mg COD/L	R^2	S_{max}^* mg COD/L	V_t^{**} mL/gVSS/day	V_{max}/K_m
AP-HAc-BMP-AOI	3.32	34.2	959	0.9968	6,011	32.8	35.7
AP-HAc-BMP-HuiYu	5.14	37.8	974	0.9985	5,583	37.4	38.8
DH-HAc-BMP-HuiYu	3.33	40.2	389	0.9668	4,809	40.5	103.3
AP-WAS-BMP-HuiYu	1.68	45.6	65,973	0.9880	31,570	16.4	0.7
DH-WAS-BMP-HuiYu	2.33	22.2	18,197	0.9654	28,449	15.9	1.2

* Maximum substrate concentration.

** Terminal specific methane production rate.

1. 兩污水廠污泥消化槽厭氧菌群分解乙酸基質之甲烷產汽速率(V_{max})皆相近快速，且半飽和常數(K_m)皆低，表現親何姓家可承受高濃度乙酸。

2. 兩場厭氧菌群分解廢氣活性污泥基質之水解酸化速率不同，而致甲烷化速率(V_{max})明顯差異。小型消化槽(AP)厭氧菌群活性高可快速利用一分解之生質污泥，但極緩達到半最大速率之污泥濃度(5%VSS)。大型消化槽運轉五年後，厭氧菌群穩定化，水解酸化甲烷化速率平穩(1.5%VSS)。



DH污水廠厭氧消化槽 Michaelis-Menten Parameters of Each Batch Test

Table 7. The relationship of Michaelis-Menten parameters of each batch test.

Batch test	V_0 mL/gVSS/ day	V_{max} mL/gVSS/day	K_m mg COD/L	R^2	S_{max}^* mg COD/L	V_t^{**} mL/gVSS/day	V_{max}/K_m
AP-HAc-BMP-Kotaro	5.13	43.7	383	0.9803			
CL-HAc-BMP- I -YuMin	10.16	40.2	45.9	0.9716			
CL-HAc-BMP- II -YuMin	9.06	122.8	1,830	0.9294			
AP-HAc-BMP-AOI	3.32	34.2	959	0.9968	6,011	32.8	35.7
AP-HAc-BMP-HuiYu	5.14	37.8	974	0.9985	5,583	37.4	38.8
DH-HAc-BMP-HuiYu	3.33	40.2	389	0.9668	4,809	40.5	103.3
AP-WAS-BMP-Kotaro	1.29	19.3	7,483	0.7017			
CL-PS-BMP- I -YuMin	8.25	9.8	1,093	0.9704			
CL-PS-BMP- II -YuMin	2.79	39.4	4,747	0.9197			
AP-WAS-BMP-HuiYu	1.68	45.6	65,973	0.9880	31,570	16.4	0.7
DH-WAS-BMP-HuiYu	2.33	22.2	18,197	0.9654	28,449	15.9	1.2

* Maximum substrate concentration.

** Terminal specific methane production rate.

Michaelis-Menten Model
$$V = V_0 + \frac{V_{max} \cdot S}{S + K_m} -23-$$



3-6 污水處理廠厭氧污泥消化槽實廠操作參數與厭氧菌群活性評估

- 小型污水廠現況AP Anaerobic Digester
- 廢水量 132,000 CMD
 - PS/WAS量 496 CMD，AD Vol. 4,000 X2 m³
 - $VS_{in} = 15\sim 20 \text{ g/L}$ ； $VS_{eff} = 12\sim 17 \text{ g/L}$ (Ryoko)
 - $VSS/SS = 0.55\sim 0.65$ ---無機含量高，有機負荷不足
 - VS loading $\sim 1.24 \text{ kg VS/m}^3\text{-day}$
 - 餘裕量=14 t VS/day CMD(3 kg VS/m³-day)約KW-130 m³
 - KW VS $\sim 108 \text{ g/L}$ (凱尹，2001)
 - 2-stage pilot verified- KW消化時，甲烷相HRT約20 day，與目前消化槽相當，故共消化可行。
- 厭氧消化槽活性測試
 - $K_m = 974 \text{ mg-COD/L(HAc)}$ ； $65,973 \text{ mg-COD/L(WAS)}$ ； $2,296 \text{ mg-COD/L(KW)}$
 - $\nu_{max} = 37.8 \text{ mL/gVSS/day}$ ； $45.6 \text{ mL/gVSS/day(WAS)}$ ； $116 \text{ mL/gVSS/day(KW)}$



DH Anaerobic Digester

- 大型污水廠現況
 - 廢水量 400,000~460,000 CMD
 - PS/WAS 700~800CMD，AD Vol. 13,000 ×3 m³
 - HRT=18 day-----2座併聯
 - VSin ~ 33± 5 g/L；VSeff ~ 17± 3 g/L；VSS/SS = 0.7
- VS loading ~ 0.97 kg VS/m³-day
 - AD Performance
 - 0.3 CH₄ m³ /m³-day即1kg CODr/m³-day 屬正常操作
 - F/M < 0.1
- 厭氧消化槽活性測試
 - Km = 389 mg-COD/L(HAc)；18,197 mg-COD/L(WAS)
 - ν_{\max} = 40.2 mL/gVSS/day；22.2 mL/gVSS/day(WAS)



3-7 厭氧污泥消化槽(AD)厭氧菌群菌相結構

DH 厭氧消化槽污泥SEM照片

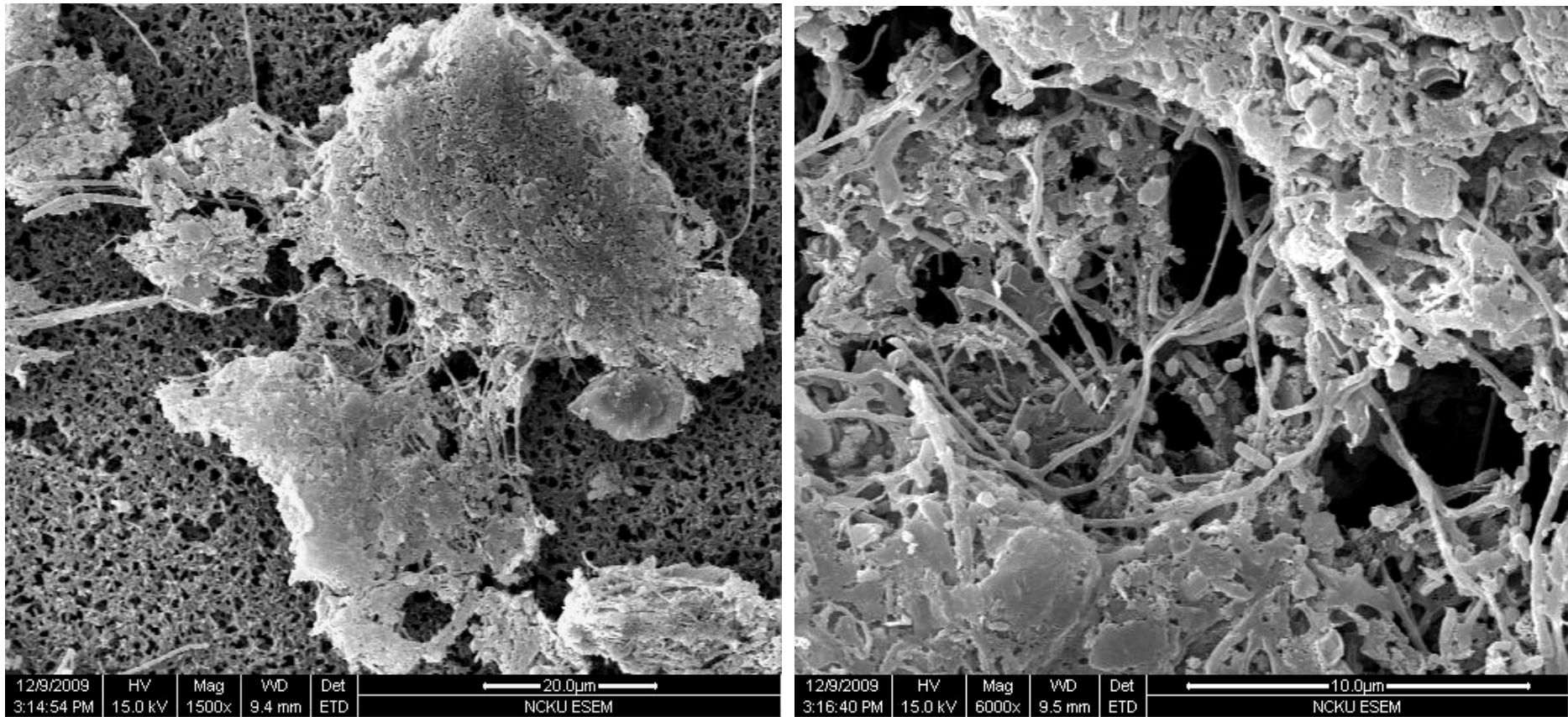


Figure AD污泥以絲狀的桿菌糾結成團，成團的污泥也可以發現長度1um~2um的短桿菌及大小為1um的球菌（2009.12）

DH 厭氧消化槽污泥SEM照片

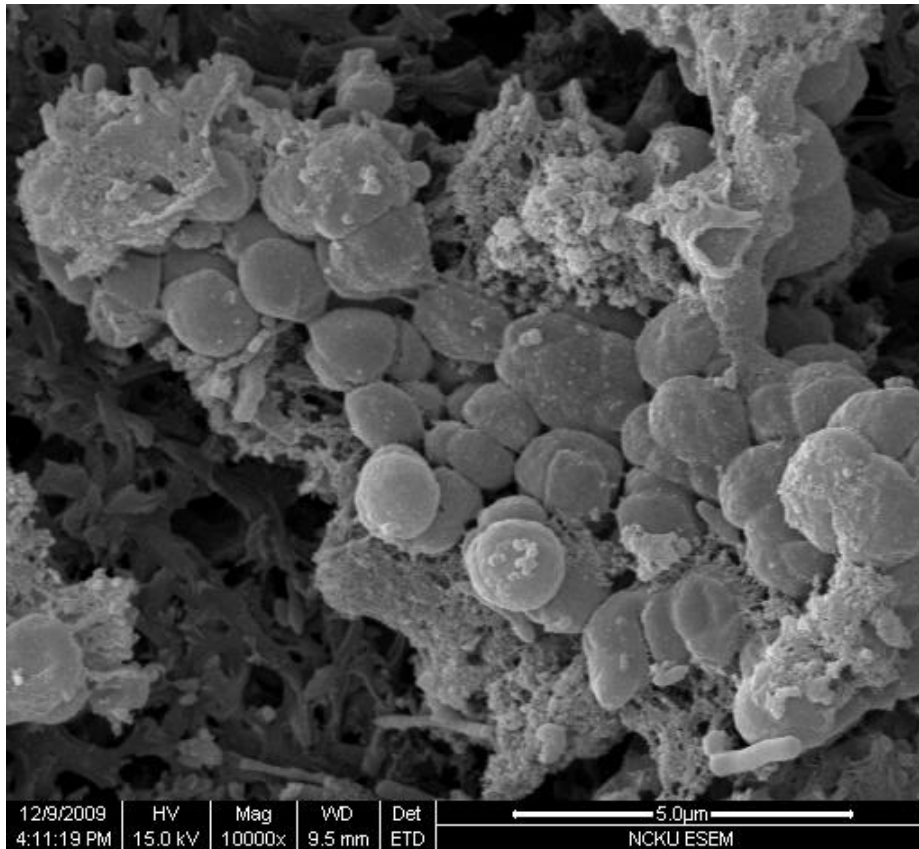


Figure AD 污泥中可以發現成團的球菌，以4個對生的球菌每個大小約為1um
(2009.12)

DH 厭氧消化槽污泥SEM照片

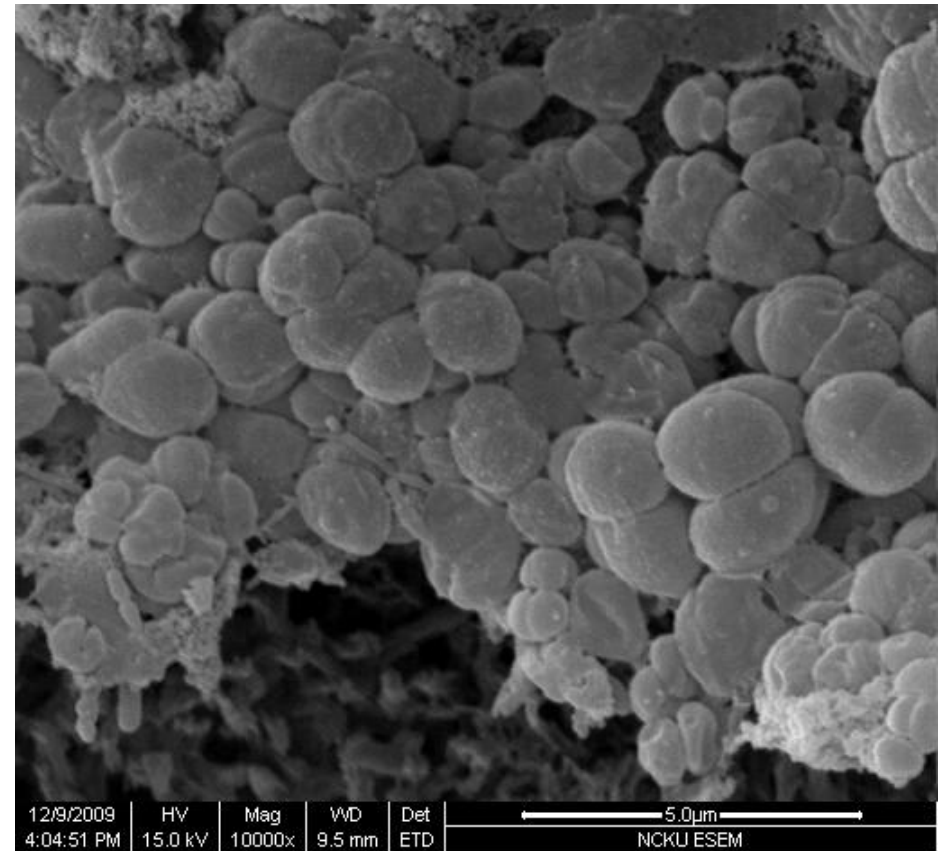
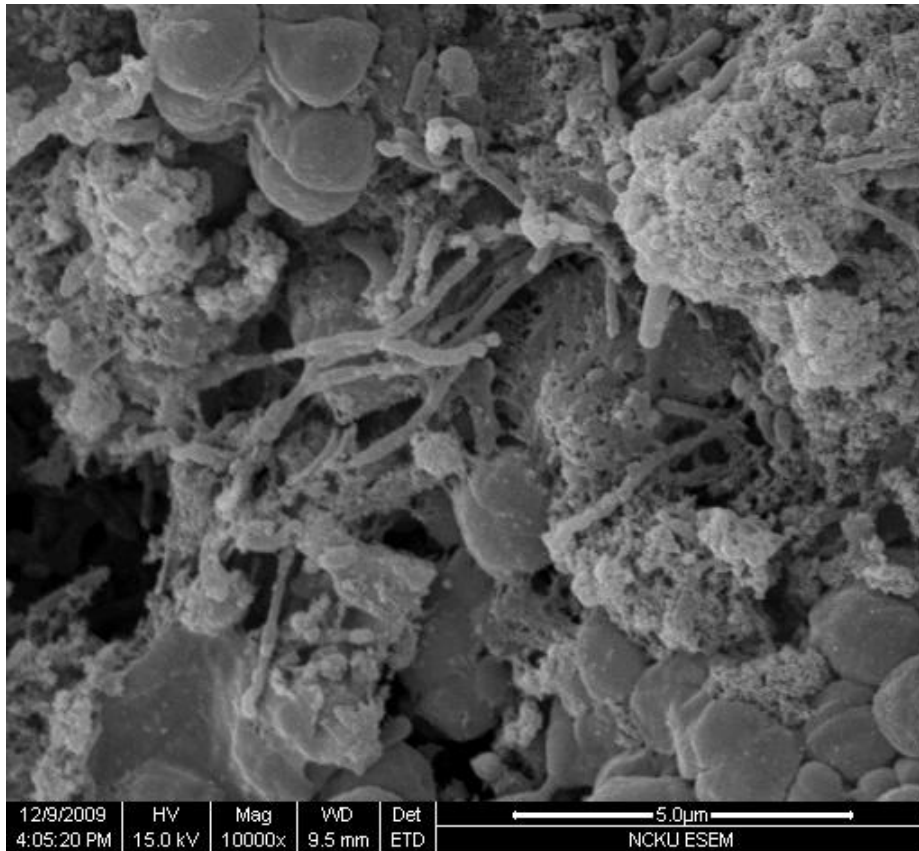


Figure 長桿菌與球菌皆可容易發現在AD污泥中，球菌是以團聚的方式生長
(2009.12)

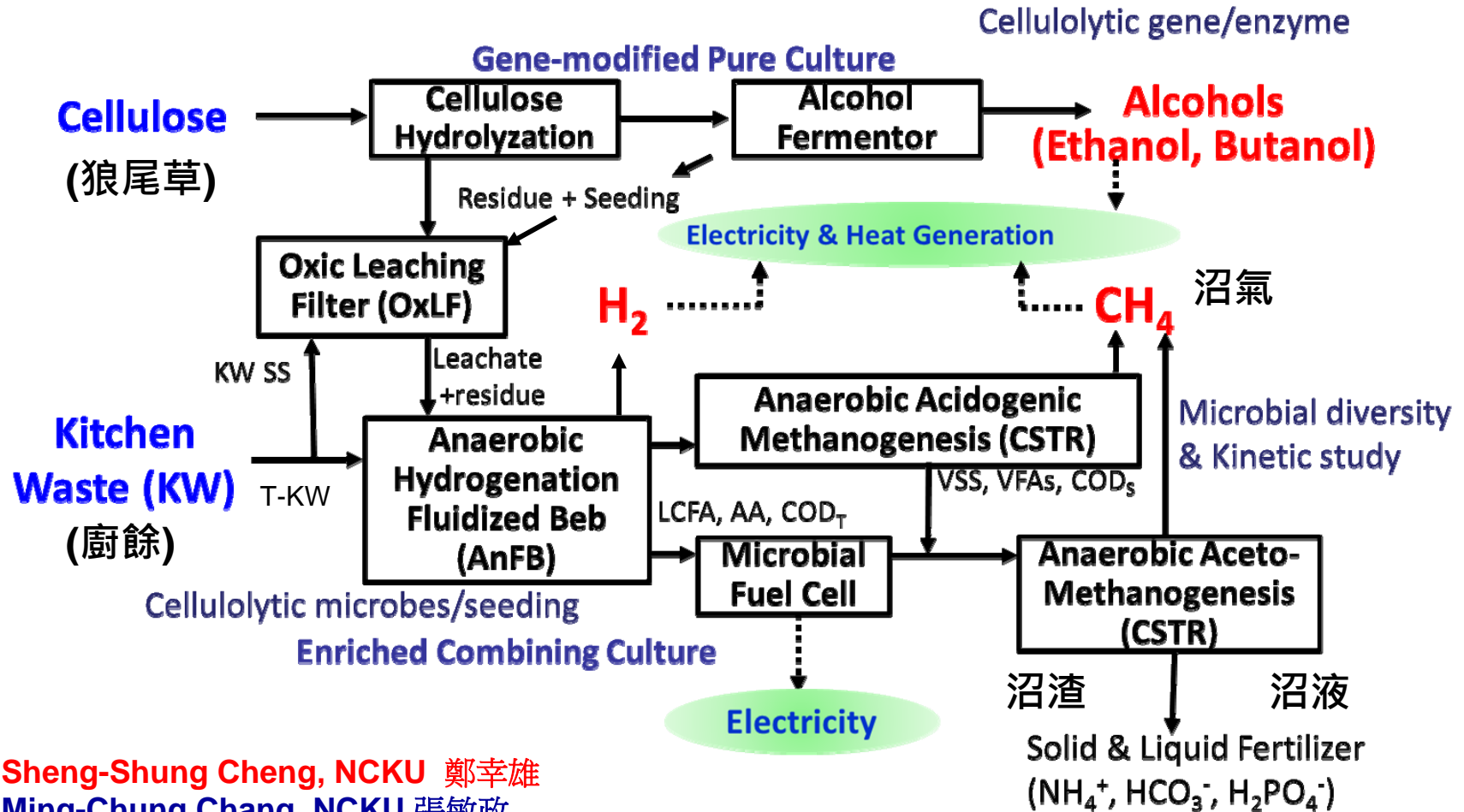
結語

1. 參訪的厭氧消化程序以D-H廠功能最佳，每日可產生12,000m³的生質氣體生成。
2. 參訪的AD在進料污泥的前置處理上，以PS與WAS先進污泥濃縮槽後的方式較佳，可以提高進料的污泥濃度。
3. F-T廠的串聯操作目前HRT過長，若要提昇其功能需調高進流物料濃度及進料量。
4. 以分光光度計分析抽取DNA的品質可以提高PCR產物成功的機會，稀釋倍率達1,000有助於降低干擾提高PCR產物的生成，可提高分子生物檢測技術之準確性。
5. D-H廠，處理水量設計500,000 CMD，目前平均日處理水量400,000-460,000m³，每年可以產生32,000噸的污泥，每日約100 m³/day 污泥，每噸污水生產污泥乾重為69 g/m³，其污泥厭氧消化設備總共有3個，每槽積為11,590 m³，目前使用兩個槽體，每日兩個消化槽總產氣量可達12,000 m³的產氣量，甲烷比例達60%以上。以單位槽積甲烷的產量來看，每日有0.3 m³CH₄ /m³-day的產率，相當有機負荷去除速率 1kg CODr/m³-day，屬正常操作功能，仍有提昇負荷之空間（2-3倍）。
6. 厭氧污泥消化槽所採污泥，經分子生物檢測技術T-RFLP分析結果，皆以 *Methanosaetaceae* sp. 長桿狀甲烷菌為主角，係厭氧消化槽低體積負荷量最普遍存在的菌種。



3-8 高溫厭氧醱酵程序處理廚餘與木質纖維之共消化機制研究

II-1 NSC Integrated Program: Integrating study on high effective biofuel energy recovery with multistage biorefinery process (Phase V, 2009-2012)



Sheng-Shung Cheng, NCKU 鄭幸雄
 Ming-Chung Chang, NCKU 張敏政
 Chieh-Chen Huang, NCHU 黃介辰
 Yu-Hong Wei, YZU 魏毓宏
 Wen-Chien Kuo, NPUST 郭文健



3-8 高溫厭氧醱酵程序處理廚餘與木質纖維之共消化機制

II-1 NSC Integrated Program: Integrating study on High Effective Biofuel Energy Recovery with Multistage BioRefinery Process (Phase V, 2009-2012)



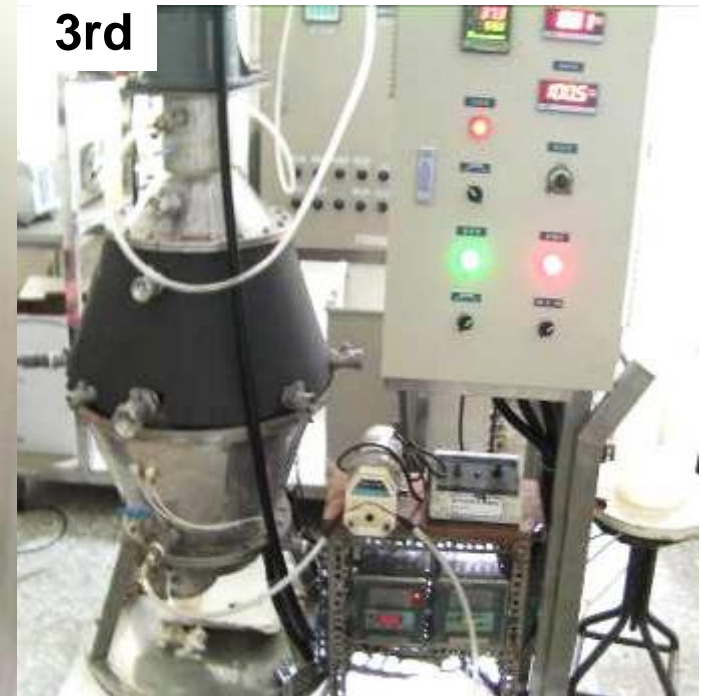
好氧真菌水解木質纖維
1st

Aerobic Leaching
Filter (95 L)



厭氧水解酸化產氫反應
2nd

Anaerobic
Acidogenesis 55°C
(8 L) -31-



厭氧古老菌產甲烷反應
3rd

Anaerobic
Methanogenesis 55°C
(85 L)

Lab of Professor Sheng-Shung Cheng
Department of Environmental Engineering,
NCKU



厭氧生物處理程序

Organics Conversion in Anaerobic System

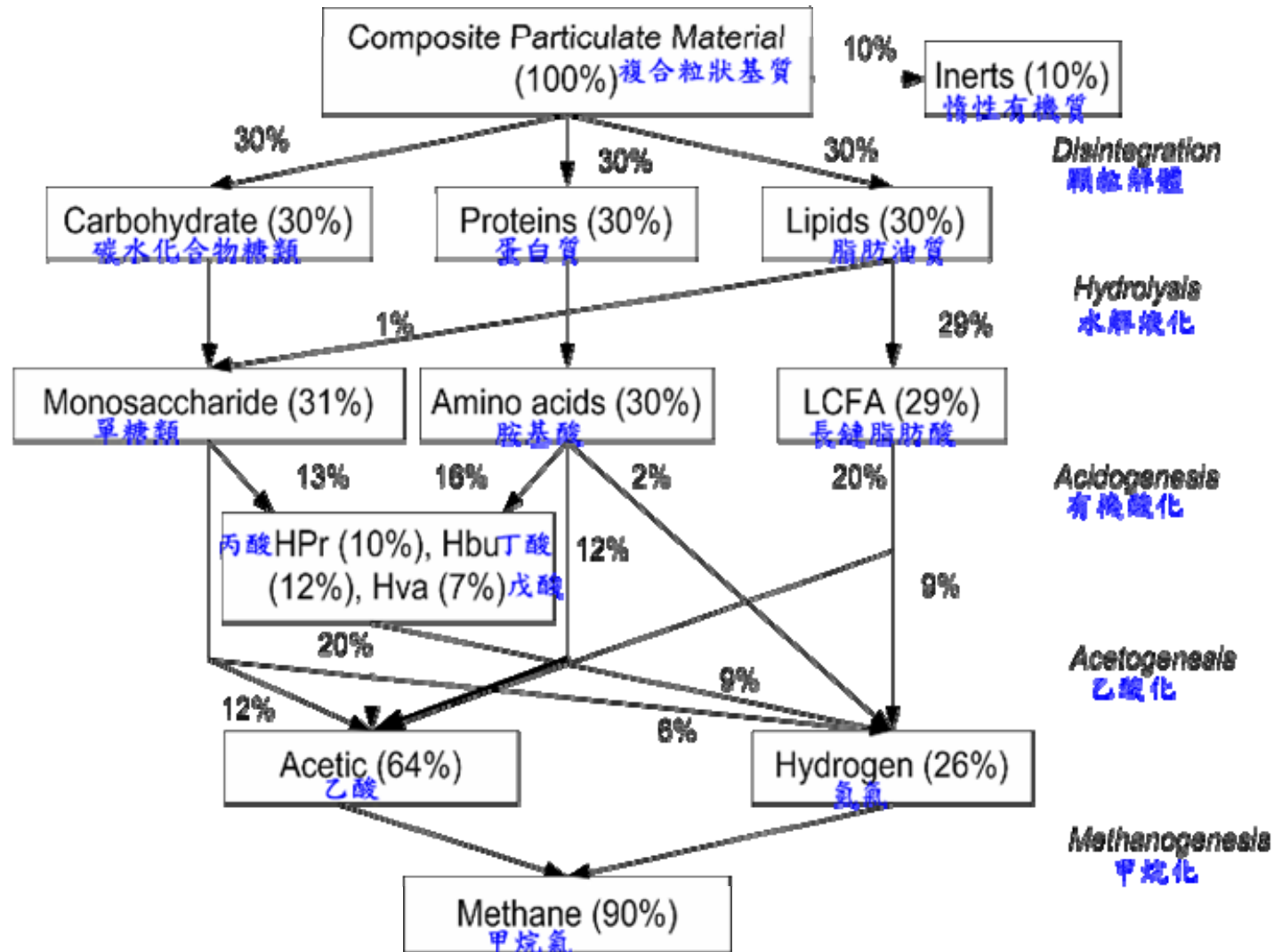


Figure COD flux for a particulate composite comprised of 10% inerts, 30% each of carbohydrate, proteins and lipids (in terms of COD). (Batstone *et al.*, 2002).-32-



Multiple Substrate Fermentation:

Kitchen Waste Characteristics

Table Kitchen wastes collected from metropolis in Taiwan (S.S. Cheng2006~2011)

Kitchen Waste Characteristics		Taipei	Kaohsiung	Tainan (starch)	Tainan (vegetable)	Tainan (KWSL)	mixed KW *
Monitoring Year		2006~2007		2007~2008		2009	2010~2012
COD	total	82 ± 28	106 ± 30	350 ± 82	78 ± 20	104 ± 18	357 ± 71
	soluble	31 ± 10	35 ± 1.2	150 ± 1	24 ± 8	76 ± 8	102 ± 53
	TOC			139 ± 5	28 ± 6	42 ± 5	134 ± 21
COD/TOC				2.52	2.83	2.50	2.66
Solid	TS	50 ± 16	55 ± 10	267 ± 21	46 ± 12	87 ± 11	199 ± 20
	TVS	40 ± 15	50 ± 10	257 ± 66	40 ± 11	73 ± 11	185 ± 20
	SS	40 ± 12	42 ± 9	212 ± 46	36 ± 9	26 ± 5	111 ± 12
	VSS	37 ± 13	41 ± 9	206 ± 45	33 ± 9	24 ± 5	106 ± 10
	Moisture				-	-	80 ± 2
Carbohydrate	Total	5 ± 3	15 ± 5	144 ± 50	14 ± 8	27 ± 6	48 ± 21
	Soluble	0.7 ± 0.4	4.0 ± 3	68 ± 31	5 ± 4	24 ± 6	26 ± 16
	cellulose				4 ± 1		-
N-compounds	Org-N _T	2 ± 0.5	2.5 ± 0.6	5.5 ± 3	1.5 ± 0.4	2.5 ± 0.3	7 ± 3
	Org-N _S	1 ± 0.3	0.8 ± 0.4	1.2 ± 1	0.8 ± 0.1	1.8 ± 0.2	3 ± 1
	NH ₄ ⁺ -N	0 ± 0.2	0.2 ± 0.1	0.07 ± 0	0.06 ± 0.04	0.2 ± 0.04	N.D.
Lipid	Oil & Grease	8 ± 2	11 ± 4	23 ± 10	13 ± 5	8 ± 3	62 ± 35
pH		5 ± 0.1	4.5 ± 0.2	4.5 ± 0	4.0 ± 0.2	4.3 ± 0.5	4.3 ± 0.5
VFAs	HLa	6 ± 2.1	10.7 ± 4.6	8 ± 3	2.8 ± 0.8	14.5 ± 2.5	12 ± 3
	HAc	1 ± 0.3	1 ± 0.5	2 ± 1	0.4 ± 0.2	2.5 ± 0.3	6 ± 2
	HPr	0 ± 0.1	0.06 ± 0.03	N.D.	N.D.	N.D.	N.D.
	HBu	0 ± 0.2	0.05 ± 0.03	N.D.	N.D.	N.D.	N.D.
	HVa	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Units: g/L, *Units: mg/g, except pH -33-

Lab of Professor Sheng-Shung Cheng
Department of Environmental Engineering,



台南市廚餘特性分析

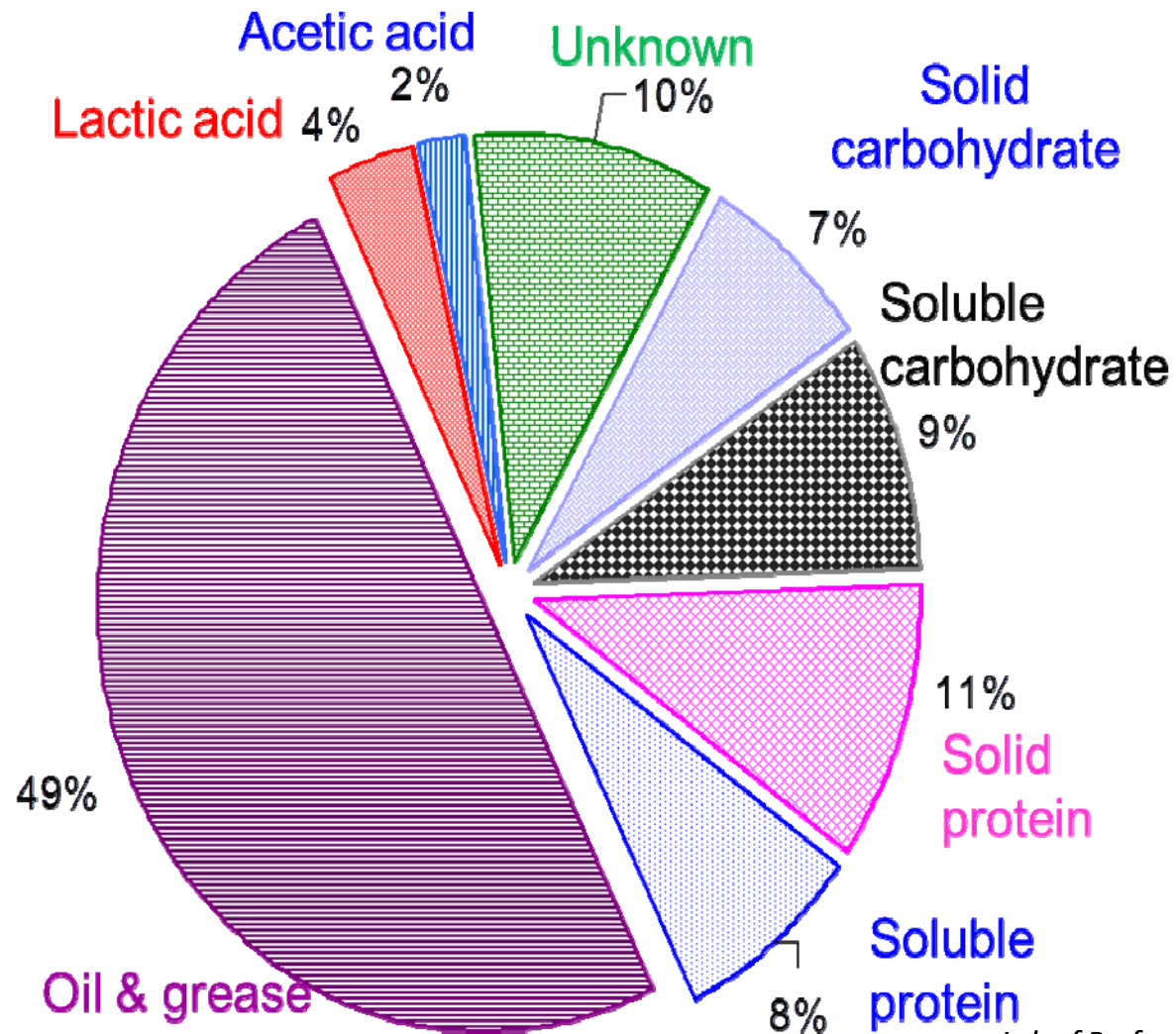
Characteristics		Tainan KW (2007 .10~2008.2 n=7)		
COD	CODt(mg/L)	271,265	±	4,497
	CODs(mg/L)	112,678	±	942
Solid	TS(mg/L)	166,525	±	978
	TVS(mg/L)	161,318	±	875
	SS(mg/L)	136,008	±	2,714
	VSS(mg/L)	132,368	±	2,855
	Carbohydrate t(mg/L)	111,043	±	2,746
Carbohydrate	Carbohydrate s(mg/L)	57,527	±	801
	Reducing Sugar(mg glucose/L)	26,064	±	760
Protein	Org-Nt(mg N/L)	5,172	±	341
	Org-Ns(mg N/L)	803	±	35
	NH ₄ ⁺ -N(mg N/L)	45	±	8
Lipid	Oil&Grease(mg/L)	12,508	±	380
	HLa(mg/L)	4,427	±	103
VFA	HAc(mg/L)	1,636	±	54
	HPr(mg/L)			N.D.
	HBu(mg/L)			N.D.
	HVa(mg/L)			N.D.
Solid COD/VSS		1.20		
Solid Carbohydrate/VSS		0.40		
O&G/VSS		0.09		
Solid Org-N/VSS		0.03		

鄭幸雄、王郁萱、李澤坤等(2008)



Multiple Substrate Fermentation: Kitchen Waste Characteristics

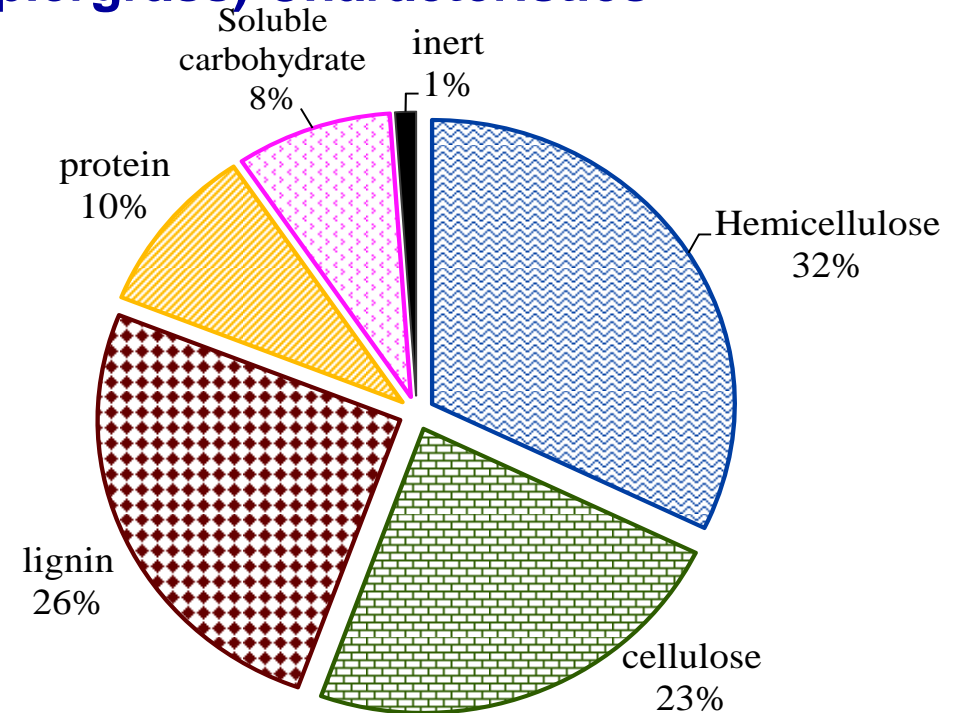
Electron distribution of mixed kitchen wastes in Tainan (S.S. Cheng *et al.*, 2011~2012)



Multiple Substrate Fermentation: Cellulosic Feedstock(Napiergrass) Characteristics



(Provided by Dr. Cheng, LRI, 2010)



The ADVANTAGES of napiergrass for bioenergy

Easy planting	
High growth rate	harvested every 8 or 10 weeks
High yield density	60~80 ton-dry matter/hectare/year
Inexpensive	\$ 52~97 /ton-dry matter
High electron density	COD/TOC = 2.44 g-COD/g-TOC

Multiple Substrate Fermentation: ACIDOGENESIS PHASE– I-CSTR of HYDROGEN FERMENTER

Feedstock:

Kitchen waste & Napiergrass

Control Factor:

Volumetric loading rate

1st – 10 g COD/L-day

2nd– 15 g COD/L-day

3rd – 20 g COD/L-day

Table. Substrate & Microbes / Environmental Factors

Substrate		Kitchen waste : Napier grass = 4 : 1		
Inoculation		Cow dung & NPUST Pilot		
Reactor		1	2	3
Item	unit			
Total Volume	L		10	
Working Volume	L		8	
HRT	day		8	
pH			6	
Temperature	°C		55	
Stirring Speed	rpm		100	
Inoculation	g VSS/L		30	
VLR	g COD/L-day	10	15	20

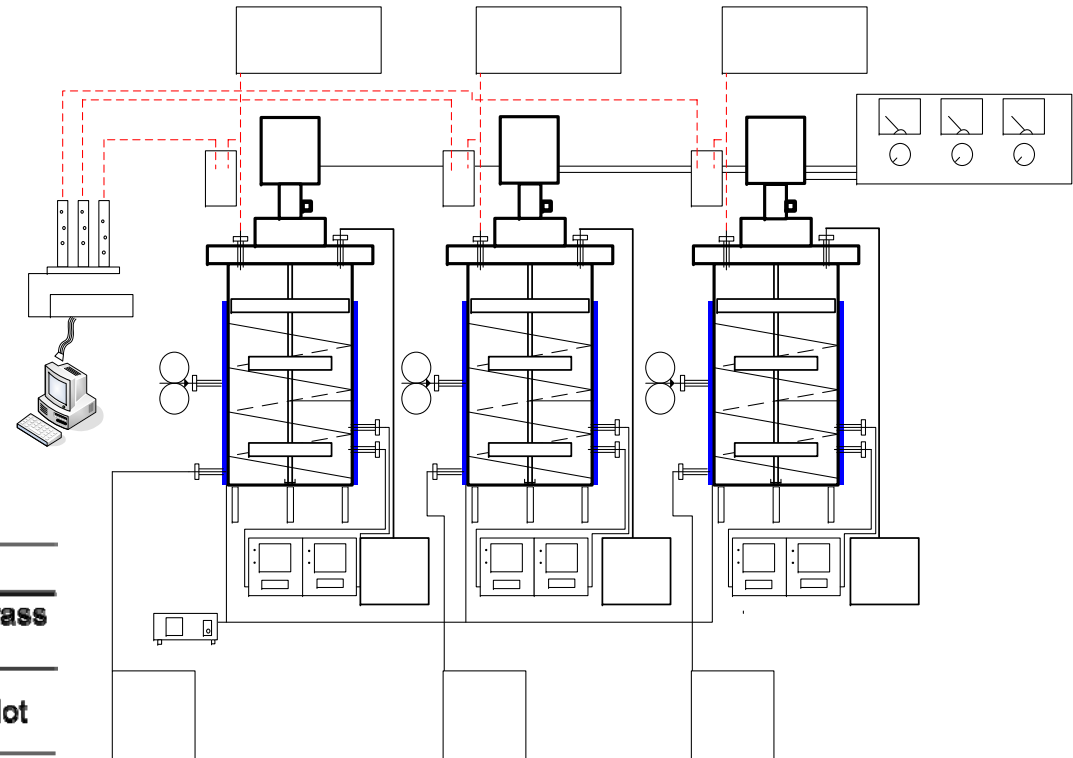


Fig. Schematic diagram of I-CSTR anaerobic hydrogen fermenter fed with kitchen waste.

(Bo-Kuang Chen *et al.*, 2010)

Multiple Substrate Fermentation: METHANOGENESIS PHASE– EGG-SHAPED ANAEROBIC DIGESTER

Feedstock:
Effluent of hydrogen fermenters

Advantages of Egg-shaped Digester:

- 1) Minimum grit accumulation
- 2) Reduced scum formation
- 3) Higher mixing efficiency
- 4) Lower operating and maintenance cost

Table. Substrate & Microbes / Environmental Factors		
Substrate		Effluent of hydrogen fermentor
Inoculation		Sludge from Di-hua and Fu-tien wastewater treatment plant
Reactor		Egg-shaped anaerobic digester
Item	unit	
Total Volume	L	90
Working Volume	L	85
HRT	day	22 to 40
Turnover time	min.	22
Temperature	°C	55
Stirring Speed	rpm	100
Inoculation	g VSS/L	30
VLR	g COD/L-day	2.5

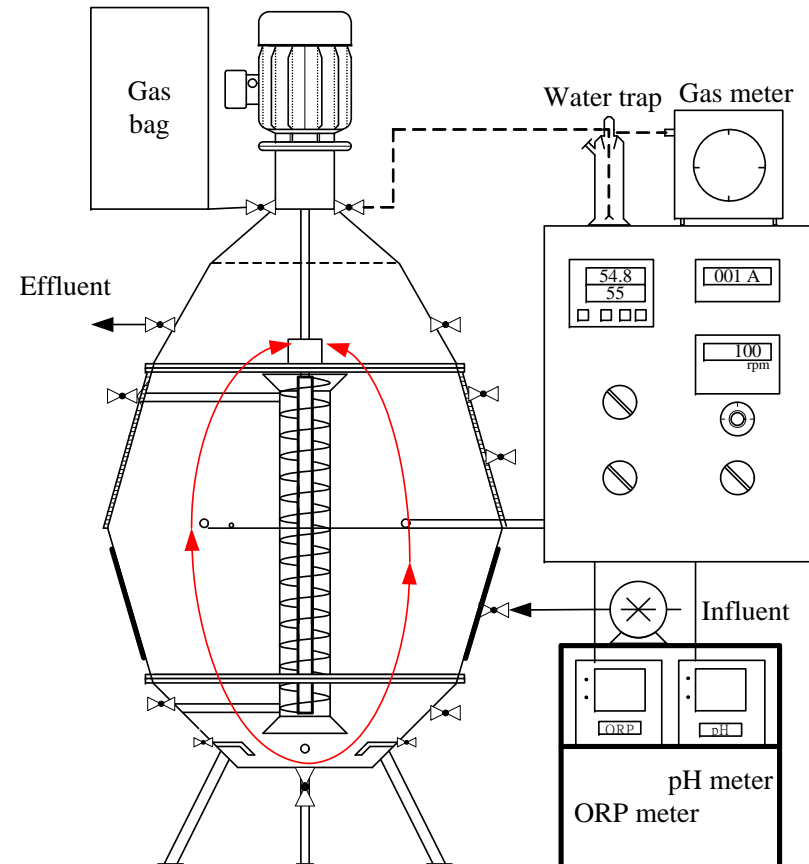
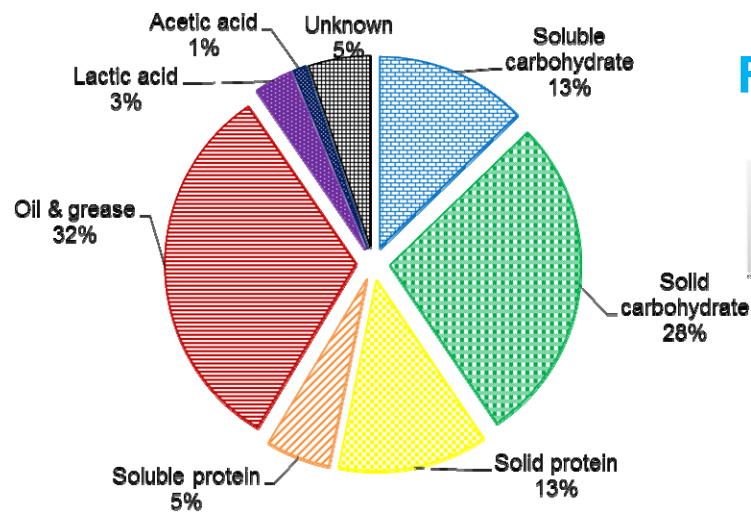


Fig. Schematic diagram of egg-shaped anaerobic methane digester fed with first-phase effluent.
(Bo-Kuang Chen *et al.*, 2010)

Multiple Substrate Fermentation: Performance indicators of hydrolysis-acidogenesis phase

INFLUENT



H₂
Fermenter

EFFLUENT

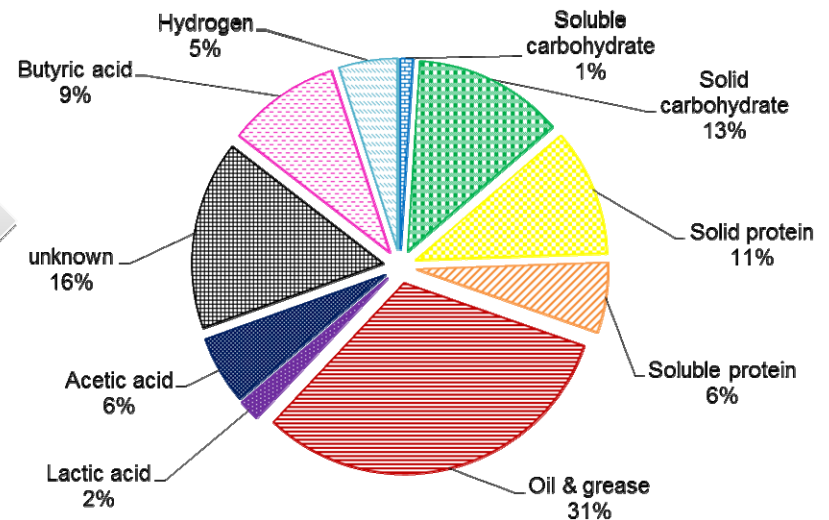
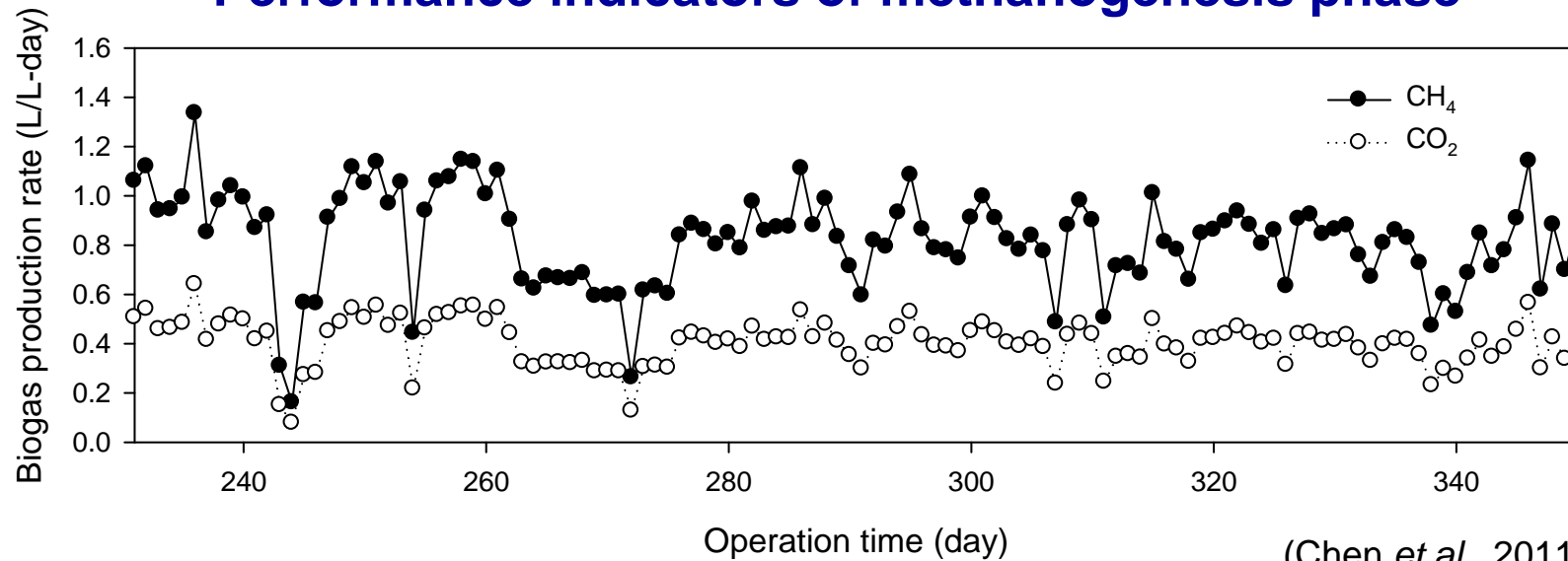


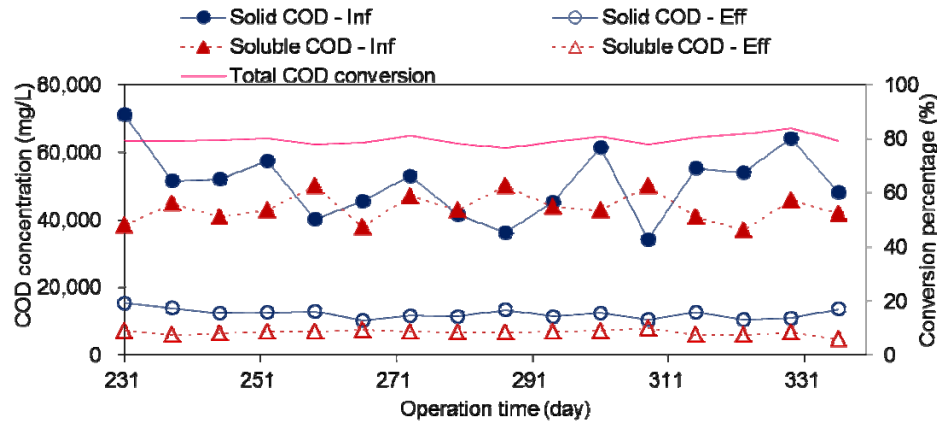
Fig. Electron distribution of feedstock and acidified effluent from hydrogen fermenter in terms of COD (Chen *et al.*, 2011)

Multiple Substrate Fermentation: Performance indicators of methanogenesis phase

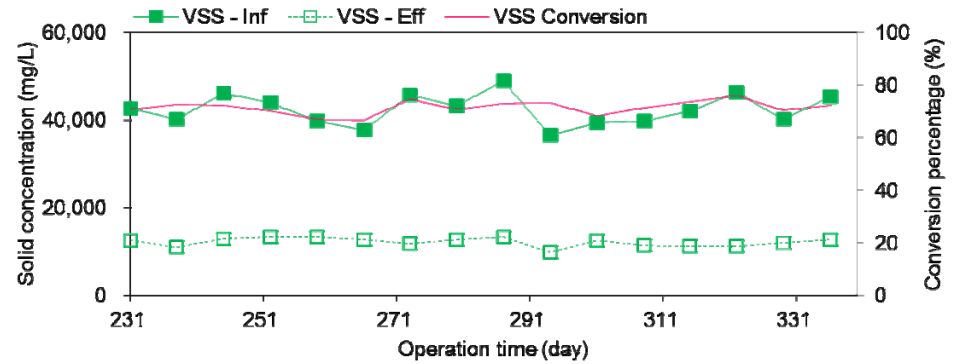


Parameter	Unit	Operation time (day)			
		54 - 152	153 - 184	185 - 200	231 - 359
Volumetric loading rate Feedstock	g COD/L-day	1.4 ± 0.8	3.2 ± 0.3	4 ± 0.3	2.5 ± 0.2
		Effluent of acidogenic phase			
Methane production rate	L CH₄/L-day	0.6 ± 0.26	1.13 ± 0.1	1.2 ± 0.08	0.8 ± 0.4
Methane percentage	%	73 ± 4.2	70.0 ± 1.4	69 ± 2.2	70.0 ± 1.0
Methane production yield	mmol CH₄/g CODremoved	39 ± 44	17 ± 1.3	16 ± 2.8	14.2 ± 3.3
Total COD conversion	%	63 ± 20	81 ± 1	80 ± 3	79 ± 6
VSS conversion	%	46 ± 22	72 ± 4	69 ± 4	71 ± 3
Solid carbohydrate conversion	%	N.A.	N.A.	N.A.	93 ± 7
Soluble carbohydrate conversion	%	N.A.	N.A.	N.A.	92 ± 3
Cellulose conversion	%	68 ± 17	89 ± 9	89 ± 9	94 ± 8
Solid org-N conversion	%	-19 ± 51	24 ± 21	6 ± 35	56 ± 5
Soluble org-N production	mg N/L	65 ± 10	42 ± 16	63 ± 12	39 ± 5
Oil & grease conversion	%	89 ± 6	85 ± 10	89 ± 4	88 ± 3

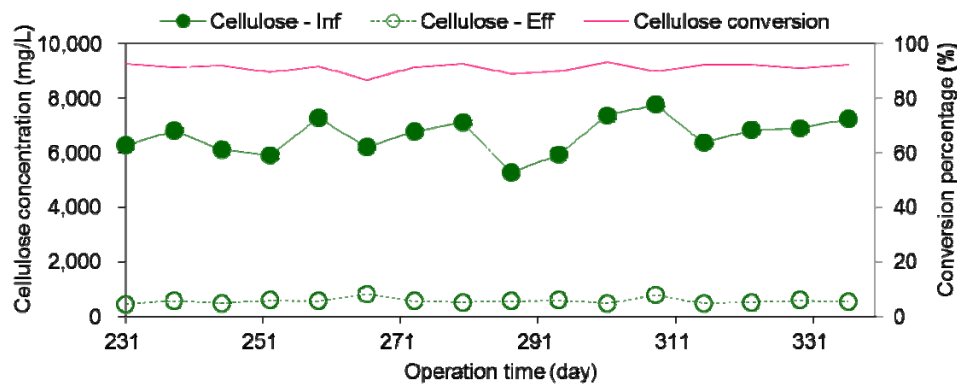
Multiple Substrate Fermentation: Cellulose and oil & grease degradation in methanogenesis phase



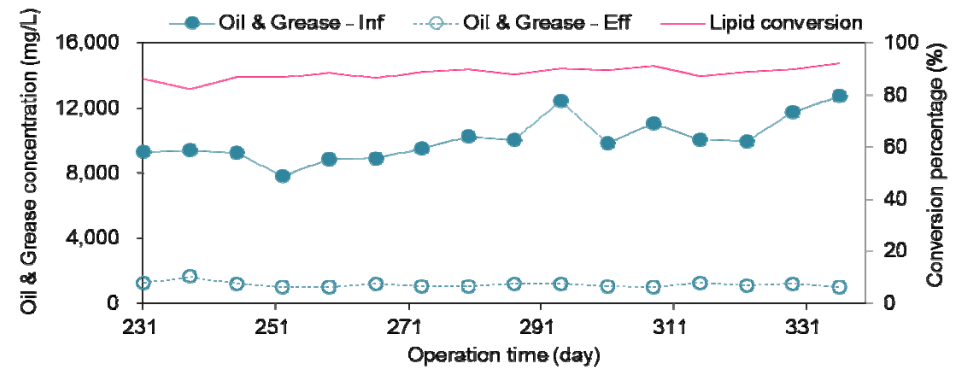
COD conversion: 80 % → CH₄



VSS conversion: 70 %



Cellulose conversion: 90 %



Lipid conversion: 90 %

Recalcitrant material such as cellulose and lipid from fermentative effluent of hydrogen fermenters can be effectively converted into methane during 340 operating days. (Chen *et al.*, 2011)

兩段式高溫厭氧生物共消化程序處理廚餘及下水污泥之操作條件與功能指標(鄭幸雄等,2008)

Pilot	VLR Kg COD/m ³ - day	COD_removal %	Biogas Production L/L/day	Biogas Composition	Biofuel Yield, mole/Kg COD- day
AR1	46.0	34.5	5,250	H ₂ ,28%	0.37
AR2	3.7	61.8	1,169	CH ₄ ,65%	13.4

55°C 2-stage Anaerobic Thermophilic Digesters(pilot study, 2008-2009)

厭氧污泥生化甲烷產能實驗之食微比生物活性比較表(鄭幸雄等,2008)

Batch test 35°C	F/M(40% transf.) g COD/g VSS/day	References
AP-Hac-BMP	0.23	Hui Yu & AOI(2008)
DH-Hac-BMP	0.25	HuiYU(2008)
AP-WAS-BMP	0.29	HuiYU(2008)
DH-WAS-BMP	0.14	HuiYU(2008)
AP-KW/WAS-BHP-	0.35	AOI(2008)



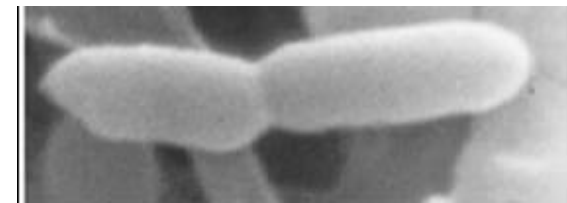
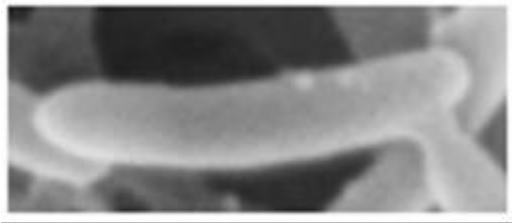
3-9 高溫厭氧發酵程序有機基質馴養菌群社會結構

III. Microbial Community Analyses:

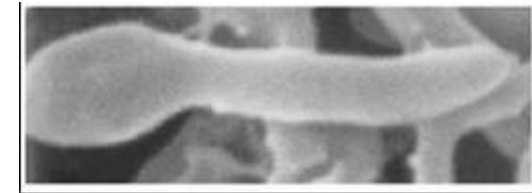
III-1. Morphology of Hydrogen Producing Bacteria Using Peptone as Substrate

Type I short rod-shape

Clostridium species



Type II Bacteria with endospore



The bacteria from a wastewater treatment plant of food industry was pretreated with heat and acclimated with peptone in a batch reactor.(Bai et al., 2004)

Microbial Community Analyses: SEM photo in Starch-peptone fed CSTR system

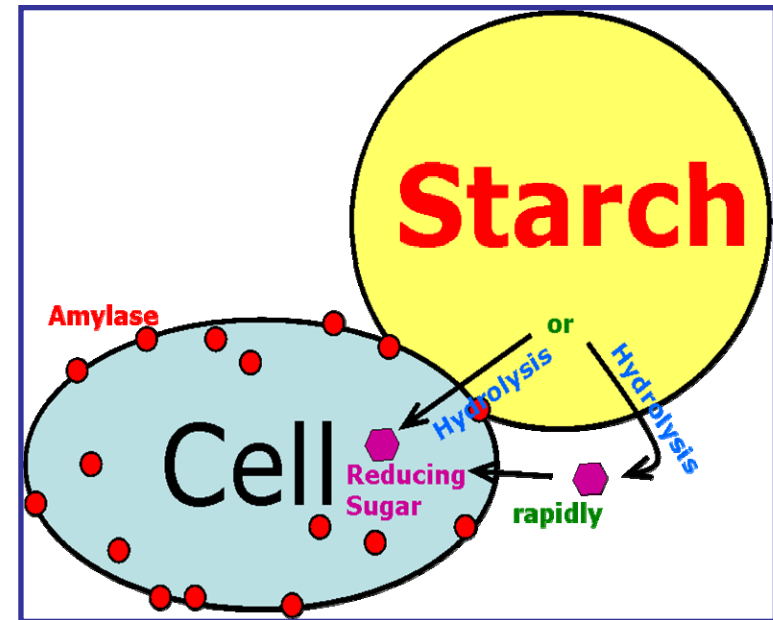
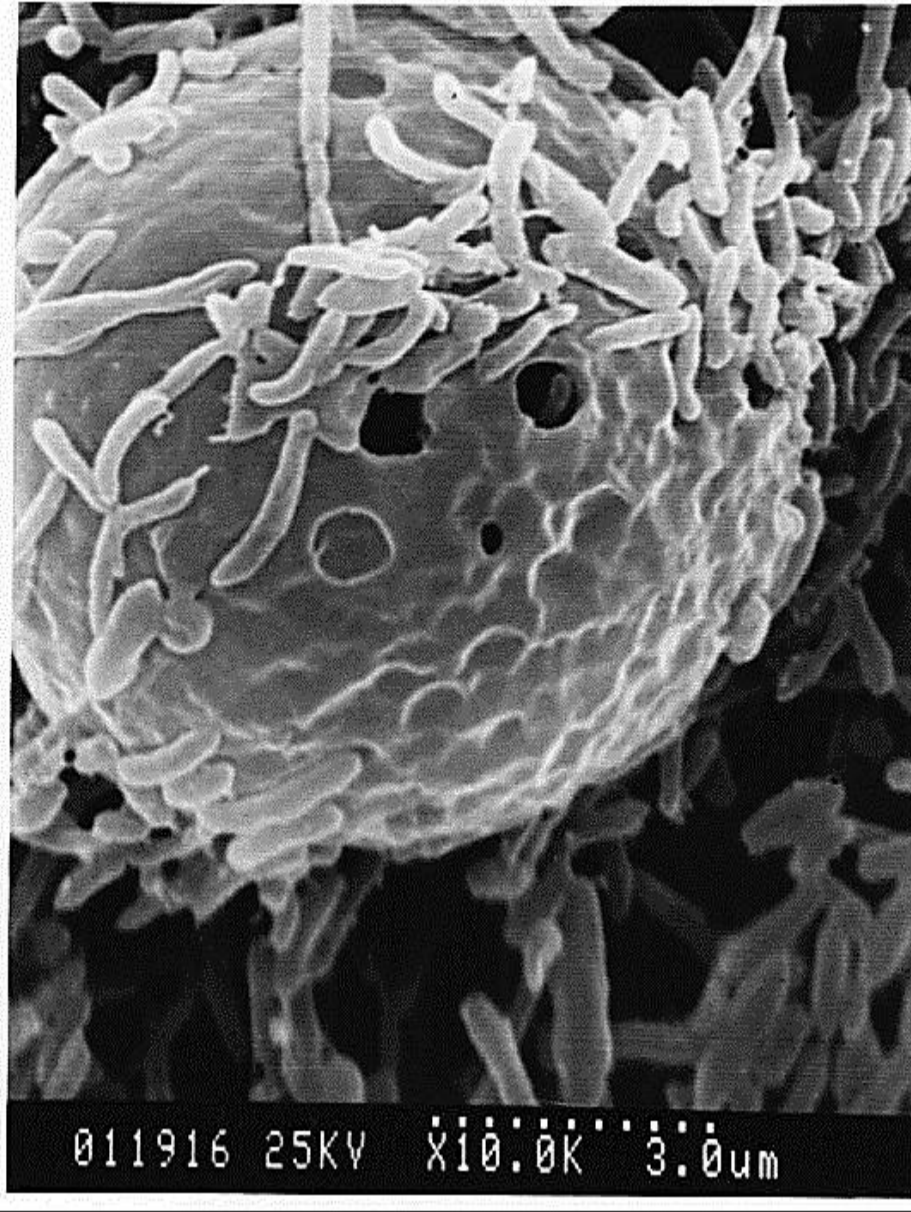
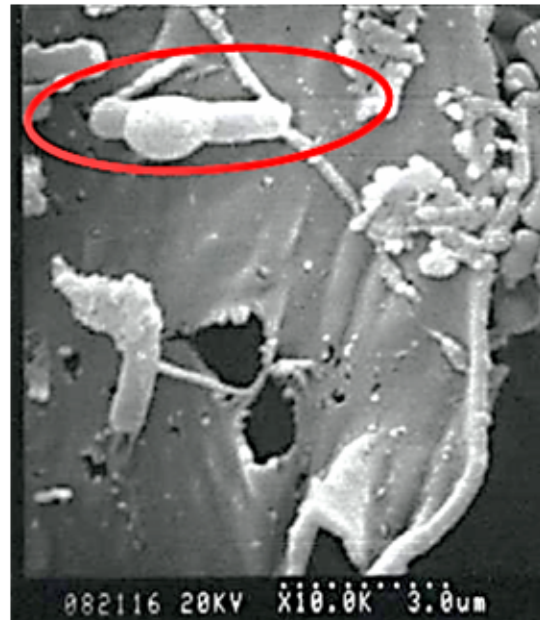
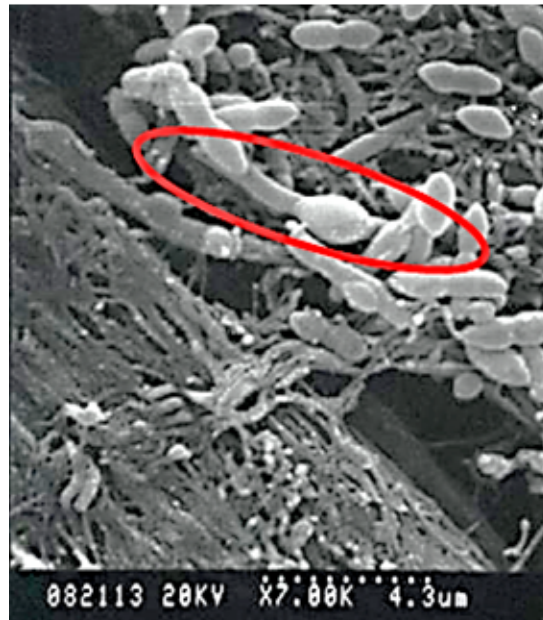
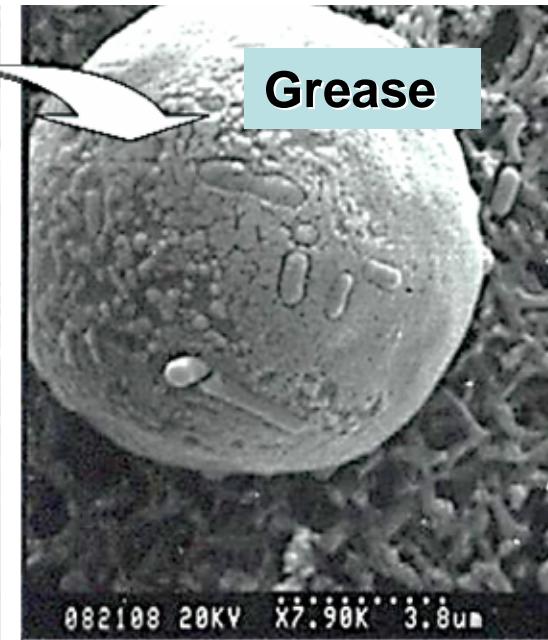
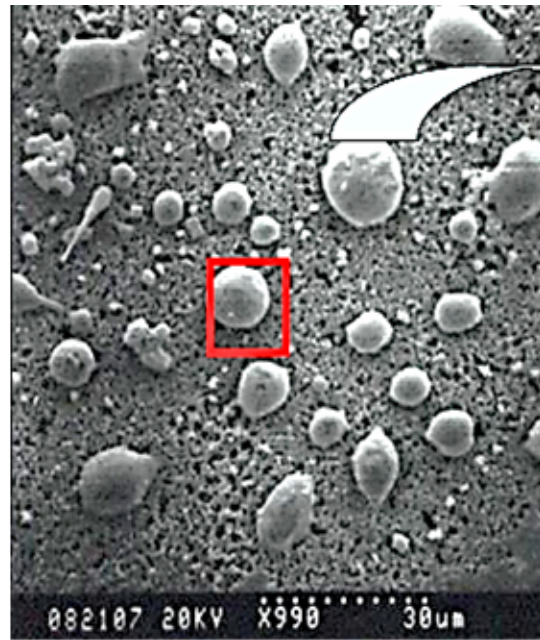


Fig. SEM morphology of the sludge in the membrane bio-hydrogen reactor which the starch is prepared without heated. (Chao et. al, 2004)

Microbial Community Analyses: SEM Photos in Kitchen Waste Fermenting System



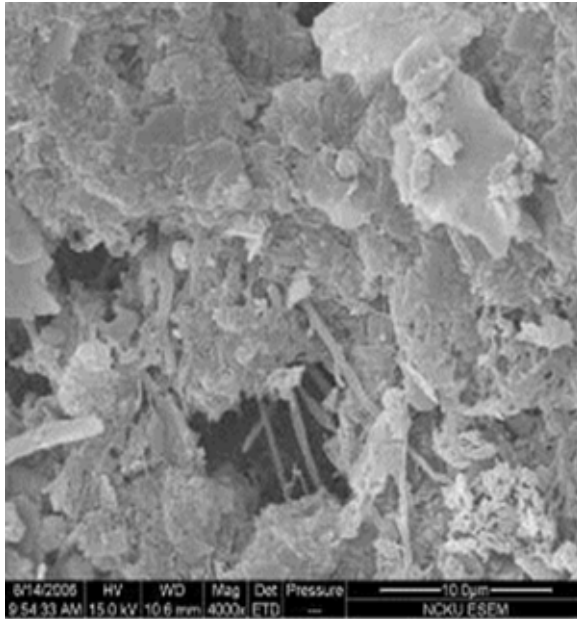
- 1-2 μm **short-rod bacteria** is the most dominant species
- 5-20 μm spherical object might be **micelle** of oil and grease

• The sporulation and germination of ***Clostridium*** (S.S. Cheng et al., 2005)
bacteria Professor Sheng-Shung Cheng

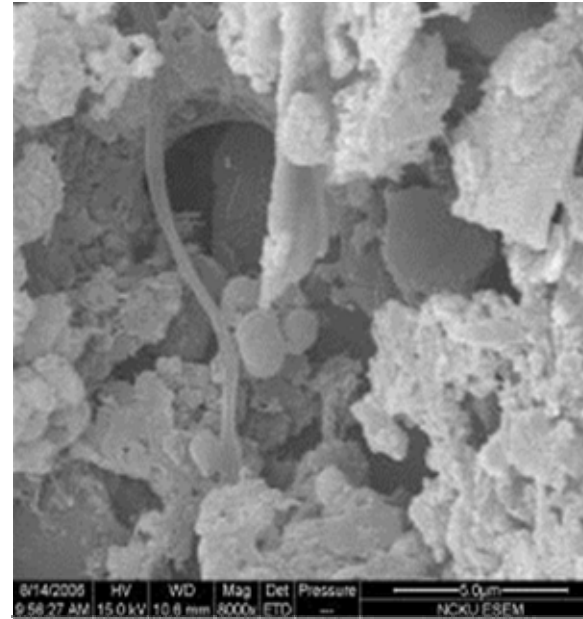
Department of Environmental Engineering,
NCKU



電子顯微鏡微生物菌相觀察

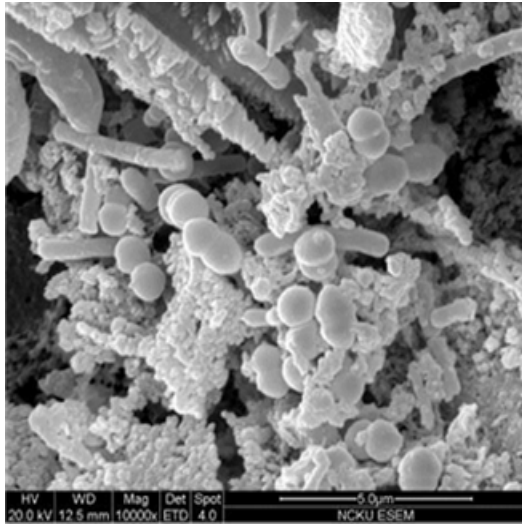


實廠消化槽污泥中可以找到以醋酸為基質的絲狀菌 *Methanosaeta like* 存在。
8000X (鄭幸雄等,2008)

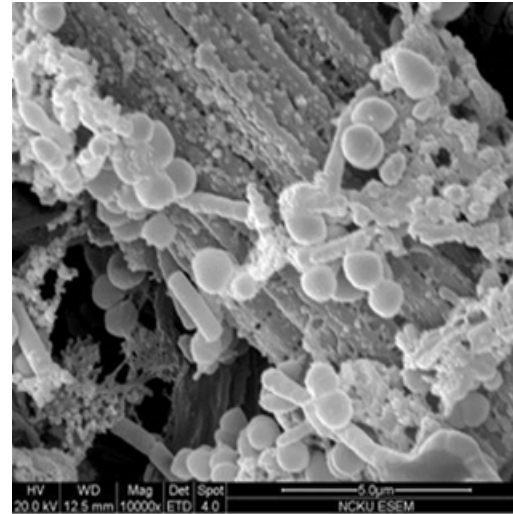


實廠消化槽污泥層中，有利用醋酸的絲狀菌外還有利用氫氣的 *Methanosarcina like* 存在。
8000X (鄭幸雄等,2008)

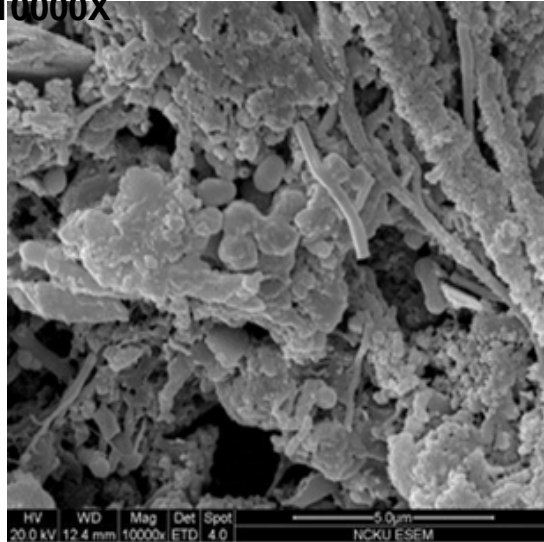
電子顯微鏡微生物菌相觀察



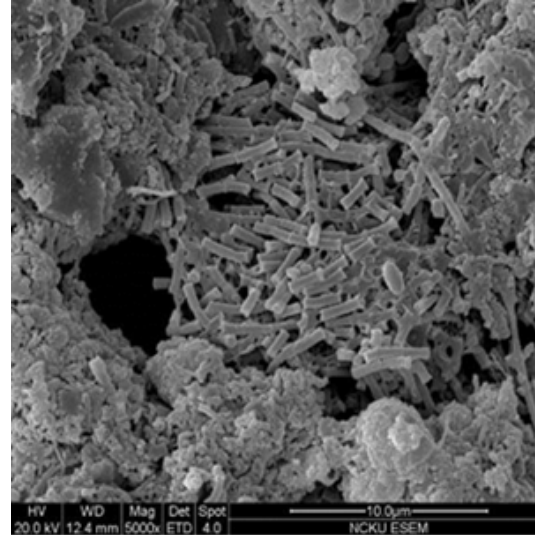
模場酸化槽球菌的大小直徑約為1µm，很多球菌都兩個相連生長。**10000X**



模場酸化槽中纖維素上有許多球菌及短桿菌沾附在上面生長**10000x**



模場甲烷槽的污泥較為厚實，球菌及桿菌都包覆在污泥內生長。**5000X**(鄭幸雄等,2008)



模場甲烷槽短桿菌聚集在一起生長，多半為一節或是兩節的桿菌所組成，長度約為**2µm-4µm**

3-10 高溫蛋型厭氧消化槽進流廚餘與木質纖維之菌群生態檢測 Microbial Community Analyses:

Clone library of egg-shaped anaerobic digester **BACTERIA**
(Chang-Chun Chen *et al.*, 2011)

Table. Similarity values of 16S rRNA sequences retrieved from egg-shaped digester

OTU NO.	Nearest relative (GenBank Accession NO.)	Similarity	Isolated environment of nearest relative
eg-36	<i>Anaerobaculum mobile</i> strain NGA	97%	Anaerobic digester
eg-1	Uncultured bacterium clone	99%	Anaerobic digester
eg-49	<i>Bacillus infernus</i> TH-22	95%	Anaerobic digester
eg-83	<i>Anaerobaculum</i> sp. OS1	99%	Oil production water
eg-6	<i>Clostridium thermocellum</i> strain JCM 9323	99%	Cow dung LCFAs degrader
eg-25	<i>Syntrophomonas palmitatica</i> strain MPA	100%	Methanogenic sludge
eg-56	<i>Clostridium</i> sp. FG4	100%	Biocompost
eg-70	<i>Clostridium</i> sp. 6-31	90%	Biocompost
eg-67	Uncultured bacterium clone C55_D6_L_B_A11	99%	Anaerobic digester
eg-20	<i>Syntrophaceticus schinkii</i> strain Sp3	93%	Methanogenic sludge

- 1) **Lignocellulolytic** bacteria such as *Clostridium thermocellum* and other *Clostridium* species were found in anaerobic digester. Moreover, microorganisms which conduct **β -oxidation** also existed in syntrophic methanogenic phase.
- 2) Through maintaining a **syntrophic** condition, recalcitrant material which are difficult to be utilized can be converted by diverse but stable microbial community.

Microbial Community Analyses:

Archaeal diversity shift in egg-shaped digester

ARCHAEA

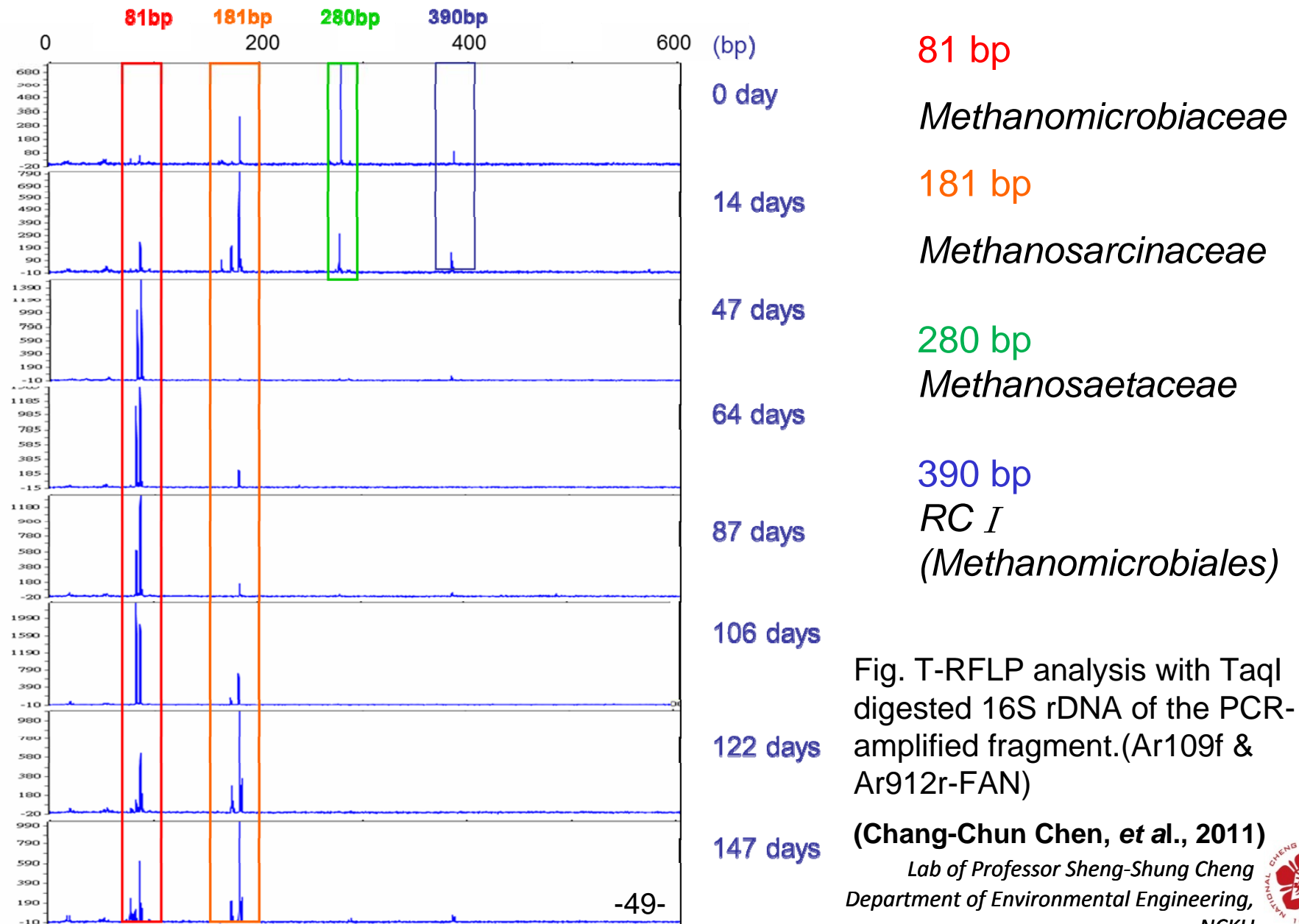


Fig. T-RFLP analysis with TaqI digested 16S rDNA of the PCR-amplified fragment.(Ar109f & Ar912r-FAN)

(Chang-Chun Chen, et al., 2011)

Lab of Professor Sheng-Shung Cheng
Department of Environmental Engineering,



NCKU

Kitchen Waste廚餘
Dr.Wen-Ching Chen

陳文欽博士

Dr.Wen-Chi Chen

陳文卿博士

Dr. Sheng-Shung Cheng

鄭幸雄博士

Dr.Wen-Chien Kuo

郭文健博士



Pilot Scale H₂-CH₄ Processes in Taiwan

台灣廚餘生物污泥厭氧產氫產甲烷能源化程序

模型場址

Hsinchu

Garbage composting

Kitchen Waste + Waste Activated Sludge

Dr. Sheng-Shung Cheng 鄭幸雄博士

Dr.Wen-Chien Kuo 郭文健博士



Tainan

Fodder + sugar 麵麩廢水

Dr. Ming-Der Bai 白明德博士

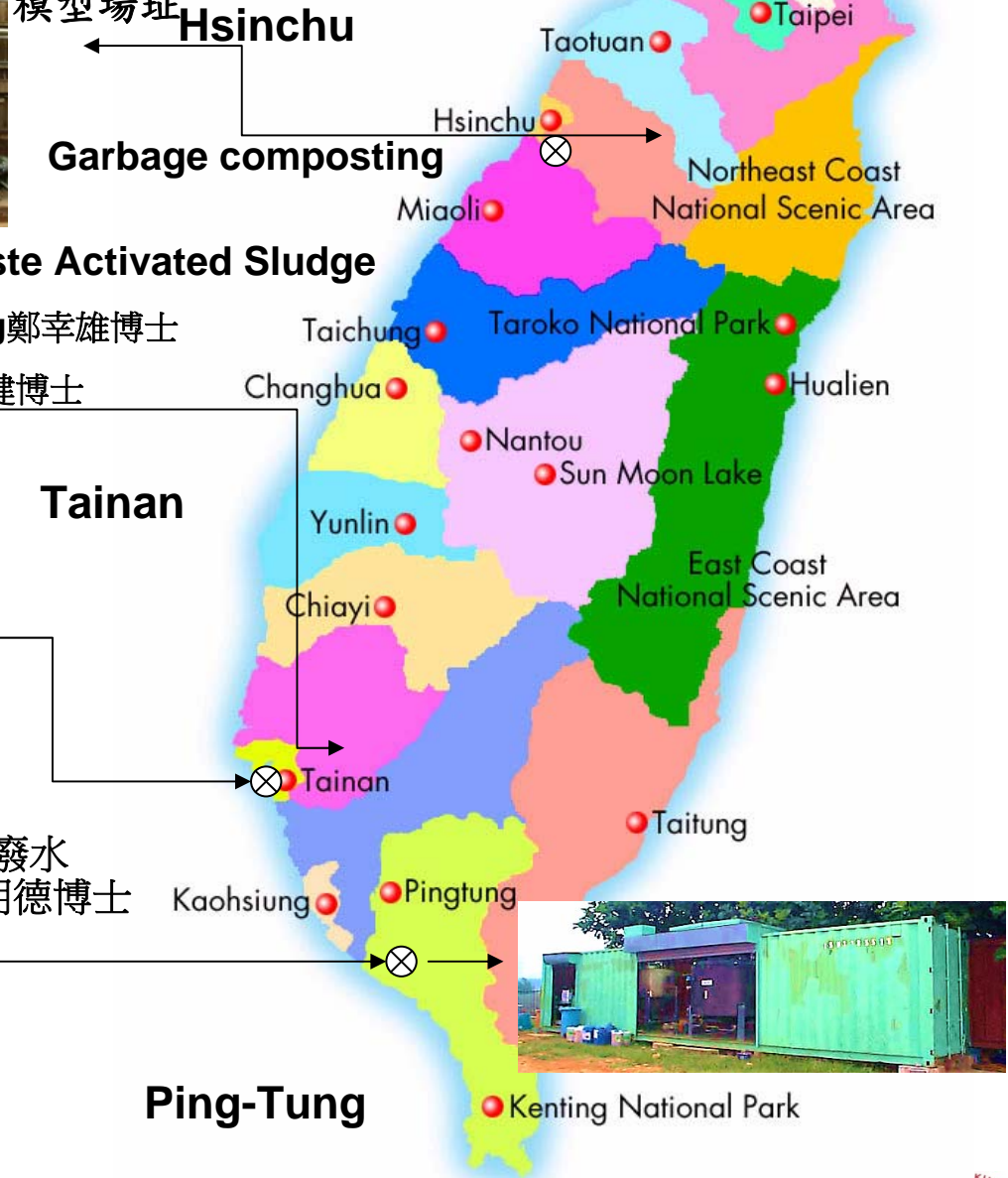
Kitchen Waste + Agriculture Waste

Dr. Wen-Chien Kuo

郭文健博士(屏科大)



Ping-Tung



新北市八里污水處理廠厭氧污泥消化槽



照片 八里污水處理廠蛋型厭氧污泥消化槽 (9,900m³×6槽) 2005.01.20