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APPENDICES

Appendix A Calibration Curves

Table A1 Calibration curve for hydrogen (H_2)

Volume of hydrogen (ml)	Peak area
0.02	16,313
0.04	58,770
0.08	180,674
0.1	226,743
0.2	427,198
0.4	778,509

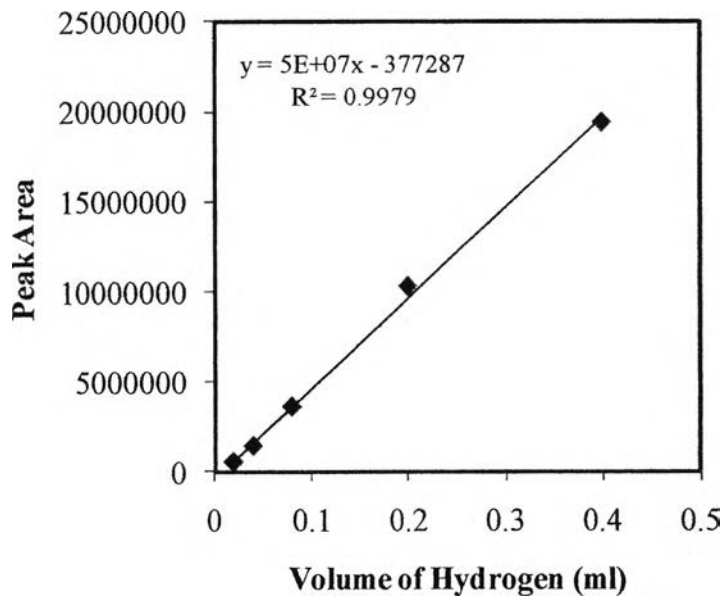

Figure A1 The relationship between volume of hydrogen (H_2) and peak area.

Equation

$$\text{Amount of hydrogen} = \frac{\text{Peak area} + 377287}{5 \times 10^7}$$

Table A2 Calibration curve for nitrogen

Volume of nitrogen (ml)	Peak area
0.02	34,210
0.04	128,767
0.08	305,287
0.1	393,916
0.2	809,433
0.4	1,602,475

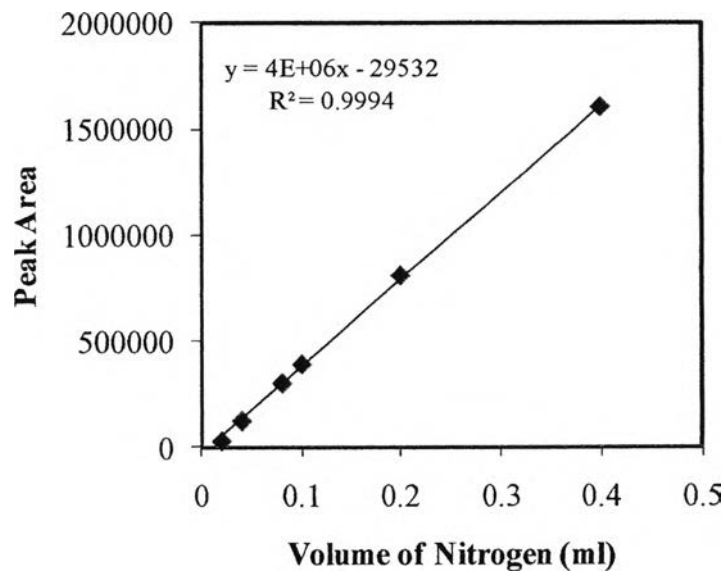

Figure A2 The relationship between volume of nitrogen (N_2) and peak area.

Equation

$$\text{Amount of nitrogen} = \frac{\text{Peak area} + 29532}{4 \times 10^6}$$

Table A3 Calibration curve for oxygen

Volume of oxygen (ml)	Peak area
0.02	12,286
0.04	43,995
0.08	104,342
0.1	135,546
0.2	280,220
0.4	562,001

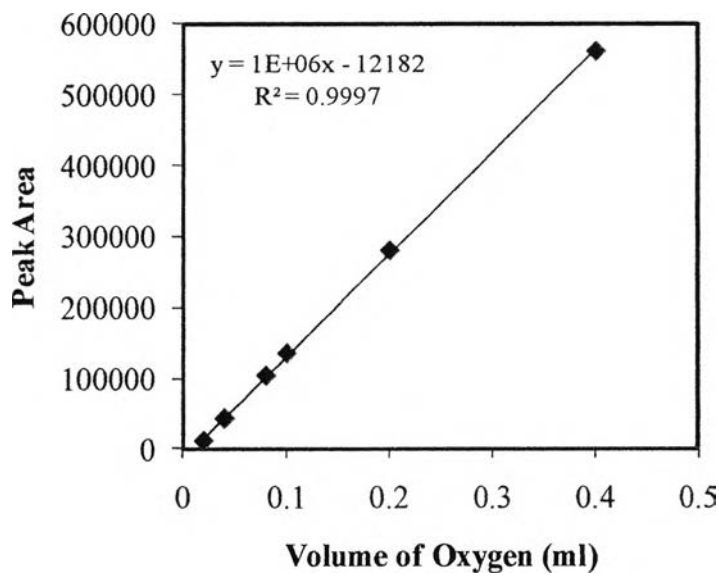

Figure A3 The relationship between volume of oxygen (O_2) and peak area.

Equation

$$\text{Amount of oxygen} = \frac{\text{Peak area} + 12182}{1 \times 10^6}$$

Table A4 Calibration curve for methane (CH_4)

Volume of methane (ml)	Peak area
0.02	92,517
0.04	381,106
0.1	1,293,552
0.2	2,674,654

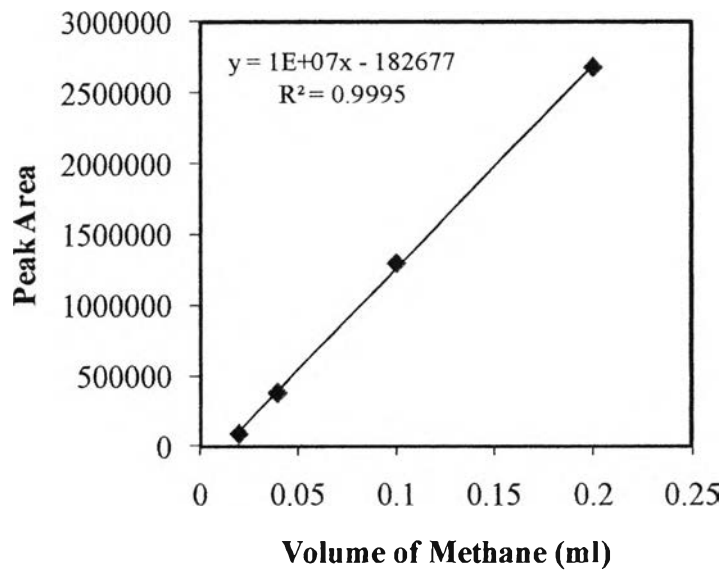

Figure A4 The relationship between volume of methane (CH_4) and peak area.

Equation

$$\text{Amount of methane} = \frac{\text{Peak area} + 182677}{1 \times 10^7}$$

Table A5 Calibration curve for carbon dioxide (CO_2)

Volume of carbon dioxide (ml)	Peak area
0.02	26,118
0.04	97,539
0.08	220,122
0.2	596,414
0.4	1,315,885

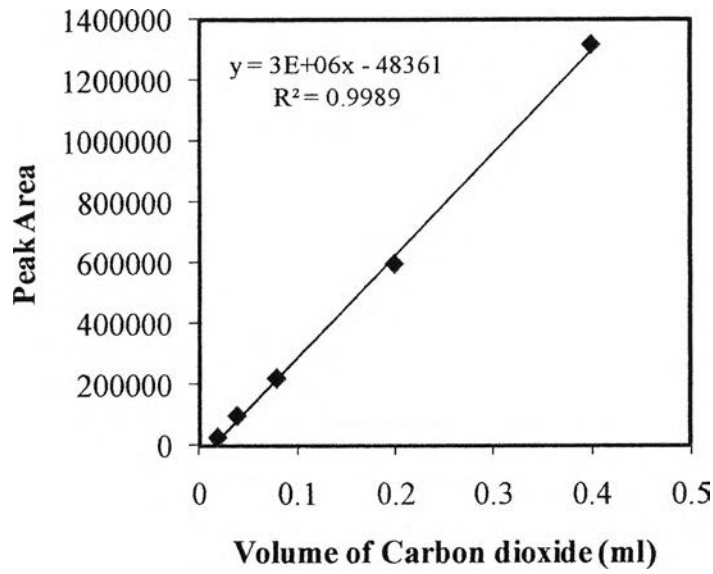

Figure A5 The relationship between volume of carbon dioxide (CO_2) and peak area.

Equation

$$\text{Amount of carbon dioxide} = \frac{\text{Peak area} + 48361}{3 \times 10^6}$$

Appendix B Preparation of 5 wt./vol.% NaOH Solution for pH-controlled System

Preparation of NaOH at concentration of 5 wt./vol.%

$$= \frac{5}{100}\frac{g}{ml} = 50\frac{g}{l}$$

Appendix C Volatile Fatty Acids (VFA) Quantification by Distillation Method

C 1. Acetic Acids Stock Solution Preparation for Recovery Factor (f) Determination

Concentration of fresh acetic acid (liquid) = 99.7%

Density of acetic acid = 1.07 g/ml

Molecular weight of acetic acid = 60

Determination of fresh acetic acids concentration in term of molar

$$= \frac{0.997 \text{ L of acetic acid}}{\text{L of solution}} \text{ x } \frac{1.07 \text{ g of acetic acid}}{\text{mL of acetic acid}} \text{ x } \frac{1 \text{ mol of acetic acid}}{60 \text{ g of acetic acid}}$$

$$= 17.78 \text{ M}$$

Preparation of acetic acid at concentration of 2,000 mg/L

$$= 2{,}000 \frac{\text{mg of acetic acid}}{\text{L of solution}} \text{ x } \frac{1 \text{ mole of acetic acid}}{60 \text{ g of acetic acid}}$$

$$= 0.0333 \text{ M}$$

Dilution of acetic acid

$N_1V_1 = N_2V_2$

$V_1 = N_2V_2/N_1$

$= (0.0333 \text{x} 1)/17.78$

$= 1.873 \text{x} 10^{-3}$ L

C 2. Standard Sodium Hydroxide (0.1 M) Preparation

Concentration of fresh NaOH (solid) = 99%

Molecular weight of acetic acid = 40

Preparation of acetic acid at concentration of 0.1 M

$$= \frac{0.1 \text{ mol}}{1 \text{ L}} \text{ x } \frac{40 \text{ g}}{1 \text{ mol}} x \frac{100}{99}$$

$$= 4.04 \text{ g}$$

C 3. Recovery Factor (f) Determination

Distill 150 ml of 0.0333 M of acetic acid in distillation apparatus

Calculate the recovery factor

$$f = \frac{a}{b}$$

where

a = volatile acid concentration recovered in distillate, mg/L

b = volatile acid concentration in standard solution used, mg/L

Find volatile acid concentration recovered in distillate by titration with 0.1 M of NaOH (MW of acetic acid = 60.5)

1) Distillate 50 ml NaOH 11.7 ml

Used NaOH	=	$11.7 \times 10^{-3} \times 0.1$
	=	1.17×10^{-3} mol
Acetic acid in distillate	=	1.17×10^{-3} mol
	=	$1.17 \times 10^{-3} \times 60.5$
	=	0.07 g
Concentration of acetic acid in distillate		
	=	0.07/50
	=	1.405×10^{-3} g/ml
	=	1,405 mg/l

2) Distillate 25 ml NaOH 5.7 ml

Used NaOH	=	$5.7 \times 10^{-3} \times 0.1$
	=	5.7×10^{-4} mol
Acetic acid in distillate	=	5.7×10^{-4} mol
	=	$5.7 \times 10^{-4} \times 60.5$
	=	0.034 g
Concentration of acetic acid in distillate		
	=	0.034/25
	=	1.368×10^{-3} g/ml
	=	1,368 mg/l
Average	=	1,387 mg/l
Recovery factor (f)	=	1,387/2,000
	=	0.693

Appendix D Raw Data of Effect of COD Loading Rate on Hydrogen Production

D 1. COD loading rate = 23 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	H_2	CO_2		H_2	CO_2
1	0.01	0.05	0.06	13.99	86.01
2	0.01	0.05	0.06	13.82	86.18
3	0.01	0.06	0.07	11.91	88.09
4	0.02	0.08	0.10	15.69	84.31
5	0.01	0.09	0.11	12.97	87.03
6	0.02	0.08	0.09	17.06	82.94
7	0.02	0.07	0.09	18.14	81.86
8	0.02	0.07	0.08	18.83	81.17
9	0.02	0.07	0.09	19.18	80.82
10	0.02	0.08	0.09	18.97	81.03
AVG.	0.02	0.07	0.09	18.44	81.56

Gas production rate	=	25.6	l/d
Hydrogen production rate	=	4.7	l/d
Specific hydrogen production rate	=	178.3	ml H_2/g MLVSS d
VFA Concentration	=	6,316	mg/l as acetic acid
Hydrogen yield	=	57.5	ml H_2/g COD removed
COD removal efficiency	=	11.4	%
MLVSS	=	6,646	mg/l
VSS	=	1,750	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	1,100
Acetic acid	1,126
Propionic acid	1,064
Butyric acid	2,299
Valeric acid	2,032

D 2. COD loading rate = 31 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	H2	CO2		H2	CO2
1	0.015	0.055	0.07	21.55	78.45
2	0.015	0.062	0.08	20.06	79.94
3	0.017	0.057	0.07	22.78	77.22
4	0.018	0.078	0.10	18.36	81.64
5	0.019	0.064	0.08	22.38	77.62
6	0.018	0.045	0.06	28.70	71.30
7	0.016	0.053	0.07	23.14	76.86
8	0.016	0.053	0.07	23.05	76.95
9	0.016	0.052	0.07	23.39	76.61
10	0.016	0.054	0.07	22.77	77.23
AVG.	0.020	0.052	0.07	23.09	76.91

Gas production rate	=	29.6	l/d
Hydrogen production rate	=	6.6	l/d
Specific hydrogen production rate	=	316.22	ml H_2/g MLVSS d
VFA concentration	=	8,002	mg/l as acetic acid
Hydrogen yield	=	62.12	ml H_2/g COD removed
COD removal efficiency	=	13.5	%
MLVSS	=	5,253	mg/l
VSS	=	2,746	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	1,219
Acetic acid	1,291
Propionic acid	1,148
Butyric acid	2,750
Valeric acid	2,562

D 3. COD loading rate = 46 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	H2	CO2		H2	CO2
1	0.013	0.059	0.072	18.270	81.730
2	0.017	0.067	0.083	19.882	80.118
3	0.009	0.049	0.058	15.700	84.300
4	0.016	0.044	0.060	26.827	73.173
5	0.016	0.043	0.059	26.980	73.020
6	0.018	0.069	0.087	20.549	79.451
7	0.017	0.043	0.060	27.978	72.022
8	0.017	0.044	0.060	27.854	72.146
9	0.016	0.043	0.060	27.609	72.391
10	0.017	0.044	0.060	27.586	72.414
AVG.	0.020	0.049	0.066	27.757	72.243

Gas production rate	=	63.9	l/d
Hydrogen production rate	=	17.7	l/d
Specific hydrogen production rate	=	1,076	ml H_2/g MLVSS d
VFA concentration	=	9,689	mg/l as acetic acid
Hydrogen yield	=	125.11	ml H_2/g COD removed
COD removal efficiency	=	27.26	%
MLVSS	=	4,113	mg/l
VSS	=	3,153	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	2,296
Acetic acid	2,316
Propionic acid	2,268
Butyric acid	2,938
Valeric acid	2,702

D4. COD loading rate = 62 kg/ m^3d pH = 5.5 Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	H2	CO2		H2	CO2
1	0.021	0.059	0.080	26.738	73.262
2	0.019	0.079	0.098	19.355	80.645
3	0.021	0.083	0.104	20.537	79.463
4	0.020	0.065	0.085	23.770	76.230
5	0.020	0.064	0.085	23.710	76.290
6	0.022	0.068	0.090	24.718	75.282
7	0.008	0.023	0.031	24.920	75.080
8	0.015	0.045	0.060	24.924	75.076
9	0.017	0.050	0.068	25.862	74.138
10	0.024	0.074	0.098	24.198	75.802
AVG.	0.020	0.052	0.069	24.976	75.024

Gas production rate	=	50.09	l/d
Hydrogen production rate	=	12.4	l/d
Specific hydrogen production rate	=	613.18	ml H_2/g MLVSS d
VFA concentration	=	12,631	mg/l as acetic acid
Hydrogen yield	=	58.87	ml H_2/g COD removed
COD removal efficiency	=	14.87	%
MLVSS	=	5,073	mg/l
VSS	=	2,436	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	2,312
Acetic acid	3,211
Propionic acid	3,196
Butyric acid	5,618
Valeric acid	4,942

Appendix E Raw Data of Effect of COD Loading Rate on Methane Production

E1. COD loading rate = 4.5 kg/ m^3d pH = uncontrol Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	CH4	CO2		CH4	CO2
1	0.019	0.008	0.034	57.439	24.186
2	0.020	0.005	0.025	79.911	20.089
3	0.020	0.004	0.024	83.191	16.809
4	0.020	0.004	0.024	82.653	17.347
5	0.020	0.004	0.024	83.776	16.224
6	0.020	0.006	0.026	75.821	24.179
7	0.024	0.015	0.039	61.847	38.153
8	0.024	0.015	0.039	61.840	38.160
9	0.025	0.015	0.040	61.423	38.577
10	0.024	0.015	0.039	61.221	38.779
AVG.	0.020	0.013	0.037	61.583	38.417

Gas production rate	=	24.22	l/d
Methane production rate	=	9.31	l/d
Specific methane production rate	=	2,326	ml CH_4/l d
VFA concentration	=	4,066	mg/l as acetic acid
Methane yield	=	582.6	ml CH_4/g COD removed
COD removal efficiency	=	26.4	%
MLVSS	=	4,040	mg/l
VSS	=	1,860	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	Concentration (ppm)
Ethanol	782
Acetic acid	776
Propionic acid	874
Butyric acid	405
Valeric acid	394

E2. COD loading rate = 6.2 kg/ m^3d pH = uncontrol Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	CH4	CO2		CO2	CH4
1	0.046	0.037	0.083	44.439	55.561
2	0.062	0.021	0.083	25.521	74.479
3	0.041	0.020	0.061	32.830	67.170
4	0.048	0.022	0.070	30.942	69.058
5	0.044	0.026	0.070	37.233	62.767
6	0.040	0.024	0.065	37.747	62.253
7	0.043	0.025	0.068	36.646	63.354
8	0.041	0.025	0.066	37.624	62.376
9	0.044	0.026	0.069	37.045	62.955
10	0.039	0.024	0.063	37.848	62.152
AVG.	0.040	0.025	0.066	37.357	62.643

Gas production rate	=	29.2	l/d
Methane production rate	=	10.9	l/d
Specific methane production rate	=	2,729	ml CH_4/g MLVSS d
VFA concentration	=	3,547	mg/l as acetic acid
Methane yield	=	638.3	ml CH_4/g COD removed
COD removal efficiency	=	31.03	%
MLVSS	=	3,513	mg/l
VSS	=	2,673	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	637
Acetic acid	639
Propionic acid	744
Butyric acid	301
Valeric acid	345

E3. COD loading rate = 8.8 kg/ m^3d pH = uncontrol Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	CH4	CO2		CO2	CH4
1	0.074	0.280	0.354	79.133	20.867
2	0.097	0.033	0.129	25.141	74.859
3	0.058	0.033	0.091	35.947	64.053
4	0.067	0.033	0.100	33.017	66.983
5	0.061	0.029	0.090	32.385	67.615
6	0.058	0.029	0.087	33.791	66.209
7	0.066	0.034	0.100	33.528	66.472
8	0.064	0.032	0.096	33.397	66.603
9	0.069	0.037	0.106	34.592	65.408
10	0.069	0.035	0.104	33.847	66.153
AVG.	0.067	0.033	0.099	33.841	66.159

Gas production rate	=	60.6	l/d
Methane production rate	=	20.37	l/d
Specific methane production rate	=	5,091	ml CH_4/l d
VFA concentration	=	2,638	mg/l as acetic acid
Methane yield	=	1,172	ml CH_4/g COD removed
COD removal efficiency	=	50.4	%
MLVSS	=	2,960	mg/l
VSS	=	3,060	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	386
Acetic acid	312
Propionic acid	378
Butyric acid	286
Valeric acid	254

E4. COD loading rate = 11.6 kg/ m^3d pH = uncontrol Temperature = 37°C

Days	Amount of each component (ml)		Total amount (ml)	Produced gas composition (%)	
	CH4	CO2		CO2	CH4
1	0.021	0.007	0.029	25.6851	74.3149
2	0.021	0.008	0.029	27.3525	72.6475
3	0.066	0.012	0.078	14.7757	85.2243
4	0.022	0.013	0.035	36.0510	63.9490
5	0.024	0.013	0.037	35.6038	64.3962
6	0.024	0.013	0.037	35.0298	64.9702
7	0.024	0.013	0.038	35.3249	64.6751
8	0.025	0.014	0.038	35.7750	64.2250
9	0.025	0.014	0.038	35.8879	64.1121
AVG.	0.024	0.013	0.038	35.663	64.476

Gas production rate	=	36.7	l/d
Methane production rate	=	13.05	l/d
Specific methane production rate	=	3,262	ml CH_4/l d
VFA concentration	=	2,509	mg/l as acetic acid
Methane yield	=	470.4	ml CH_4/g COD removed
COD removal efficiency	=	40.3	%
MLVSS	=	2,840	mg/l
VSS	=	2,860	mg/l

Distillated sample 100 µl + Internal standard (n-propanol 3,000 ppm) 100 µl

VFA	**Concentration (ppm)**
Ethanol	368
Acetic acid	415
Propionic acid	450
Butyric acid	341
Valeric acid	323

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Proceedings:

1. Poontaweegeratigarn, T., Chavadej, S., and Rangsunvigit, P. (2012, April, 11-13) Hydrogen Production from Alcohol Wastewater by Upflow Anaerobic Sludge Blanket Reactors under Mesophilic Temperature. Proceedings of ICCBEE 2012 : International Conference on Chemical, Biological and Environmental Engineering, Venice, Italy
2. Poontaweegeratigarn, T., Chavadej, S., and Rangsunvigit, P. (2012, April, 24) Hydrogen and Methane Production from Alcohol Wastewater by Upflow Anaerobic Sludge Blanket Reactors. Proceedings of The3rd Research Symposium on Petroleum, Petrochemicals, and Advanced Materials and The 18th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.