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APPENDIX A : THE DETAIL OF ALL SAMPLES

Sample no.	Characteristics of sample	Sampling Site	Date
Soil			
1.	Rough gray	around of the chemistry II building	28 Jan. 97
3.	Black, wet	In village	30 Jan. 97
5.	Gray, dry	Mining plant	30 Jan. 97
7.	Black, wet	Sewage treatment plant, Hauy Kwang	4 Mar. 97
18.	Blue, dry	Mining plant	28 Jun. 97
23.	Mud, brown	Farm in province of Pratumthanee	7 Apr.98
24.	Dry, soil	Farm in Thonburi	9 Apr. 98
26.	Rough, brown	Mining plant	10 Apr. 98
27.	Mud, brown	Chemical plant	10 Apr. 98
28.	Black, wet	Mining plant	10 Apr. 98
29.	Brown, wet	Bang-pre industry	10 Apr. 98
31.	Brown, wet	Chicken farm in Samut-Songkarm	20 Apr. 98
32.	Brown, wet	Duck farm	20 Apr. 98
33.	Black, wet	Industrial plant	20 Apr.98
34.	Black, wet	Industrial plant	20 Apr.98
Water			
10.	White, turbidity	ICI paint plant, Co.	19 Mar. 97
13.	Black	Nawanakorn Industrial community	27 Jun.97
25.	Turbidity	Canal in Bangkok	7 Apr. 98
Sediment			
2.	Mud with duck weed	Pool front General Science building	28 Jan. 97
4.	Turbidity	Canal at Victory Monument	30 Jan. 97
9.	Black, putrid smell	Thai-ping industry, Co.	19 Mar. 97
11.	Black, mixed oils	Side Vipawadee Rangsit road	19 Mar. 97
12.	Rough	Nawanakorn Industrial community	27 Jun. 97
14.	Brown	Paint plant	27 Jun. 97
15.	Brown	Ladkrabung Industrial community	27 Jun. 97

Sample no.	Characteristics of sample	Sampling Site	Date
	Sediment		
17.	Brown	Ladkrabung Industrial community	27 Jun. 97
19.	Brown	Canal in Bangkok	15 Nov. 97
20.	Black	Canal in Bangkok	15 Nov. 97
21.	Brown	Canal in Bangkok	16 Nov. 97
30.	Black	Oil industrial plant	20 Apr. 98
	Activated Sludge		
6.	Brown	Sewage treatment plant, Haui Kwang	4 Mar. 97
8.	Brown	Sewage treatment plant, Si-Phya	4 Mar. 97
16.	Black	Ladkrabung Industrial Community	27 Jun. 97

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX B : MEDIA

1. MacConkey agar

For isolating and differentiating lactose-fermenting from lactose-non fermenting gram negative enteric bacilli.

Formula : ingredient per liter

Bacto Peptone	17 g
Bacto Proteose Peptone	3 g
Bacto Lactose	10 g
Bacto Bile Salt No.3	1.5 g
Sodium Chloride	5 g
Bacto Agar	13.5 g
Neutral Red	30 mg
Bacto Crystal Violet	1 mg

Final pH 7.1 ± 0.2 at 25 °C

Direction : suspend 50 grams in 1 liter, distilled or deionize water and boil to dissolve completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure. Avoid overheating.

2. MacConkey-Inositol-Potassium Tellurite (MCIK)

Formula : ingredient per liter

MacConkey agar	40 g
Myo-inositol	1.8 g
Potassium tellurite	0.003 g

Direction : Suspend MacConkey and warm to dissolve the medium completely. Autoclaved at 121 °C for 15 min and then cooled to

50 °C, filter-sterilized myo-inositol and potassium tellurite were added. After mixing well, the medium was poured into sterile petri plates.

3. Minimal Medium

Formula : ingredient per liter

(NH ₄) ₂ SO ₄	2	g
KH ₂ PO ₄	13.6	g
FeSO ₄ 7H ₂ O	0.5	mg
1 M MgSO ₄ 7H ₂ O	1	ml
20% Glucose	10	ml
Vitamin B	0.1	ml
20% cas amino acid	5	ml

The glucose, MgSO₄ 7H₂O, and cas amino acid were separated autoclave at 110 °C, 10 min. The another was normal sterilize at 121°C for 15 min.

4. Motility test medium

Formula : ingredient per liter

Beef extract	3	g
Peptone	10	g
NaCl	5	g
Agar	4	g

Final pH = 7.3

5. MR/VP broth

Formula : ingredient per liter

Polypeptone	7	g
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Glucose	5	g
Dipotassium phosphate	5	g

Final pH = 6.9 ± 0.2

6. Pseudomonas Selective Isolation Agar (PSIA)

Formula : ingredient per liter

Tryptic Soy	30	g
5% (w/v) nitrofurantoin	7	ml in N,N-dimethylformamide
0.1%(w/v) crystal violet	2	ml in deionized water
Agar	15	g

Two ml of crystal violet were added in 990 ml of Tryptic Soy Agar (TSA). After, autoclaving and cooling the TSA to 50 °C adding 7 ml nitrofurantoin.

7. Salmonella-Shigella Agar (SS)

Formula : ingredient per liter

Bacto Beef Extract	5	g
Bacto Proteose Peptone	5	g
Bacto Lactose	10	g
Bacto Bile Salt No.3	8.5	g
Sodium Citrate	8.5	g
Sodium Thiosulfate	8.5	g
Ferric Citrate	1	g
Bacto Agar	13.5	g
Brilliant Green	0.33	mg
Neutral Red	25	mg

Final pH 7.0 ± 0.2 at 25 °C

Direction : Suspend 60 grams in 1 liter distilled or deionized water and boil carefully for no more than 2-3 min to dissolve completely. Avoid overheating. Do not autoclave.

8. Simmons Citrate Agar

Formula : ingredient per liter

Magnesium Sulfate	0.2	g
Ammonium Dihydrogen Phosphate	1	g
Dipotassium Phosphate	1	g
Sodium Citrate	2	g
Sodium Chloride	5	g
Bacto Agar	15	g
Bacto Brom Thymol Blue	0.08	g

Final pH 6.8 at 25 °C

Direction : To rehydrate the medium, suspend 24.2 grams in 1L, cold freshly distilled water and heat to boiling to dissolve the medium completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121 °C).

9. Triple Sugar Iron Agar (TSI)

Formula : ingredient per liter

Bacto Beef Extract	3	g
Bacto Yeast Extract	3	g
Bacto Peptone	15	g
Proteose Peptone	5	g
Bacto Dextrose	1	g
Bacto Lactose	10	g

Saccharose	10 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Bacto Agar	12 g
Bacto Phenol Red	24 mg

Final pH 7.4 at 25 °C

Direction : To rehydrate the medium, suspend 65 grams in 1000 ml, cold freshly distilled water and heat to boiling to dissolve the medium completely. Sterilize in the autoclave for 15 minutes at 15 pounds pressure. Allow the tubes to solidify in a slanting position in a manner which will give a generous butt.

10. Tryptic Soy Broth (TSB)

Formula : ingredient per liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g
(Bacto Agar	15 g; TSA)

Final pH 7.3 ± 0.2 at 25 °C

Direction : Suspend 30 grams in 1 liter distilled or deionized water and warm slightly to dissolve completely. Sterilize at 121-124 °C for 15 min.

APPENDIX C : PHENOL-SULFURIC ACID METHOD

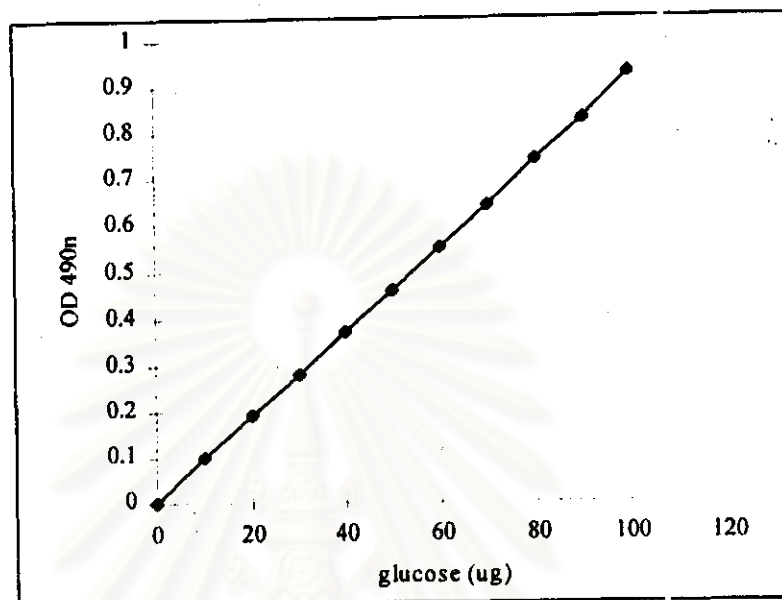
Reagent :

- (i) 67% conc. (V/V) Sulfuric acid
- (ii) Phenol reagent : Added 5 grams reagent-graded phenol to
100 ml distilled water

Procedure

- (i) To prepare the stock standard, dissolved 100 mg glucose in 100 ml distilled water (conc. 1 mg/ml). Diluted 1:10 in distilled water just before use to give a solution containing 100 μ g glucose per ml. Prepared standards (10-100 μ g/ml) from the diluted solution.
- (ii) 1.0 ml glucose solution added 1.0 ml of the phenol reagent; mixed rapidly and thoroughly
- (iii) Added 5.0 ml of concentrated sulfuric acid, mixed rapidly, and let stand for 10 min.
- (iv) Placed the tubes in a water bath at 25 C for 15 min.
- (v) Read the absorbance of each tube at 490 nm against the blank without glucose using the spectrophotometer.
- (vi) Determined the concentration of carbohydrate in the samples from a standard curve prepared by plotting the absorbances of the standards versus the concentration of glucose.

Figure C-1 A linear standard curve of glucose detected by this method



Calculation Method

$$\text{Glucose } (\mu\text{g}) = \frac{\text{Absorbance read}}{\text{Slope constant}}$$

Where slope constant equals to 0.0092

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APPENDIX D : LOWRY'S METHOD

Reagent :

- (i) 2 mg/ml BSA (Bovine Serum Albumin)
- (ii) Folin-Ciocalteu reagent (lowry's reagent) composite with:

Reagent A

- a. 10 ml of 2.0% (w/v) $\text{NaKC}_4\text{H}_4\text{O}\cdot 4\text{H}_2\text{O}$
- b. 10 ml of 1.0% (w/v) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
- c. 200 ml of 2.0%(w/v) Na_2CO_3 in 0.1N NaOH

For reagent A, mix 2 ml of (a), 2 ml of (b) and 196 ml of (c).

Reagent A must be freshly prepared.

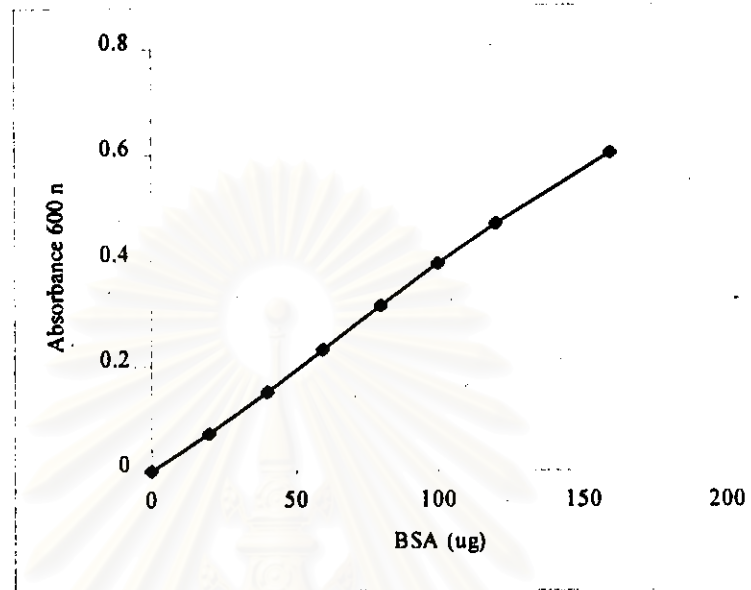
Reagent B (Phenol reagent)

Make 1:3 dilution before used.

Serial dilution standard technique

Prepare a set of 5 serial dilutions (1:2) of standard BSA in the following manner. Pipet 0.5 ml of distilled water into each of five test tube (no.1-5). Pipet 0.5 ml of stock BSA solution (2.0 mg/ml) into tube no.1. Mix gently, and then transfer 0.5 ml of this diluted solution to tube no.2. Continue this dilution to tube no.5. From each tube in this serial dilution set, pipet 0.1 ml solution to another set of 5 test tubes. Add reagent A 3.0 ml, mix well and wait 10 minutes before adding 0.3 ml of reagent B. After 30 minutes, measure the absorbance at 600 nm of every tube. Plot the BSA standard calibration curve (OD vs μg BSA).

Figure D-1 A linear standard curve of Bovine Serum Albumin detected by this method



Calculation Method

$$\text{BSA } (\mu\text{g}) = \frac{\text{Absorbance read}}{\text{Slope constant}}$$

Where slope constant equals to 0.00346

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**APPENDIX E : INFLUENCE OF pH, TEMPERATURE, TIME
AND CAMIUM CONCENTRATION TO EPS PRODUCTION**

Table E-1 Influence of pH factor on EPS production of each strain.

Strains	pH	Dried weight EPS (mg)	Dried weight cells (mg)	Proportion of EPS (mg) per cell (mg)
87	5	8.46	8.8	0.96
	6	10.68	9.9	1.08
	7	15.41	13.3	1.16
	8	24.70	14.95	1.65
	9	18.64	13.0	1.43
98	5	10.85	11.25	0.96
	6	9.50	10.15	0.94
	7	16.29	10.9	1.49
	8	17.15	13.0	1.32
	9	18.06	12.75	1.42
205	5	7.19	8.35	0.86
	6	13.45	9.75	1.38
	7	13.92	9.75	1.43
	8	24.70	11.05	2.23
	9	28.91	12.2	2.37
207	5	5.25	8.8	0.60
	6	7.40	10.45	0.71
	7	7.73	11.1	0.70
	8	18.90	13.05	1.45
	9	23.55	10.15	2.32

Table E-1 (continuous)

Strains	pH	Dried weight EPS (mg)	Dried weight cells (mg)	Proportion of EPS (mg) per cell (mg)
273	5	4.45	16.35	0.31
	6	14.26	16.45	0.87
	7	16.40	14.4	1.00
	8	14.80	12.0	1.23
	9	6.70	11.05	0.61

To duplicates, temperature 37 °C, for 24 hr.

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Table E-2 Influence of temperature factor on EPS production of each strain.

Strains	Temp.	Dried weight EPS (mg)	Dried weight cells (mg)	Proportion of EPS (mg) per cell (mg)
87	30	14.26	14.4	0.99
	37	19.77	18.65	1.06
	40	11.25	9.87	1.14
	45	6.12	7.95	0.77
	50	0	8.95	0
98	30	12.36	12.00	1.03
	37	12.67	12.30	1.03
	40	17.39	12.70	1.37
	45	12.77	12.40	1.03
	50	0	7.00	0
205	30	17.47	10.85	1.61
	37	15.63	11.75	1.33
	40	20.77	13.40	1.55
	45	18.41	13.95	1.32
	50	0	6.05	0
207	30	9.55	10.85	0.88
	37	9.80	12.10	0.81
	40	16.58	14.80	1.12
	45	5.98	6.95	0.86
	50	0	6.50	0
273	30	12.58	13.10	0.96
	37	13.75	12.50	1.10
	40	14.61	13.65	1.07
	45	10.88	11.33	0.96
	50	0	7.50	0

To duplicates, adjust pH 7.0, 24 hr.

Table E-3 Influence of time factor on EPS production of each strain.

Strains	Time (hr)	Dried weight EPS (mg)	Dried weight cells (mg)	Proportion of EPS (mg) per cell (mg)
87	24	72.80	62.8	1.16
	48	156.20	62.6	2.49
	72	274.80	55.9	4.92
98	24	76.60	52.5	1.46
	48	117.50	61.0	1.93
	72	246.10	77.3	3.18
205	24	104.70	42.8	2.45
	48	206.60	57.4	3.56
	72	252.10	61.2	4.12
207	24	70.90	41.8	1.70
	48	115.90	49.6	2.34
	72	243.50	62.8	3.88
273	24	82.18	53.5	1.93
	48	158.86	58.0	2.74
	72	229.04	63.8	3.59

To duplicates, adjust pH 7.0, 37 °C.

Table E-4 Influence of Cd concentration factor on EPS production of each strain.

Strains	Cd conc. (mg/l)	Dried weight EPS (mg)	Dried weight cells (mg)	Proportion of EPS (mg) per cell (mg)
87	0	6.29	4.6	1.37
	10	3.84	7.2	0.53
	20	6.23	12.8	0.49
	100	6.07	10.7	0.57
98	0	9.43	6.0	1.57
	10	10.18	10.0	1.02
	20	1.05	8.6	0.12
	100	0.40	7.7	0.05
205	0	12.52	8.9	1.41
	10	5.13	7.3	0.70
	20	9.00	8.7	1.03
	100	9.77	11.7	0.83
207	0	5.28	7.1	0.74
	10	3.26	6.9	0.47
	20	5.32	9.7	0.55
	100	0.74	9.2	0.08
273	0	7.21	6.1	1.18
	10	8.83	7.7	1.15
	20	8.44	8.0	1.05
	100	0.81	9.2	0.09

To duplicates, adjust pH 6.0, 37 °C, for 24 hr.

**APPENDIX F : COMPARISION OF UPTAKE AND ADSORPTION
CADMIUM BY VIABLE CELLS AT DIFFERENT
CONCENTRATION**

Strains	Initial Cd conc.(mg/l)	Amount of Cd adsorption (mg/l)	%adsorption	Uptake (nM/mg)
CdR-87	10.39	8.56	82.4	75.79
	50.12	30.225	60.3	269.15
	102.50	35.4	34.1	314.92
CdR-98	10.39	8.987	86.5	79.95
	50.12	36.366	72.55	323.51
	102.50	29.50	28.39	262.43
CdR-205	10.39	8.353	80.4	74.31
	50.12	37.49	74.79	333.51
	102.50	23.40	22.52	20.817
CdR-207	10.39	8.826	84.95	78.52
	50.12	38.20	76.21	339.83
	102.50	27.95	26.9	248.64

$$\% \text{adsorption} = \frac{\text{amount of adsorption}}{\text{initial concentration}} \times 100$$

$$\text{Uptake value ; } Q = V(c_i - c_f) / 1000m$$

Where Q is the Cd content (mg/g biomass), V the volume of metal solution (ml), c_i the initial concentration of Cd in solution, c_f the final concentration of Cd in solution and m is the mass of biomass (g).

(Puranik, Chabukswar and Paknikar, 1995)

**APPENDIX G : CADMIUM AND OTHER METALS BY
VIABLE CELLS, DEAD CELLS AND EPS.**

**Table G-1 Comparison the adsorption of cadmium by viable cells,
dead cells and EPS**

Strains	Initial Cd conc.(mg/l)	Amount of Cd adsorption (mg/l)		
		Viable cells	Dead cells	EPS
CdR-87	10.39	8.56±0.52 (82.40%)	8.30±0.43 (79.90%)	8.65±0.47 (83.25%)
CdR-98	10.39	8.99±0.57 (86.50%)	8.49±0.43 (81.70%)	9.19±0.46 (88.45%)
CdR-205	10.39	8.35±0.41 (80.40%)	7.57±0.51 (72.90%)	7.70±0.61 (74.15%)
CdR-207	10.39	8.83±0.50 (84.95%)	8.20±0.49 (78.95%)	8.30±0.48 (79.90%)
CdR-273	10.39	8.26±0.50 (79.55%)	7.59±0.51 (73.07%)	8.03±0.48 (77.25%)

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Table G-2 Comparison the adsorption of copper by viable cells, dead cells and EPS

Strains	Initial Cu conc.(mg/l)	Amount of Cu adsorption (mg/l)		
		Viable cells	Dead cells	EPS
CdR-87	10.49	1.22±0.47 (11.64%)	3.93±0.60 (37.48%)	3.62±0.78 (34.57%)
CdR-98	10.49	2.81±0.42 (26.78%)	5.56±0.43 (53.06%)	2.83±0.51 (27.03%)
CdR-205	10.49	3.94±0.60 (37.60%)	6.39±0.51 (60.74%)	2.89±0.45 (27.52%)
CdR-207	10.49	4.10±0.39 (39.11%)	6.59±0.42 (62.84%)	3.38±0.58 (32.21%)
CdR-273	10.49	6.196±0.61 (59.09%)	9.48±0.53 (90.45%)	5.11±0.53 (48.76%)

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Table G-3 Comparison the adsorption of manganese by viable cells, dead cells and EPS

Strains	Initial Mn conc.(mg/l)	Amount of Mn adsorption (mg/l)		
		Viable cells	Dead cells	EPS
CdR-87	10.09	3.91±0.43 (38.73%)	3.31±0.39 (32.76%)	0.68±0.48 (6.71%)
CdR-98	10.09	4.12±0.55 (40.86%)	3.56±0.52 (35.28%)	3.12±0.41 (30.88%)
CdR-205	10.09	7.89± 0.74 (78.16%)	3.24±0.51 (32.11%)	7.87±0.48 (78.00%)
CdR-207	10.09	6.27±0.38 (62.13%)	4.09±0.45 (40.55%)	3.79±0.48 (37.65%)
CdR-273	10.09	3.76±0.43 (37.26%)	5.84±0.47 (57.86%)	3.12±0.52 (30.95%)

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**APPENDIX H : EFFECT OF CADMIUM CONCENTRATION
TO pH OF MEDIA**

Cd conc.(mg/l)	pH in media*	PH in distilled water	pH at soluble Cd in media
0	7.3	8.2	-
100	7.3	5.6	5.5
200	7.2	6.0	5.2
300	7.2	6.0	5.1
400	7.1	6.1	5.0
500	7.1	6.1	5.0
600	7.1	6.1	4.9
700	7.0	6.1	4.8
800	6.8	6.1	4.8
900	6.8	6.1	4.8
1000	6.5	6.1	4.5

* Tryptic Soy Broth

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BIOGRAPHY

Miss Anicha Luengchaichawang was born in Ratchaburi on the 11 th of November, 1973. She was graduated with Bachelor of Science (Microbiology) in 1994 from Burapha University, Bangsan, Chonburi. A year later, 1995, she was started study in Biotechnology, Faculty of Science, Chulalongkorn University.



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