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APPENDICES

APPENDIX A

BACTERIAL SOURCES

Fifty samples were chosen as bacterial source. They were collected during December 1997 to November 1998, and were categorized into various groups as follows :

Soil

The soil samples were collected from:

Sampling Sites	Date of Collection
Chromium plating, area I, Bangkok	December 6, 1997
Chromium plating, area II, Bangkok	December 6, 1997
Printing machinery, area II, Bangkok	December 7, 1997
Wood, area I, Bangkok	February 3, 1998
Metal plating, area I, Bangkok	February 9, 1998
Painting, area II, Bangkok	February 10, 1998
Klong Taweewattana, area I, Bangkok	April 8, 1998
Klong Bangbua, area IX, Nonthaburi	April 8, 1998
Oil refinery, area VI, Samutprakarn	April 9, 1998
Duck farm (1), area VII, Nakornprathom	April 10, 1998
Duck farm (2), area VII, Nakornprathom	April 10, 1998
Chicken farm (1), area VII, Nakornprathom	April 10, 1998
Chicken farm (2), area VII, Nakornprathom	April 10, 1998
Chicken farm (3), area VII, Nakornprathom	April 10, 1998
Pig farm (1), area VII, Nakornprathom	April 10, 1998
Pig farm (2), area VII, Nakornprathom	April 10, 1998
Pig farm (3), area VII, Nakornprathom	April 10, 1998

Sampling Sites	Date of Collection
Wastewater treatment, area VI, Samutprakarn	April 10, 1998
Chemical production, area V, Samutprakarn	May 9, 1998
Can production industry, area VI, Samutprakarn	May 9, 1998
Industrial sector, area V, Samutprakarn	May 9, 1998
Rice field, area VIII, Pathumtani	June 8, 1998
Orange orchard, area III, Bangkok	June 8, 1998
Treatment sector, area IV, Bangkok	June 8, 1998
Area (1), Chulalongkorn University	October 3, 1998
Garbage heap, area (1), Chulalongkorn University	October 3, 1998
Chaopraya River (1), area II, Bangkok	October 3, 1998
Chaopraya River (2), area II, Bangkok	October 3, 1998
ferric company (1), area X, Karnchanaburi	November 3, 1998
ferric company (2), area X, Karnchanaburi	November 3, 1998

Wastewater

The samples were collected from:

Sampling Sites	Date of Collection
Chromium plating, area I, Bangkok	December 6, 1997
Chromium plating, area II, Bangkok	December 6, 1997
Printing machinery, area II, Bangkok	December 7, 1997
Wood, area I, Bangkok	February 3, 1998
Metal plating, area I, Bangkok	February 9, 1998
Painting, area II, Bangkok	February 10, 1998
Oil refinery, area VI, Samutprakarn	April 9, 1998
Wastewater treatment, area VI, Samutprakarn	April 10, 1998
Chemical production, area V, Samutprakarn	May 9, 1998
Industrial sector, area V, Samutprakarn	May 9, 1998
Treatment sector, area IV, Bangkok	June 8, 1998

Sludge

The samples were collected from:

Sampling Sites	Date of Collection
Chromium plating, area I, Bangkok	December 6, 1997
Wood, area I, Bangkok	February 3, 1998
Painting, area II, Bangkok	February 10, 1998
Oil refinery, area VI, Samutprakarn	April 9, 1998
Chemical production, area V, Samutprakarn	May 9, 1998

Natural Water

The samples were collected from:

Sampling sites	Date of Collection
Klong Taweewattana, area I, Bangkok	April 8, 1998
Klong Bangbua, area IX, Nonthaburi	April 8, 1998
Chaopraya River (1), area II, Bangkok	October 3, 1998
Chaopraya River (2), area II, Bangkok	October 3, 1998

APPENDIX B

CULTURE MEDIA

1. Nutrient Broth (NB) (Difco laboratories, Detroit, Michigan, U.S.A.) (Shen and Wang, 1995a)

Formula in gram per 1 liter

- Beef extract 3
- Bacto peptone 5

Final pH 7.1

2. Nutrient Agar (NA) (Difco)

Formula in gram per 1 liter

- Beef extract 3
- Bacto peptone 5
- Agar 15

Final pH 7.1

The NB medium was prepared by suspending 8 gram of NB in 1 L of distilled water and added 15 gram of agar when prepare NA medium and boil to dissolve completely by microwave. Then, the medium was autoclaved at 121°C, 1 atmosphere for 15 min. All media were dispended in plates and before used, plate was incubated over night for checking of sterilization.

3. Pseudomonas Selective Isolation Agar (PSIA)
(adapted from Krulger and Sheikh, 1986)

Formula in milliliter and gram per 1 liter

- Nitrofurantoin (5% solution)	7
- Crystal violet (0.1 % solution)	2
- TSB	30
- Agar	15
- Distilled Water	990

Pseudomonas selective isolation agar (PSIA) was prepared as follows. A stock solution of 5 % (wt/vol) nitrofurantoin (Sigma, Steinheim, Germany), was prepared in N,N-dimethyformamide (Merck, Darmsatadt, Germany). A stock solution of 0.1 % (wt/vol) crystal violet (Merck, Darmsatadt, Germany) was prepared in deionized water. The stock solution were stored at room temperature, and nitrofurantoin solution was protected from exposure to light. The medium (PSIA) was prepared by suspending 30 gram of TSB and 15 gram of agar in 990 ml distilled water and added 2 ml of crystal violet stock solution. After the mixture was autoclaved at 121 ° C for 15 min and then cooled to 50°C 7 ml of nitrofurantoin stock solution were added (adapted from Krulger and Sheikh, 1986). All media were dispended in plates and before used plates was incubated over night for checking of sterilization.

4. MacConkey-inositol-potassium tellurite (MCIK) agar
(adapted from Toman, Cirerana and Jofre, 1986)

Formula in milliliter and gram per 1 liter

- MacConkey Agar 40
- Myo-inositol 10 mM
- Potassium tellurite 0.003

The medium (MCIK) was prepared by suspending 40 gram of MacCongey agar in 1 L of distilled water. After the mixture was autoclaved at 121 ° C for 15 min and then cooled to 50 ° C, myo-inositol (Fluka, Messerschmittstr, Switzerland), final concentration 10 mM. and potassium tellulite (Merck, Darmsatadt, Germany), final concentration, 3 µg/ml were added (adapted from Toman, Cirerana and Jofre, 1986). All media were dispended in plates and before used plates was incubated over night.

5. Shigella and Salmonella (SS) Agar (Difco)

Formula in gram per 1 liter

- Bacto Beef Extract	5
- Bacto Proteose Peptone	5
- Bacto Lactose	10
- Bacto Bile Salt No. 3	8.5
- Sodium Citrate	8.5
- Sodium Thiosulfate	8.5
- Ferric Citrate	1
- Bacto Agar	13.5
- Brilliant Green	0.33 mg
- Neutral Red	0.025

Final pH 7.0 ± 0.2 at 25°C

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

6. MacConkey Agar (Difco)

Formula in gram per 1 liter

- Bacto Peptone	17
- Bacto Proteose Peptone	3
- Bacto Lactose	10
- Bacto Bile Salts No. 3	1.5
- Sodium Chloride	5
- Bacto Agar	13.5
- Neutral Red	0.03
- Bacto Crystal Violet	0.001

The medium was prepared by suspending 50 gram of MacConkey agar in 1 L of distilled water and boil to dissolve completely. After, the medium was autoclaved at 121 °C for 15 min. Avoid overheating. All media were dispended in plates and before used plates was incubated over night.

Selective Medium

Strains	MCIK	PSIA	MacConkey	EMB	SS	EC	BHI	SRB	King A	S
CrR-2	+/pink	-	white	+/pink	-	+/growth	+	-	-/yellow	-
CrR-14	-	+/pink	pink	-	-	-	+	+	-/yellow	-
CrR-15	-	-	-	-	-	+/growth	+	+	-/yellow	-
PhR-26	+/pink	-	-	-	-	-	+	++	-/yellow	-
PhR-33	-	+/pink	pink	-	-	-	+	+	-/yellow	-
PhR-64	-	-	white	+/pink	-	+/growth	+	+	-/yellow	-
CPR-4	+/pink	+/pink	pink	+/pink	colorless	+/growth	+	+	-/yellow	+/green
CPR-16	+/pink	+/pink	pink	+/pink	pink	+/growth	+	++	+/green	+/green
CPR-17	+/pink	-	white	+/pink	colorless	+/growth	+	++	-/yellow	+/green

APPENDIX C

FORMULAR AND PREPARATION OF SOME BIOCHEMICAL TESTS AND RESULT OF SOME SELECTED BACTERIAL STRAINS

1. Motility test medium

Formula in gram per 1 liter

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

Final pH 7.3

2. MR/VP broth

Formula in gram per 1 liter

- Polypeptone	7
- Glucose	5
- Dipotassium phosphate	5

Final pH 6.9

3. Simmons Citrate Agar

Formula in gram per 1 liter

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

Final pH 6.8 at 25 °C

Preparation : 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).

4. Three Sugar Iron Agar (TSI)

Formula in gram per 1 liter

- Bacto Beef Extract	3	
- Bacto Yeast Extract	3	
- Bacto Peptone	15	
- Proteose Peptone	5	
- Bacto Dextrose	1	
- Bacto Lactose	10	
- Saccharose	10	
- Ferrous Sulfate	0.2	
- Sodium Sulfate	5	
- Sodium Thiosulfate	0.3	
- Bacto Agar	12	
- Bacto Phenol Red	24	mg

Final pH 7.4 at 25 °C

Preparation : 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 (121 °C). Allow the tubes to solidify in a slanting position in a manner which will give a generous butt.

Biochemical Tests

Strains	motile	nitrate	citrate	TSI	indole	gelatin	oxidase	P	urease	LB	KCN	MR	VP
CrR-2	+	+	-	KK+-	+	-	-	-	-	+	-	+	-
CrR-14	-	++	+	AA--	+	+	+	+	-	-	+	-	-
CrR-15	+	+	+	AA--	-	-	-	-	-	+	-	-	+
PhR-26	+	++	+	KK--	-	-	-	-	+	-	+	-	+
PhR-33	-	++	+	AA--	+	+	+	+	-	-	+	-	-
PhR-64	+	+	-	KK+-	+	-	-	-	-	+	-	+	-
CPR-4	-	++	+	AA--	+	+	+	+	-	-	+	-	-
CPR-16	+	++	+	KK++	+	-	-	+	+	-	+	+	+
CPR-17	+	+	-	KK+-	+	-	-	-	-	+	-	+	-

APPENDIX D

STANDARD CURVE OF CHROMIUM AND PHENOL

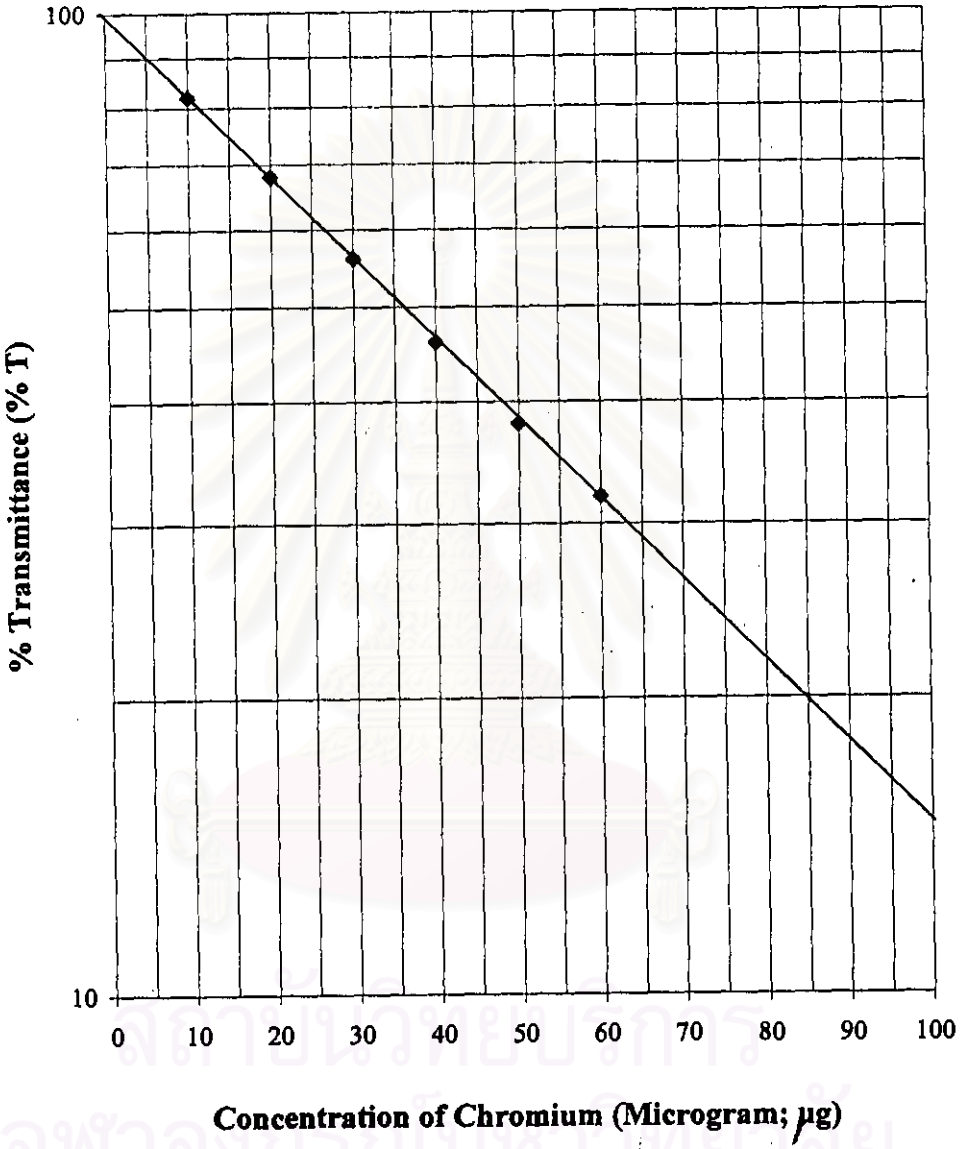

Figure D.1 Standard curve of chromium by colorimetric method (Greenberg, A.E.,et.al., 1992)

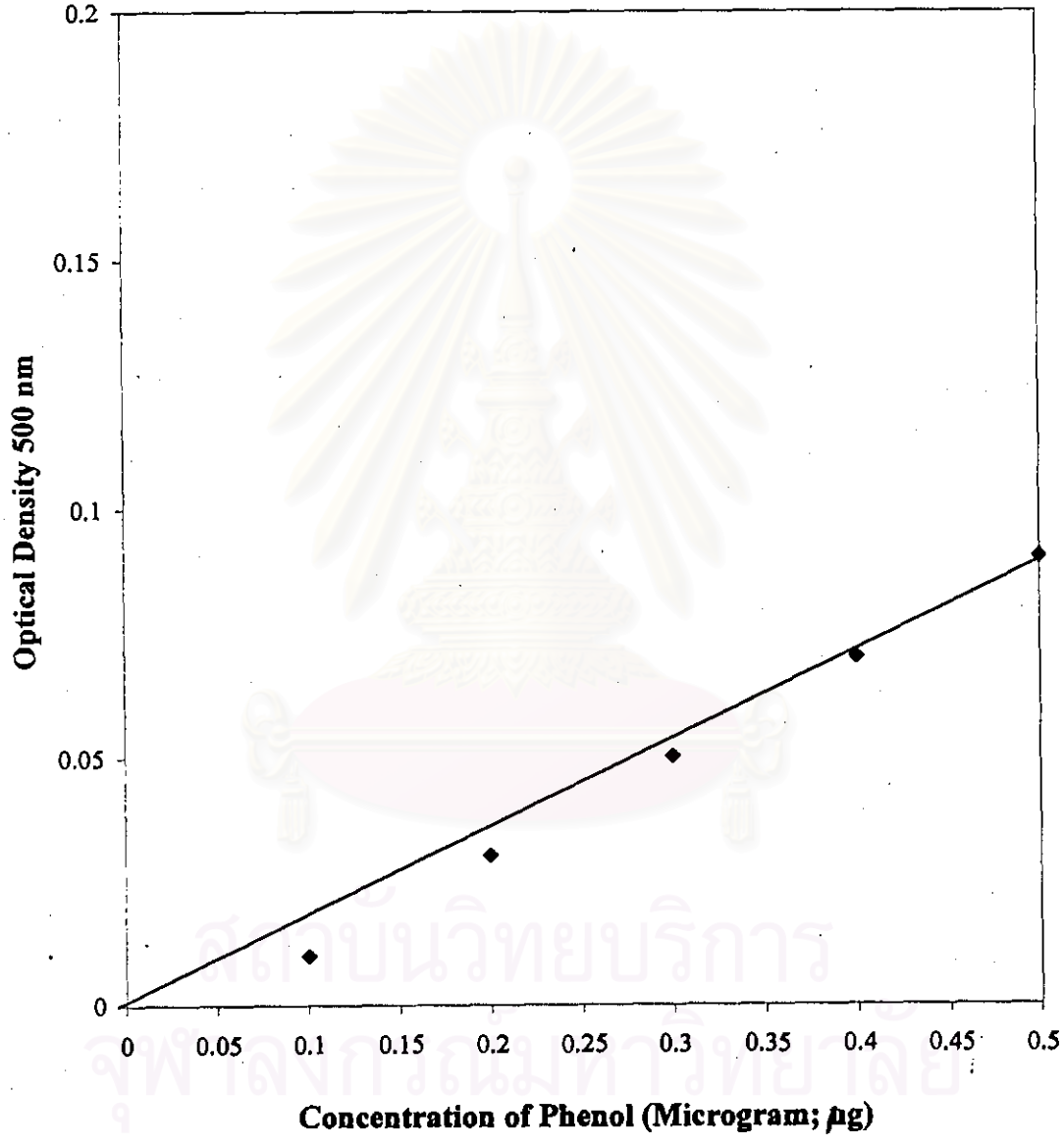

Figure D.2 Standard curve of phenol by direct photometric method (Greenberg A.E., et.al., 1992)

APPENDIX E

SOME CHARACTERISTICS OF THE SELECTED BACTERIAL ISOLATES

Strains	Identified as	Cr Resistance (μg/ml)	Ph Resistance (μg/ml)	As Resistance (μg/ml)	Zn Resistance (μg/ml)	Stability (times)[1]	pH	Temperature (°C)	Efficiency of Degradation (%)[2]		
									p-Cresol	p-Chloro-phenol	p-Nitro-phenol
CrR-2	*Escherichia sp.*	2400	1000	200	100	18	7	37	-	-	-
CrR-14	*Pseudomonas sp.*	2400	1000	200	100	18	7	37	-	-	-
CrR-15	*Enterobacter sp.*	2400	1000	200	100	18	7	37	-	-	-
PhR-26	*Klebsiella sp.*	500	2000	200	100	15	7	37	100	16.0	30.0
PhR-33	*Pseudomonas sp.*	500	2000	200	100	15	7	37	100	22.0	24.0
PhR-64	*Escherichia sp.*	500	2000	200	100	15	7	37	100	26.0	26.0
CPR-4	*Pseudomonas sp.*	1200	1200	200	100	18	7	37	100[3]	22.0	26.0
CPR-16	*Proteus sp.*	1200	1200	200	100	18	7	37	100[3]	28.0	36.0
CPR-17	*Escherichia sp.*	1200	1200	200	100	18	7	37	100[3]	34.0	36.0

[1] 20 times of repeated subculturing

[2] Initial concentration 50 μg/ml, on the third week

[3] Initial concentration 50 μg/ml, on the second week

Strains	Efficiency of Cr(VI) detoxification (%)				Efficiency of Cr(III) production (%)				Efficiency of phenol degradation (%)			
	100*	200	300	400	100	200	300	400	100	200	300	400
CrR-2+PhR-26	86.0	83.0	80.3	85.5	5.7	3.0	5.7	2.3	92.0	94.0	88.3	94.5
CrR-2+PhR-33	86.0	85.0	75.0	86.3	0.3	2.0	0.3	1.8	92.0	94.0	89.0	94.8
CrR-2+PhR-64	85.0	84.5	79.3	86.0	2.3	1.0	2.3	1.3	93.0	94.5	91.3	94.0
CrR-14+PhR-26	80.0	77.5	79.7	79.8	2.7	2.0	2.7	1.5	99.0	95.5	94.7	99.8
CrR-14+PhR-33	75.0	76.5	91.3	79.0	4.0	2.0	4.0	1.0	99.0	98.0	96.7	96.3
CrR-14+PhR-64	78.0	77.0	79.7	79.8	4.3	1.0	4.3	1.5	99.0	97.5	87.7	96.3
CrR-15+PhR-26	77.0	77.0	87.0	76.8	4.3	1.0	4.3	0.8	98.0	94.0	97.0	97.0
CrR-15+PhR-33	76.0	78.5	76.3	77.8	1.0	1.0	1.0	0.5	99.0	96.0	85.3	94.0
CrR-15+PhR-64	76.0	77.0	78.0	77.5	2.0	1.0	2.0	2.3	98.0	94.5	97.3	96.8
CPR-4	85.0	82.5	80.3	83.3	4.3	3.0	4.3	1.0	92.0	98.0	90.3	97.5
CPR-16	49.0	79.5	80.3	88.0	6.0	3.0	6.0	2.5	53.0	87.0	91.3	85.3
CPR-17	84.0	83.0	82.0	84.0	3.7	2.0	3.7	2.3	95.0	98.5	87.3	98.3

* Concentration (μg/ml)

Strains	Efficiency of Cr(VI) detoxification (%)				Efficiency of Cr(III) production (%)				Efficiency of phenol degradation (%)			
	500*	1000	1500	2000	500	1000	1500	2000	500	1000	1500	2000
CrR-2+PhR-26	78.0	92.0	95.3	97.0	20.0	8.0	5.3	3.5	92.2	78.3	58.9	38.9
CrR-14+PhR-33	78.0	90.0	94.0	96.0	20.0	10.0	6.0	4.5	98.8	76.7	57.8	41.9
CrR-15+PhR-64	80.0	91.0	94.7	97.5	18.0	9.0	5.3	5.0	91.2	91.1	53.3	47.8
CPR-4	44.0	86.0	92.0	94.5	24.0	15.0	4.7	3.0	67.6	49.3	44.1	35.5
CPR-16	48.0	82.0	90.0	94.5	4.0	4.0	3.3	2.0	80.0	61.8	47.1	35.3
CPR-17	38.0	81.0	90.7	95.0	8.0	5.0	1.3	1.5	96.0	88.9	44.5	37.8

* Concentration (μg/ml)

BIOGRAPHY

Miss Siriphon Thaweephongathikun was born in Bangkok on the 3 October 1974. She entered King Mongkut's Institute of Technology Thonburi in June 1992 and graduated a Bachelor of Science (Microbiology) in March 1996. She furthered her education at the Inter-department of Environmental Science, Graduate School of Chulalongkorn University, in 1996.