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APPENDIX

APPENDIX A

RATE OF ARSENIC AND ARSENIC COMPOUNDS IMPORTED IN THAILAND DURING 1995 TO 1999

Year	Country	Quantity/kg	Value/bath
1995	China	274200	1670723
	France	39600	904582
	Germany	13	40290
	USA	4	5360
	UK	-	238
	Total	313817	2621193
1996	China	51850	930265
	France	39600	949396
	Germany	6	20542
	Total	91456	1900203
1997	China	99000	2354048
	France	59400	1763489
	Germany	192	122136
	Total	158592	4239673

Appendix A (cont.)

Year	Country	Quantity/kg.	Value/bath
1998	China	88550	3894684
	France	19800	724440
	USA	-	27081
	Total	108350	4646205
1999	China	119150	3345426
	France	19800	844695
	Belgium	16000	739727
	Korea	3	745
	USA	6	21264
	Japan	2	18218
	Total	154961	4956051

APPENDIX B

BACTERIAL SOURCES

In this present study 50 samples were chosen as bacterial source, they were collected during May 1998 to December 1999 and were categorized into various groups as follows:

Type of samples	Site	Characteristic
Soil	S-1	Roughly sand, black
	S-2	Roughly sand, brown
	S-3	Sand, light brown
	S-4	Roughly sand, dark brown
	S-5	Clay, black
	S-6	Clay and mud, brown
	S-7	Clay, dark brown
	S-8	Clay, brown
	S-9	Clay and sand, black
	S-10	Sand, reddish brown
	S-11	Clay, dark brown
	S-12	Mud and sand, black
	S-13	Clay, black and odd odor
Sediment	Sd-1	Black mud, odd odor
	Sd-2	Black mud and humus, odd odor
	Sd-3	Clay , mud and humus
	Sd-4	Black mud , bad smell
	Sd-5	Clay and sand, brownish
	Sd-6	Black mud and humus
	Sd-7	Brown clay and black mud
	Sd-8	Mud, light brown
	Sd-9	Black mud
	Sd-10	Clay, dark brown
	Sd-11	Black mud and humus
	Sd-12	Black mud
	Sd-13	Black clay and mud
	Sd-14	Clay, brownish
	Sd-15	Black clay, oil suspension
	Sd-16	Clay, dark brown
	Sd-17	Brownish mud
	Sd-18	Clay and sand, dark brown
	Sd-19	Mud and sand, black

Type of samples	Site	Characteristic
Sediment	Sd-20	Mud and sand, black
	Sd-21	Clay and sand, black
	Sd-22	Black mud
	Sd-23	Clay and mud, dark gray
	Sd-24	Clay and mud, dark brown
	Sd-25	Mud, dark brown
	Sd-26	Sand, dark brown
	Sd-27	Mud, light brown
	Sd-28	Mud, dark brown
	Sd-29	Mud, black
	Sd-30	Mud and humus, black
	Sd-31	Clay, light brown
	Sd-32	Clay, brown
	Sd-33	Mud and sand, black
	Sd-34	Clay and sand, black
	Sd-35	Clay and sand, black
	Sd-36	Sand and small stone, black
	Sd-37	Mud, dark brown and odd odor
	Sd-38	Mud and sand, black
Waste water	Ww-1	Light brown water; mud and humus
	Ww-2	Reddish brown water and humus
	Ww-3	Black water and humus
Natural water	Nw-1	Turbidity, light brown water

APPENDIX C: MEDIA

Freshwater minimal medium

Salt

Formula in gram per liter of distilled water

KH_2PO_4	0.14
NH_4CL	0.25
KCl	0.50
$CaCl_2.2H_2O$	0.15
NaCl	1.0
$MgCl_2.6H_2O$	0.62

Trace elements

Formula in milligram per liter of distilled water

Conc. HCl	0.001 ml
$MnCl_2.4H_2O$	0.1
$CoCl_2.6H_2O$	0.12
$ZnCl_2$	0.07
H_3BO_3	0.06
$NiCl_2.6H_2O$	0.025
$CuCl_2.2H_2O$	0.015
$Na_2MoO_4.2H_2O$	0.025
$FeCl_2.4H_2O$	1.5

Vitamins

Formula in milligram per liter of distilled water

p-Aminobonzoic Acid	0.05
Biotin	0.02
Nicotinic Acid	0.05
Calcium Pantothenate	0.05
Thiamine Hydrochloride	0.05
Pyridoxine Hydrochloride	0.1
Cyanocobalamin	0.001

Sterilized by 0.45 μ millipore fitter, freezed and stored in a refrigerator in the absence of light until used.

Reducing agent : Cysteine HCl 0.05%, $NaHCO_3$ 0.085%

Carbon source: Sodium lactate 10 mM.

Preparation:

The 500 ml of salt solution of ingredients was added to a 1-liter ground joint round bottom flask[a] and heated. When the solution was boiled, color turned from blue to reddish-pink. During boiling the solution for several minutes, the pink color rather than disappeared imply, indicating reduction. The mask was suddenly stopper with a rubber stopper, covered with cloth and tied tightly, and autoclaved at 121°C for 15 minutes. After transferring to the anaerobic chamber; sodium lactate, bicarbonate cysteine HCl, trace elements and vitamins was added. Before used, any plate or vial contained certain medium was incubated over night.

[A] The round shape of the flask prevents breakage due to the sterilization pressuse.

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.

Brewer anaerobic agar

Formula: Ingredients per liter

Bacto-Tryptone	5	g
Proteose Peptone No.3, Difco	10	g
Bacto-Yeast Extract	5	g
Bacto-Dextrose	10	g
Sodium chloride	5	g
Bacto-Agar	20	g
Sodium Thioglycollate, Difco	2	g
Sodium Formaldehyde Sulfoxylate	1	g
Resazurin, Certified	0.002	g

Direction: Suspend 58 grams in 1 liter distilled or deionized water and warm slightly to dissolve completely. Sterilize at 121 - 124 °C for 15 min.

Nutrient Gelatin (Formula: Ingredients per liter)

Bacto Beef Extract	3	g
Bacto Peptone	5	g
Bacto Gelatin	120	g

PH 6.8 at 25'C

To rehydrate the medium, suspend 128 grams in 1000 ml. Cold distilled water. Warm to about 50'C to dissolve the medium completely. Sterilize in the autoclave for 15 minutes at 15 ponds pressure (121°C).

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.

Motility test medium (Formula: ingredient per liter)

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

Final pH = 7.3

MR/VP broth

Formula: ingredient per liter

Polypeptone	7	g
Glucose	5	g
Dipotassium phosphate	5	g

Final pH 6.9 ± 0.2

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.

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)

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 Sulfate	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.

APPENDIX D

	Equilibrium	Equation	pK
Arsenic (orthro)			
	$H_3AsO_4 + H_2O$	$H_3O + + H_2Aso^-_4$	3.6
	$H_2Aso^-_4 + H_2O$	$H_3O + + HAso^{2-}_4$	7.3
	$HAso^{2-}_4 + H_2O$	$H_3O + + HAso^{3-}_4$	12.5
Arsenous (meta)			
	$Haso_2 \ + H_2O$	$H_3O + + Aso^-_2$	9.2

Source : Lemmo, et. al., 1983

APPENDIX E

MOLYBDENUM BLUE METHOD

Reagents:

(i) Mixed reagent ;

- $(NH_4)_6Mo_7O_{24}.4H_2O$	3 g	in 100 ml distilled water
- $C_4H_4KO_7SbO.5H_2O$	0.068 g	in 50 ml distilled water
- Conc. H_2SO_4	38.9ml	make volume to 100 ml in distilled water
- ascorbic acid	5.4 g	in 100 ml distilled water

Mix these compound together. Prepare this reagent for use and discard any excess. Do not store for more than about 6 hr.

(ii) Reducing reagent ;

- 20 ml of 3.5 N H_2SO_4
- $Na_2S_2O_5$ 14% (w/w) in 40 ml deionized water. Prepare fres for use.
- $Na_2S_2O_3.5H_2O$ 1.4% (w/w) in 40 ml deionized water

Mix 20 ml of 3.5 N sulfuric acid solution into 40 ml of the sodium metabisulfite solution. This should be done slowly to avoid excessive bubbling caused by the liberation of SO_2. Finally, mix in 40 ml of the sodium thiosulfate solution. Again, prepare this reagent for use and discard any excess. This reagent is stable for up to 24 hr.

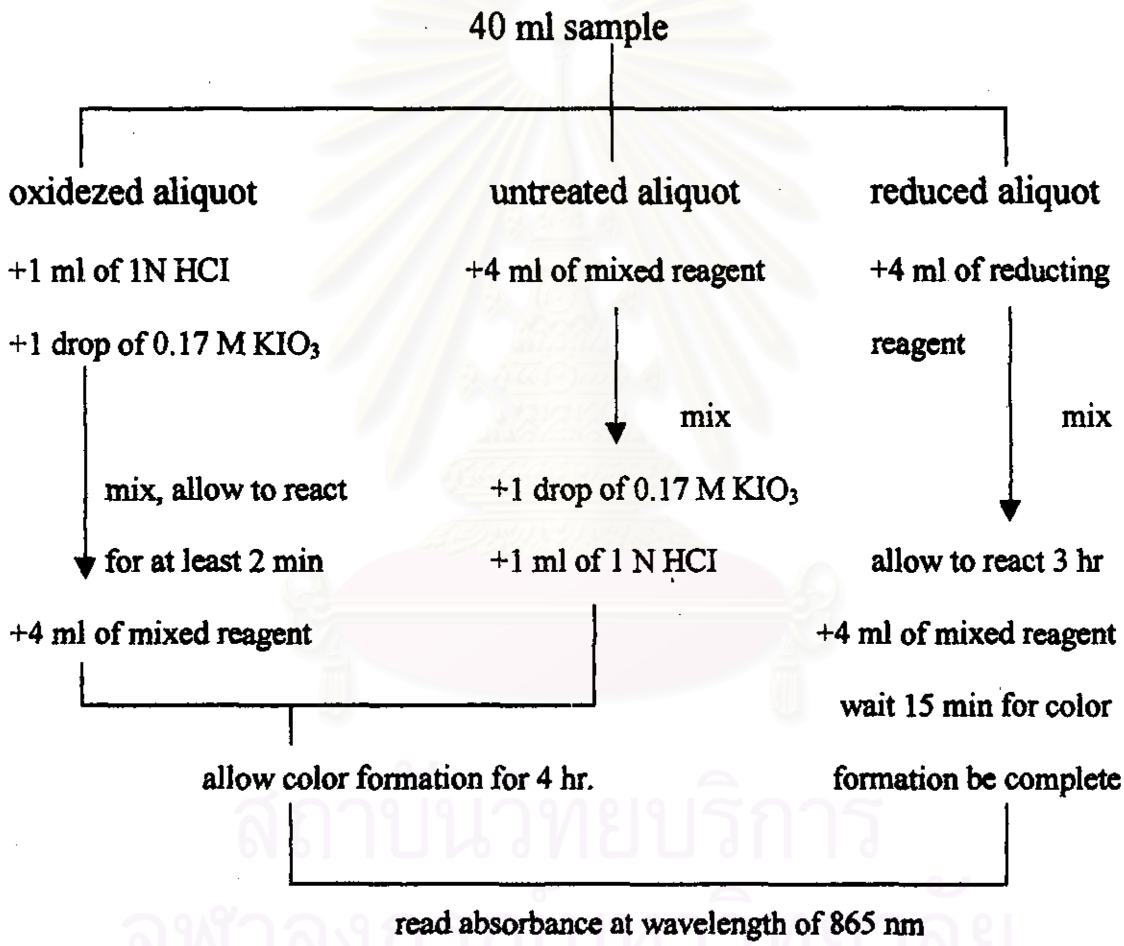
(iii) Potassium iodate solution 0.17 M.

(iv) 1 N HCI

Standard

Prepare stock solution of arsenic and phosphorus having final concentrations of 1mM from reagent grade arsenic trioxide, Sodium arsenate and potassium dihydrogen phosphate. Make appropriate dilutions of this standard solution to 0-40 μm.

Procedure



Run deionized water blank, by the above procedures, along with the samples.

Calculation

Since the final vloume of the "reduced" aliquotes is greater than that of the other aliquots, a dilution correction must be made to obtain corrected absorbances. In this procedure, the correction

$$\frac{\text{Final volume "reduced"}}{\text{Final volume "oxidized or untreated"}} = \frac{48}{45} = 1.067$$

would be applied to make the "reduced" absorbances comparable to those of the other aliquots.

After dilution correction, absorbances of the blanks are substracted to give corrected absorbance (C.A.). Absorbance factors for arsenite, arsenate and phosphate are determined from standard. Arsenite, arsenate and phosphate concentration are calculated as follows:

Arsenite = (C.A.'oxidezed' - C.A. 'untreated') x abs.factor As (III)

Arsenate = (C.A. 'untreated' - C.A. 'reduced') x abs.factor As (V)

Phosphate = C.A. 'reduced' x abs.factor PO_4^{3-}

$$\text{* absorbance factor} = \frac{\mu \text{ moles/l}}{\text{absorbance}}$$

absorbance factor of As(III) = 49.12

absorbance factor of As(V) = 45.36

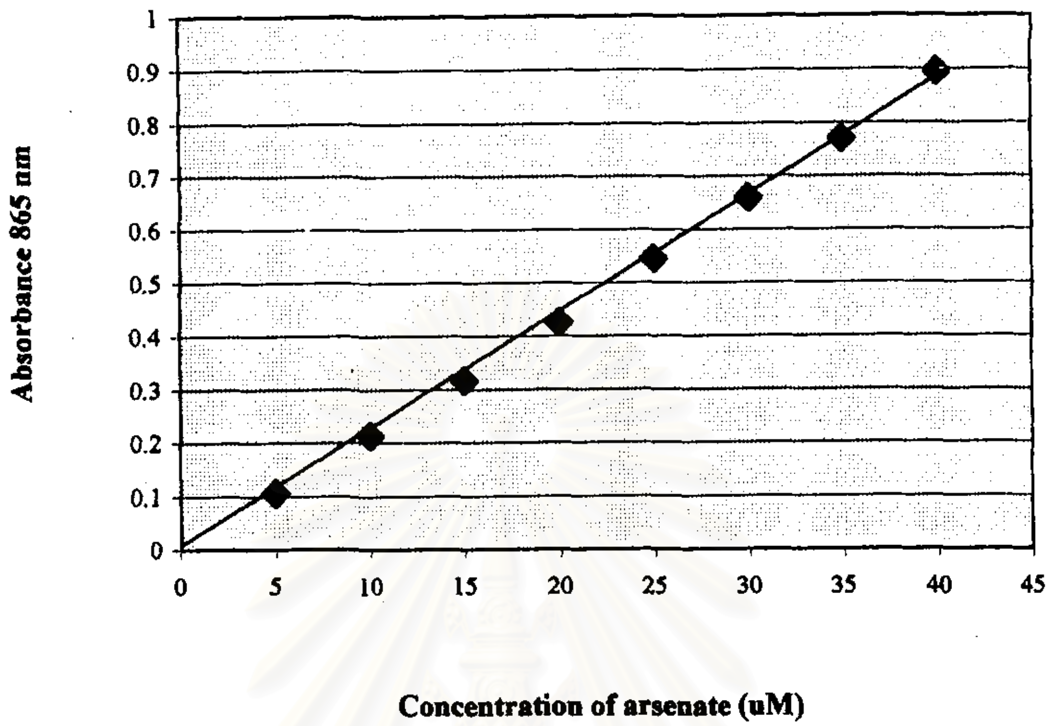
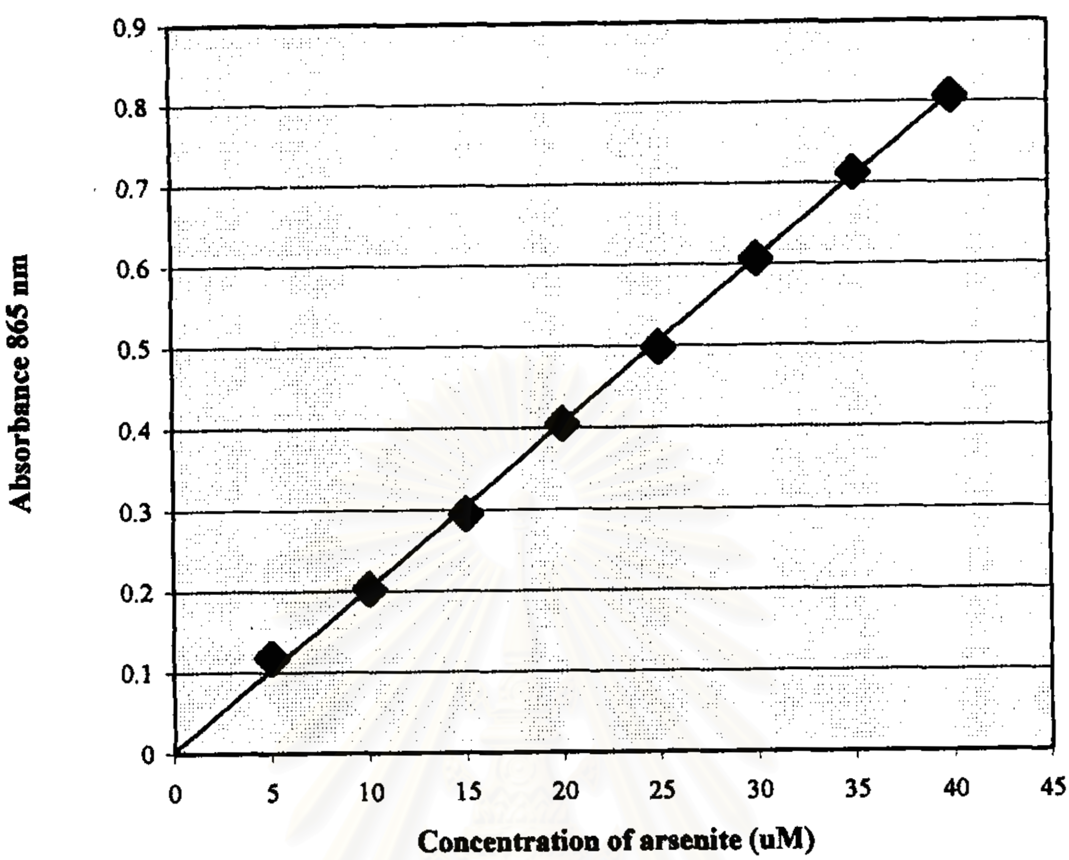


Figure E-1: Standard curve of arsenate in oxidized aliquot



0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance 865 nm
0
5
10
15
20
25
30
35
40
45
Concentration of arsenite (uM)

DETERMINATION OF HYDROGEN SULFIDE

Reagents:

- Mixed diamine reagent : 2 g N, N-dimethyl-p-phenylene diamine sulphate in 50 ml 50% (v/v) HCI + 3 g anhydrous ferric chloride in 50 ml 50% (v/v) HCI are mixed together immediately before analysis; if the solutions are not made fresh, they should be stored separately in dark bottles

Standard

Sulfide standard solution prepared in deoxygenated distilled water at range 0-1 mM.

Procedure

(i) Add 50 ml of sample to 4 ml of mixed diamine reagent, mix

(ii) Place 1 ml of sample into 50 ml deoxygenated distilled water, and wait for 20 min.

(iii) Read at 670 mm.

(iv) Run deoxygenated distilled water blank, by the above procedures, along with the samples.

Calculation

Conc. of H_2S (mM) = F x absorbance of sample

$$F; \quad \frac{\text{concentration of sulfide}}{\text{Absorbance}}$$

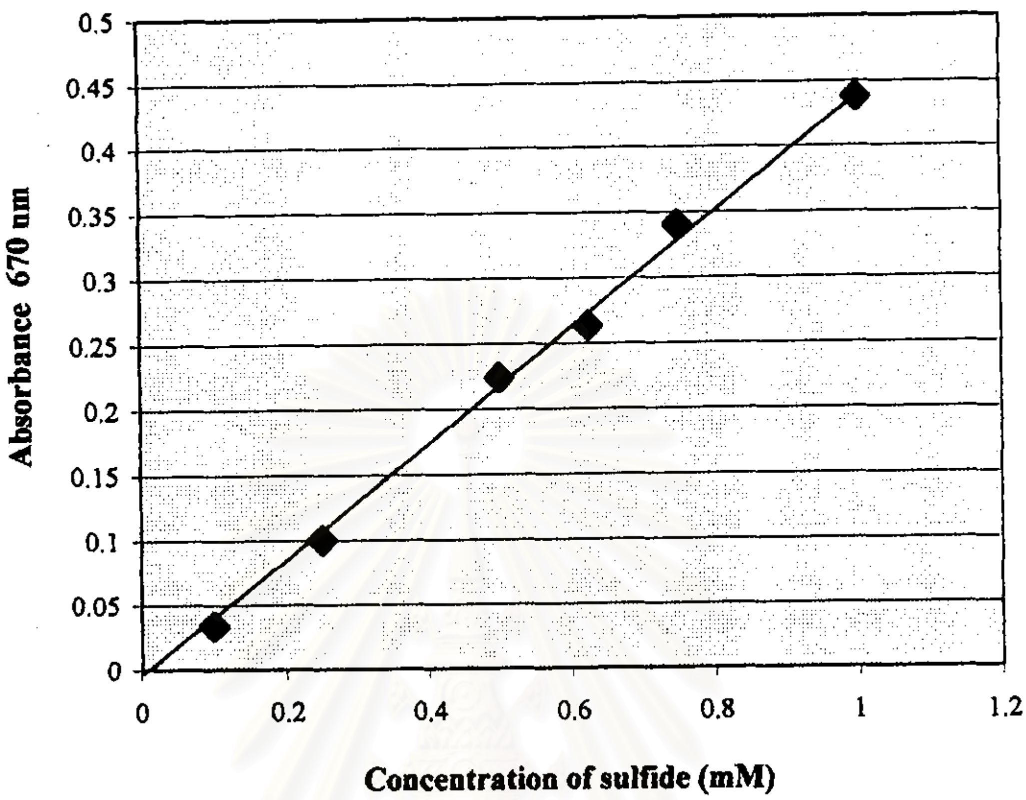


Figure E-3: Standard curve of sulfide

Appendix F

Some characteristic of the selected bacterial isolates;
AsR-17, AsR-19 and AsR-20.

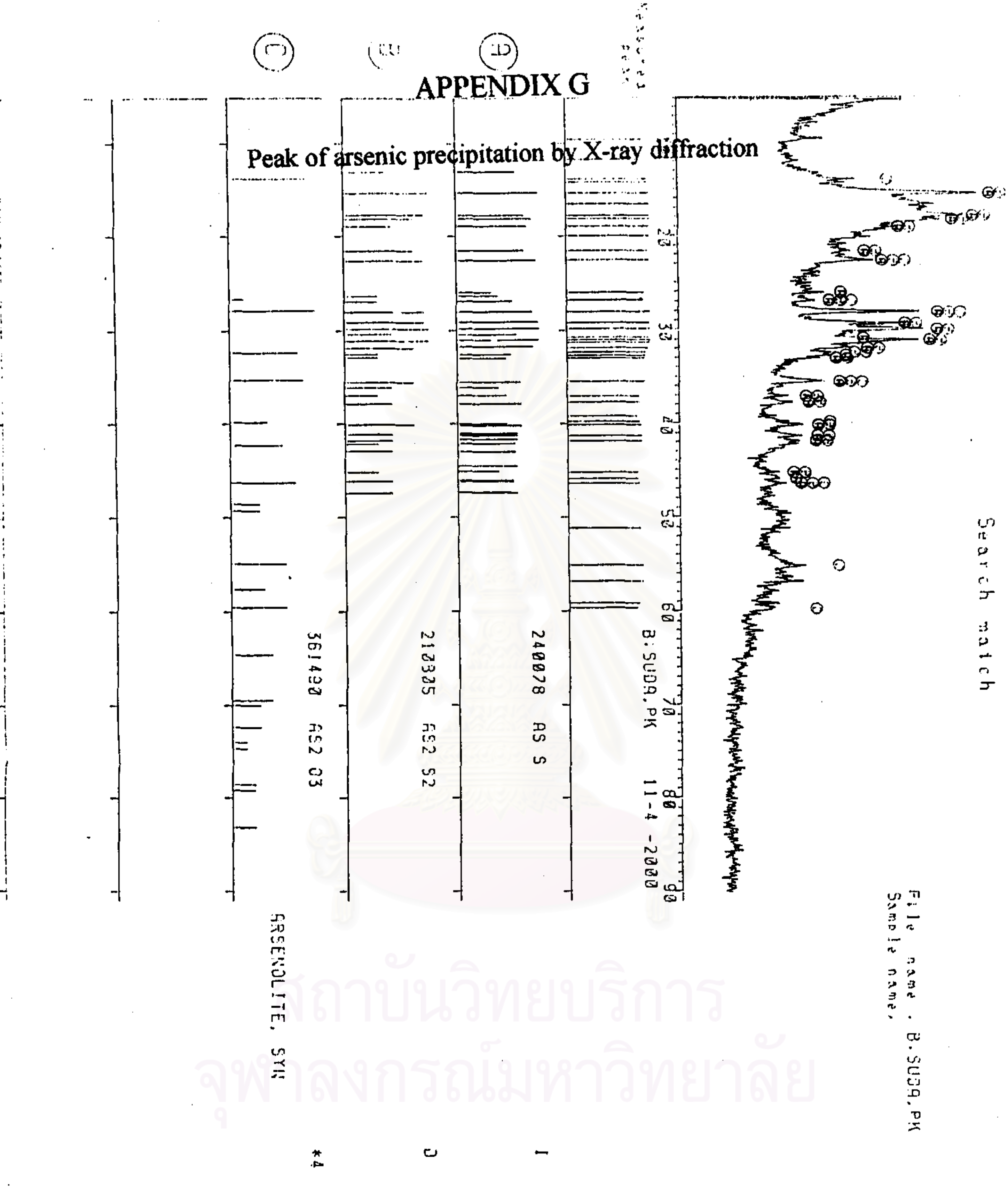
Some Characteristics	The Selected Bacterial Strains		
	AsR-17	**AsR-19**	**AsR-20**
1. Source of sample	S-2, smelting area	S-11, acrylic dye industry	
2. Type of organisms	Obligately anaerobic bacteria	Facultative anaerobic bacteria	
3. Colony	2 mm in diameter, yellowish, convex	2mm in diameter, pale to brown, clear, convex	4 mm in diameter, white , convex
4. Morphology	Rod shape, gram negative ~ 0.5 by 1.8	Rod shape, gram negative ~ 0.7 by 2.0	Rod shape, gram negative ~ 0.5 by 1.8
5. Expected genus	Unknown strain	Unknown strain	***Citrobacter***
6. Maximum resistance to arsenic: (μg/ml)			
6.1 As (V)	> 2,400	>2,400	>2,400
6.2 As (III)	> 500	< 500	<500
7. Maximum resistance to other metal ions: (μg/ml)			
7.1 Ag	ND	<100	<100
7.2 Cd	ND	<100	<100
7.3 Cr	ND	<100	<200
7.4 Cu	ND	<100	<100
7.5 Mn	ND	<800	>800
7.6 Ni	ND	<100	<100
7.7 Zn	ND	<200	<200
8. Optimum for growth			
8.1 pH	7	7	7
8.2 temperature (^oC)	35	40	35
9. Type of organism to precipitate arsenic	By itself	Consortium	

Appendix F (cont.)

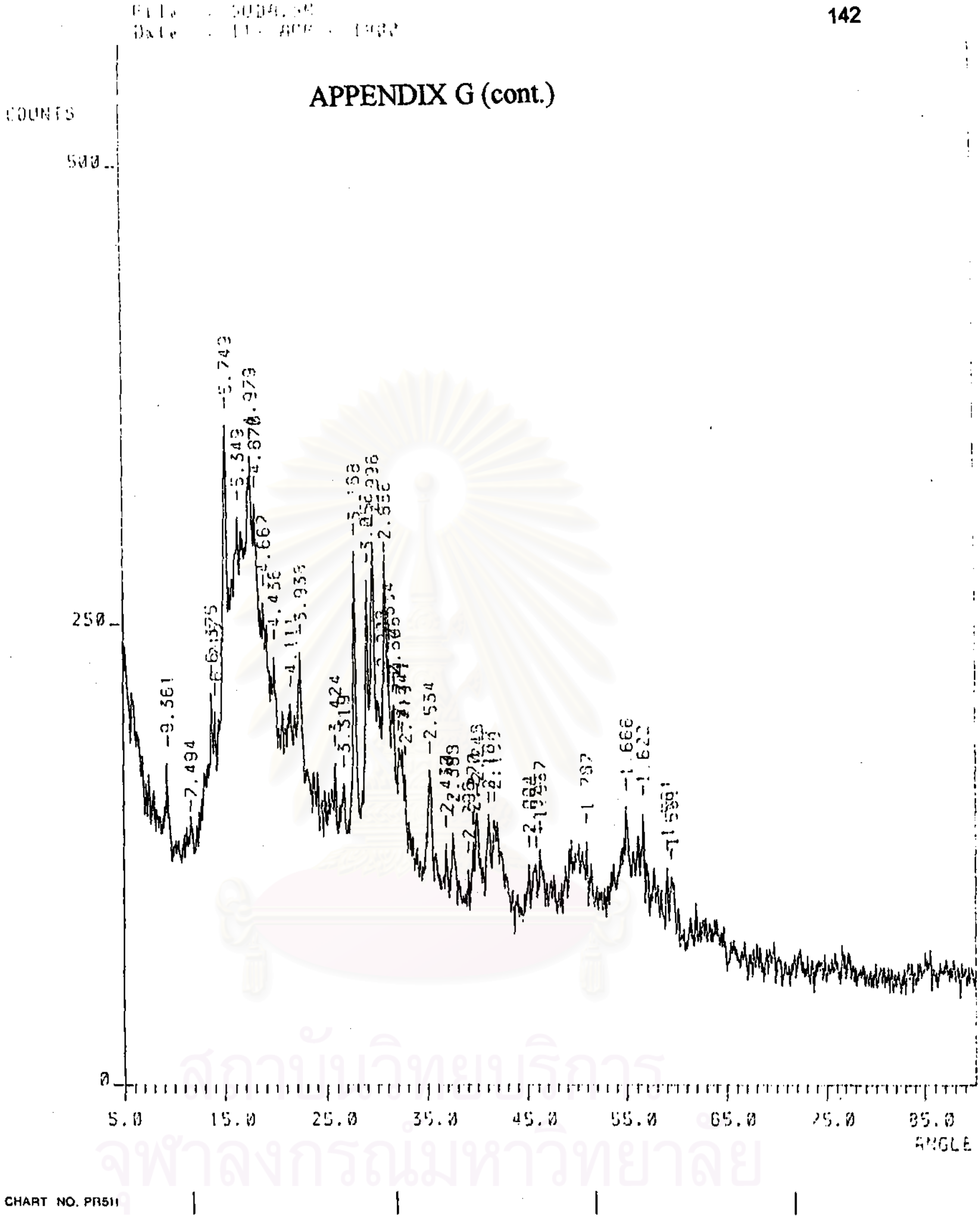
Some Characteristics	Selected Bacterial Strains		
	AsR-17	AsR-19	AsR-20
10.Optimum condition for arsenic precipitation			
10.1 arsenic, As(V) concentration; (μg/ml)	100	200	
10.2 pH	7	7	
10.3 temperature (^{o}C)	35	35	
11. Percentage of arsenic removal; (8-day of incubation)			
11.1 arsenic, As(V) concentration ; (μg/ml)			
11.1.1 100	49.21	41.60	
11.1.2 200	48.27	45.24	
11.1.3 300	-	25.86	
11.2 pH			
11.2.1 6	-	26.93	
11.2.2 7	35.02	42.21	
11.2.3 8	-	40.75	
11.3 temperature			
11.3.1 30	13.12	29.29	
11.3.2 35	45.08	46.24	
11.3.3 40	28.45	39.68	

APPENDIX G

Peak of arsenic precipitation by X-ray diffraction



APPENDIX G (cont.)



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BIOGRAPHY

Miss Suda Ittisupornrat was born in Bangkok on the 19 July 1973. She entered Kasetsart University in June 1992 and graduated a Bachelor of Science (Fisheries) in March 1995. She furthered her education at the programme of Biotechnology, Graduate School of Chulalongkorn University, in 1996.