CHAPTER 4
RESULTS

4.1 ISOLATION SCREENING AND SELECTION OF ARENIC-RESISTANT BACTERIAL ISOLATES

Two hundreds and nineteen bacterial strains resistant to 700 μg/ml of As(V) were isolated from waste waters, soils and sediments. Half of them were resistant to 1,000 μg/ml As(V), and resistance to 1,600, 1,800, 2,000 and 2,400 μg/ml As(III) found in those isolates were 3 (1.37%), 8(3.65%), 1(0.46%), 9(4.11%) and 62(8.31%), respectively (**Table 4.1**). There were 33 As-resistant bacterial strains that were capable to arsenic precipitation and varied in different As(V) concentrations as shown in **Table 4.2**. Three bacterial isolates (resistant to 2,400 μg/ml As) being capable to arsenic precipitation were chosen as the selected bacterial strains for further investigation. The result of total resistant strains and resistant & precipitate arsenic was shown in **Figure 4.1.** They were named AsR-17, AsR-19 and AsR-20. AsR-17 strain, isolated from soil of smelting area (S-2), was gram-negative, rod-shape, and obligately anaerobic bacterium, and AsR-19 and AsR-20 strains (consortium strains), isolated from sediment of acrylic dye industry (Sd-11), were gram-negative, rod-shape, and facultative anaerobic bacteria. Characteristics and biochemical tests of the selected bacterial strains were presented in **Table 4.3-4.4** and **Figure 4.2-4.10**. The last selected strain, AsR-20 was possibly identified as ***Citrobacter sp.*** and the others could not be identified by those tests.

4.2 RESISTANCE TO OTHER METAL IONS BY THE SELECTED BACTERIAL STRAINS.

In aerobic condition, the selected strains, AsR-19 and AsR-20 were found to be sensitive to a number of other heavy metals, i.e., Cd, Cr, Cu, Ni and Ag, (less than 100 μg/ml) but resistance to Zn and Mn, detailed result is summarized in **Table 4.5**.

4.3 EFFECT OF SOME ENVIRONMENTAL FACTORS GROWTH OF THE SELECTED BACTERIAL STRAINS.

The optimum pH of those selected bacterial isolates were found to be 7 and optimum temperature for AsR-17 and AsR-20 strains were shown to be 35°C but AsR-19 strain was found to be 40 °C, see **Figure 4.11-4.13.**

4.4 EFFECT OF SOME FACTORS ON PRECIPITATION CAPABILITY OF THE SELECTED BACTERIAL STRAINS.

4.4.1 EFFECT OF ARSENIC CONCENTRATION

After 8-day incubation AsR-17 that grew in medium containing 100, 200 and 300 μg/ml As. The percentages of soluble arsenic loss were 49.21, 34.74 and 0, respectively and consortium AsR-19/AsR-20 grew in medium containing 100, 200 and 300 μg/ml As. The percentages of soluble arsenic loss were 41.60, 45.24 and 29.98, respectively. It mean that the optimum As concentration for sulfide precipitation by AsR-17 and consortium, AsR-19/AsR-20 were 100 and 200 μg/ml, respectively, as shown in **Table 4.6** and **Figure 4.14-4.15**. The transformation of arsenic of each strain was presented in **Figure 4.16-4.17**.

4.4.2 EFFECT OF pH

The effect of pH on precipitate of arsenic in AsR-17 strain showed that the precipitation occurred only under pH 7 condition (35.02%) while at the pH 6 and 8, total of arsenic concentration was still remained and not occurring arsenic precipitation after 8-day incubation.

Similar effect of result of pH on arsenic precipitation AsR-19/AsR-20 coculture, was found at the level of pH 6, 7 and 8, they were able to precipitate arsenic, i. e., 26.93, 42.21 and 40.75 %, respectively, after 8-day incubation.

The effect of pH of each strain was shown in **Table 4.7-4.8** and **Figure 4.18-4.19**. The transformation of arsenic and sulfide of selected bacterial strains was presented in **Figure 4.20-4.21.**

4.4.3 EFFECT OF TEMPERATURE

After 8-day incubation, the percentages of arsenic precipitation found in AsR-17 were 13.12, 45.08 and 28.45 at 30, 35, and 40 °C, respectively. For AsR-19/AsR-20 coculture, the percentages of arsenic precipitation were 29.78, 46.24 and 39.68 at 30, 35 and 40'C, respectively after 8-day incubation (**Table 4.9-4.10** and **Figure 4.22-4.23**).
The transformation of arsenic of these strains was shown in **Figure 4.24- 4.25**.

Table 4.1 Arsenic resistance in 219 bacterial strains

As(V) conc.(μg/ml)	No. of strains	%
700	72	32.88
800	25	11.42
1000	13	5.94
1200	10	4.57
1400	16	7.3
1600	3	1.37
1800	8	3.65
2000	1	0.46
2200	9	4.11
> 2400	62	28.31
Total	219	100

Table 4.2 Arsenic resistance in 33 bacterial strains capable precipitate arsenic

As conc.(μg/ml)	No. of strains	%
700	9	27.27
800	4	12.12
1000	4	12.12
1200	7	21.21
1400	2	6.06
1600	-	-
1800	2	6.06
2000	-	-
2200	2	6.06
> 2400	3	9.09
Total	33	100

Table 4.3 Some characteristics of colony and morphology of selected bacterial isolates

Bacterial Isolates	Sources (Sampling Site)	Characteristic of		Type (Expected genus)
		Colony	Morphology	
AsR-17	**S-2** Smelting area	2 mm in diameter, yellowish and convex	Rod-shape, Gram-negative ~ 0.5 by 1.8	Obligate anaerobic bacterium (Unknown strain)
AsR-19	**Sd-11** Acrylic Dye industry	2 mm in diameter, pale to brow brown, clear and convex	Rod-shape Gram-negative ~ 0.7 by 2.0	Facultative anaerobic bacterium (Unknown strain)
AsR-20	**Sd-11** Acrylic Dye industry	4 mm in diameter, white and convex	Rod-shape Gram negative ~ 0.5 by 1.8	Facultative anaerobic bacterium *(Citrobacter)*

Table 4.4 Some selective media and biochemical tests for identification of characteristic of selected bacterial isolates.

Biochemical Test	Selected Bacterial Isolates		
	AsR-17	AsR-19	AsR-20
TSI	ND	K/N	A/A
H_2S production	+	+	-
Gas production	ND	-	+
Citrate	ND	-	+
Catalase	-	+	+
Indole	ND	-	-
NO^-_3	ND	+	+
Oxidase	-	-	-
Motility	ND	+	+
Urease	ND	-	+
MacConKey Agar	ND	growth, pale moist	growth, pink moist
SS Agar	ND	growth	growth
EMB agar	ND	-	growth, pink moist
Gelatinase	ND	+	+
MR	ND	+	+
VP	ND	-	-

Table 4.4 (cont.)

Biochemical Test	Selected Bacterial Isolates		
	As R-17	As R-19	As R-20
OF test :			
Glucose	ND	-	A/A
Dextrose	ND	-	A/A
Lactose	ND	-	A/A
Mannose	ND	-	A/A
Sucrose	ND	-	A/A

A = acid - = negative

\+ = positive ND = not determined

Table 4.5 Resistance of other metal ions by selected bacterial strains.

Bacterial Isolates	Concentration of metal ions (μg/ml)								
	As (V)	As (III)	Cd (II)	Cu (II)	Cr (VI)	Ni (II)	Mn (II)	Ag (I)	Zn (II)
AsR-17	>2400	>500	ND	ND	ND	ND	ND	ND	ND
AsR-19	>2400	<500	<100	<100	<100	<100	<800	<100	<200
AsR-20	>2400	<500	<100	<100	<200	<100	>800	<100	<200
E.coli	ND	ND	<100	<400	<400	<100	>800	<100	<200
S.macescens	ND	ND	<100	<100	<100	<100	>800	<100	<200

ND = not determined

Table 4.6 Effect of arsenic concentration on capability to precipitate arsenic in As R-17 and AsR-19/AsR-20,coculture strains.

(d)[1]	Initial conc.[2] of As(V) (μg/ml)	Test of Organism	As concentration (μg/ml) (left)		% of removal As
			Arsenate As(V)	Arsenite As(III)	
4	100	As R-17	49.42 ± 1.39	28.26 ± 0.26	~ 26.64
		As R-19 & As R-20	61.71 ± 0.58	19.65 ± 0.30	~ 21.67
		Control	74.02 ± 2.36	23.38 ± 2.46	-
	200	As R-17	125.51 ± 5.29	88.06 ± 3.61	-
		As R-19 & As R-20	96.76 ± 1.26	62.57 ± 0.30	~ 24.17
		Control	176.34 ± 3.51	25.62 ± 2.99	-
	300	As R-17	161.17 ± 3.31	169.63 ± 3.05	-
		As R-19 & As R-20	153.34 ± 1.55	69.47 ± 0.81	~ 35.76
		Control	244.72 ± 2.23	65.59 ± 2.38	-
8	100	As R-17	28.81 ± 0.52	38.69 ± 0.66	~ 49.21
		As R-19 & As R-20	34.47 ± 1.05	38.94 ± 0.09	~ 41.60
		Control	77.66 ± 1.05	28.06 ± 0.89	-
	200	As R-17	73.34 ± 0.35	68.42 ± 1.66	~ 34.74
		As R-19 & As R-20	83.39 ± 1.95	38.81 ± 0.68	~ 45.24
		Control	186.36 ± 0.19	20.15 ± 2.73	-
	300	As R-17	146.10 ± 2.11	155.70 ± 2.14	-
		As R-19 & As R-20	64.36 ± 1.09	149.63 ± 3.97	~ 29.98
		Control	248.98 ± 11.93	39.66 ± 11.81	-

(d)[1] ; Day of incubation

conc.[2] ; Concentration

Table 4.7 Effect of pH on capability to precipitate arsenic in AsR-17

(d)[1]	pH	As concentration , μg/ml (left)			% of removal Arsenic
		Arsenate As (V)	Arsenite As (III)	Sulfide S(II)	
0	7	73.50 ± 1.01	16.63 ± 1.39	-	-
4	6	22.10 ± 1.54	82.02 ± 1.43	3.81 ± 0.19	-
	7	13.15 ± 0.84	82.12 ± 0.92	4.00 ± 0.32	~ 2.88
	8	22.67 ± 0.15	83.23 ± 0.97	5.06 ± 0.29	-
	6[C]	ND	ND	ND	ND
	7[C]	74.02 ± 2.36	23.38 ± 2.46	0.19 ± 0.06	-
	8[C]	73.33 ± 1.47	32.46 ± 0.59	0.45 ± 0.03	-
8	6	14.01 ± 2.01	91.74 ± 1.16	2.53 ± 0.16	-
	7	1.12 ± 0.68	68.49 ± 1.19	3.07 ± 0.19	~ 35.02
	8	16.70 ± 0.76	90.50 ± 1.35	4.10 ± 0.19	-
	6[C]	ND	ND	ND	ND
	7[C]	73.76 ± 0.55	21.56 ± 0.92	0.19 ± 0.03	-
	8[C]	79.25 ± 0.41	17.23 ± 1.60	0.35 ± 0.03	-

(d)[1] ; Day of incubation

Table 4.8 Effect of pH on capability to precipitate arsenic in AsR-19/AsR-20 coculture

(d)[1]	pH	As concentration, µg/ml (left)			% of removal Arsenic
		Arsenate As (V)	Arsenite As (III)	Sulfide S(II)	
0	7	155.56 ± 0.06	49.92 ± 3.25	-	-
4	6	33.12 ± 2.57	140.05 ± 1.86	4.29 ± 0.54	~ 13.42
	7	32.02 ± 1.93	168.77 ± 1.57	3.65 ± 0.35	-
	8	30.50 ± 3.37	123.27 ± 2.34	2.43 ± 0.03	~ 23.12
	6[C]	ND	ND	ND	ND
	7[C]	180.82 ± 3.60	26.27 ± 3.07	ND	-
	8[C]	ND	ND	ND	-
8	6	104.72 ± 2.61	47.56 ± 1.12	3.01 ± 0.29	~ 26.93
	7	76.92 ± 9.47	47.76 ± 8.99	3.26 ± 0.38	~ 42.21
	8	78.77 ± 0.54	42.82 ± 1.22	3.52 ± 0.38	~ 40.75
	6[C]	133.95 ± 3.76	54.41 ± 3.02	-	-
	7[C]	181.75 ± 0.18	19.65 ± 2.66	ND	-
	8[C]	133.46 ± 1.15	42.52 ± 9.18	-	-

(d)[1] ; Day of incubation

Table 4.9 Effect of temperature on capability to precipitate arsenic in AsR-17

(d)[1]	Temp. (^{0}C)	As concentration, µg/ml (left)			% of removal
		Arsenate,As(V)	Arsenite, As(III)	Sulfide, S(II)	Arsenic
0	35	73.50 ± 1.01	16.63 ± 1.39	-	-
4	30	16.55 ± 0.12	79.80 ± 0.52	4.51 ± 0.45	-
	35	13.15 ± 0.84	82.12 ± 0.92	4.00 ± 0.32	-
	40	21.98 ± 2.15	87.28 ± 2.25	3.14 ± 0.22	-
	30[C]	ND	ND	ND	ND
	35[C]	74.02 ± 2.36	23.38 ± 2.46	0.19 ± 0.06	-
	40[C]	ND	ND	ND	ND
8	30	17.80 ± 0.80	69.08 ± 17.80	3.65 ± 0.19	~ 13.12
	35	27.38 ± 1.49	43.33 ± 1.66	3.33 ± 0.10	~ 45.08
	40	23.69 ± 0.96	47.86 ± 0.46	3.33 ± 0.26	~ 28.45
	30[C]	ND	ND	ND	-
	35[C]	77.66 ± 1.05	28.06 ± 0.89	-	-
	40[C]	ND	ND	ND	ND

(d)[1] ; Day of incubation

Temp. ; Temperature

Table 4.10 Effect of temperature on capability to precipitate arsenic in AsR-19/AsR-20 coculture

(d)[1]	Temp. (°C)	As concentration, µg/ml (left)			% of removal Arsenic
		Arsenate As(V)	Arsenite As(III)	Sulfide S(II)	
0	35	155.56 ± 0.06	49.92 ± 3.25	-	-
4	30	40.45 ± 0.09	136.28 ± 0.44	4.51 ± 0.45	~ 11.64
	35	38.44 ± 1.52	138.04 ± 1.21	4.00 ± 0.32	~ 16.93
	40	36.83 ± 2.79	121.29 ± 2.36	3.14 ± 0.22	~ 20.94
	30[C]	ND	ND	ND	ND
	35[C]	180.82 ± 3.60	26.27 ± 3.07	ND	-
	40[C]	ND	ND	ND	ND
8	30	93.26 ± 1.65	48.16 ± 1.52	3.39 ± 0.45	~ 29.29
	35	74.33 ± 0.30	43.02 ± 3.44	2.88 ± 0.32	~ 46.24
	40	63.80 ± 2.23	56.83 ± 1.51	2.69 ± 0.13	~ 39.68
	30[C]	ND	ND	-	ND
	35[C]	181.75 ± 0.18	19.65 ± 2.66	ND	-
	40[C]	ND	ND	ND	ND

(d)[1] ; Day of incubation

temp. ; Temperature

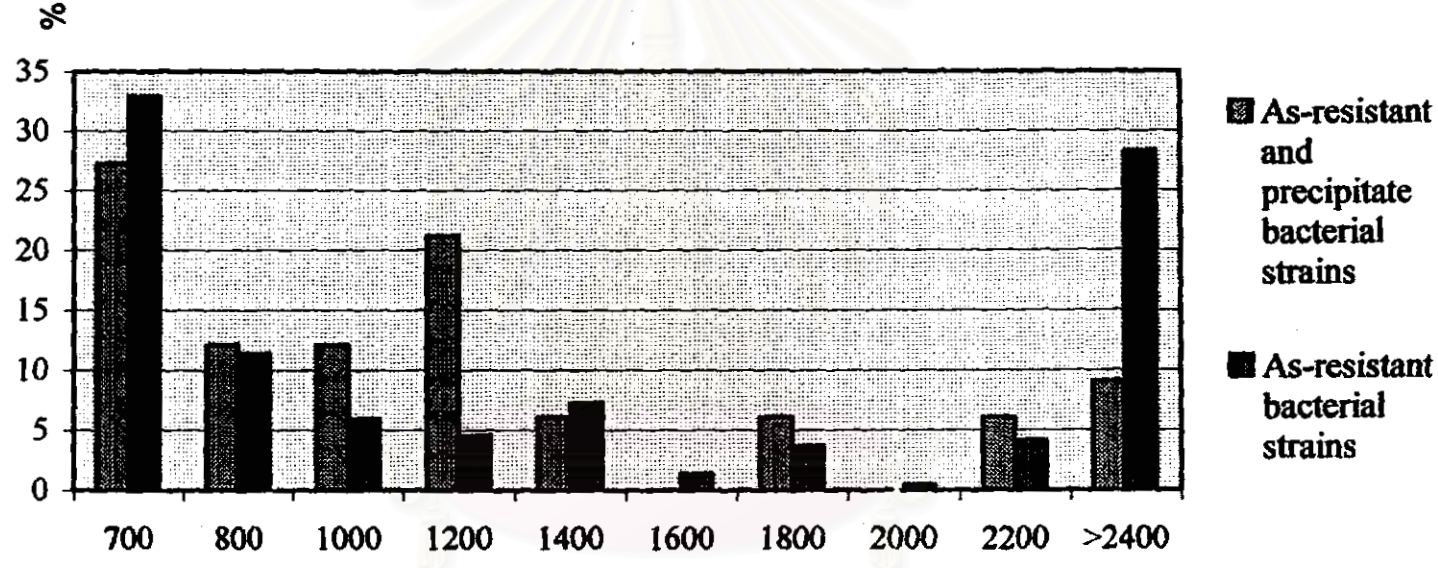
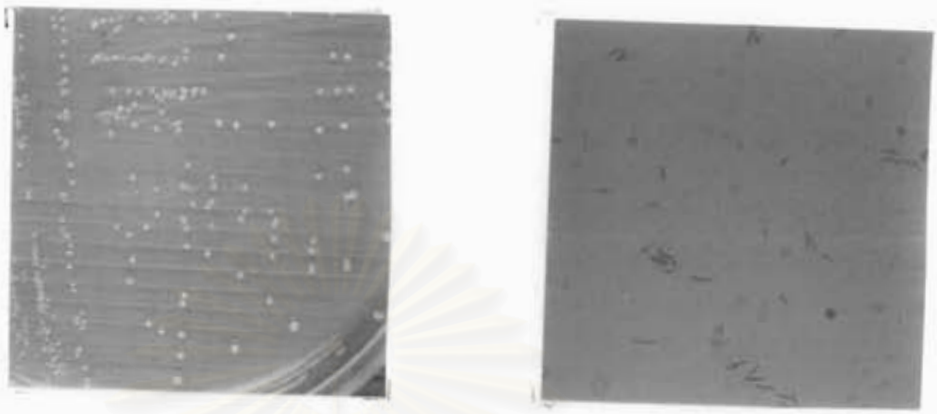
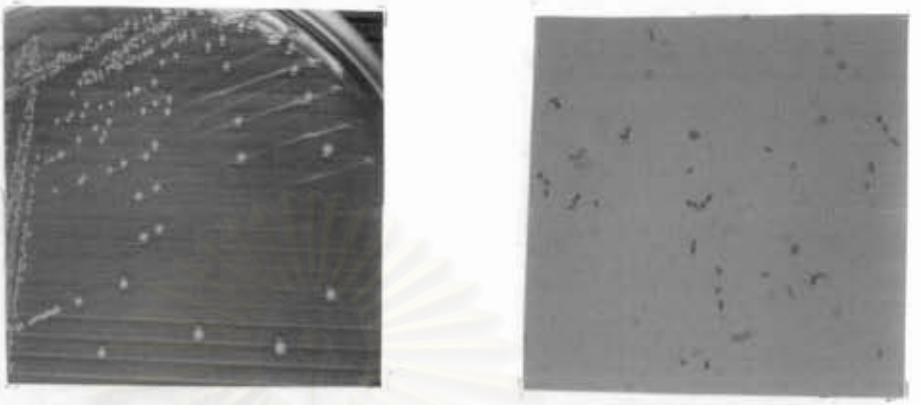


Figure 4.1 : Percentage of bacterial isolates, resistant to and precipitated arsenic and resistant to arsenic only, were compared at different arsenic



(a) (b)

Figure 4.2 Colonial characteristic (a), gram staining (b) on freshwater minimal medium and high resolution scanning electron micorgraph of AsR-17 bacteria strain (x 10,000)



(a) (b)

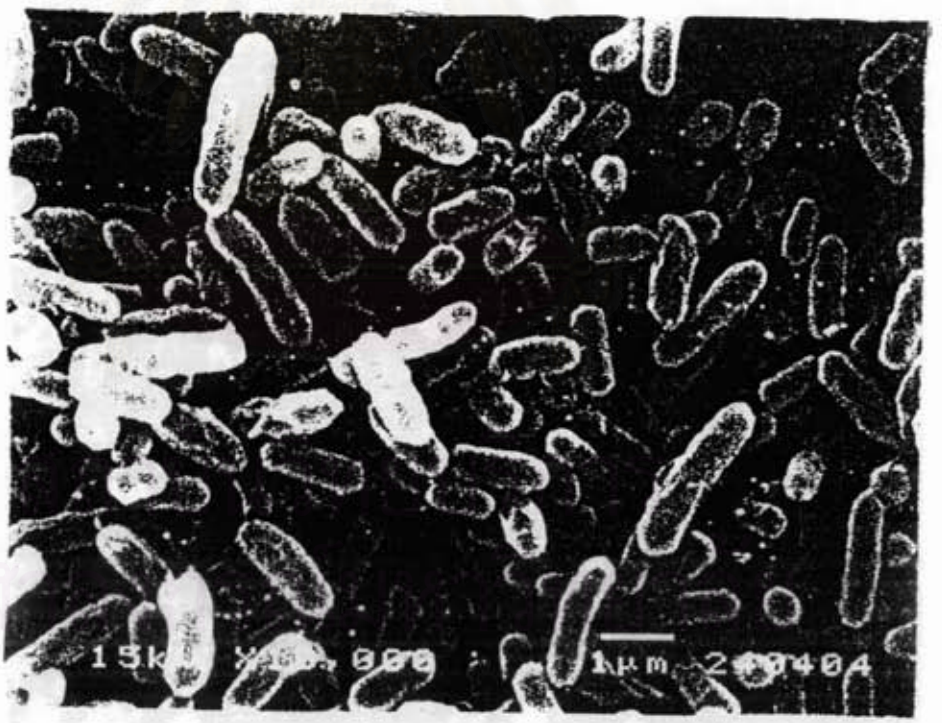


Figure 4.3 Colonial characteristic (a), gram staining (b) on freshwater minimal medium and high resolution scanning electron micorgraph of AsR-19 bacteria strain (x 10,000).

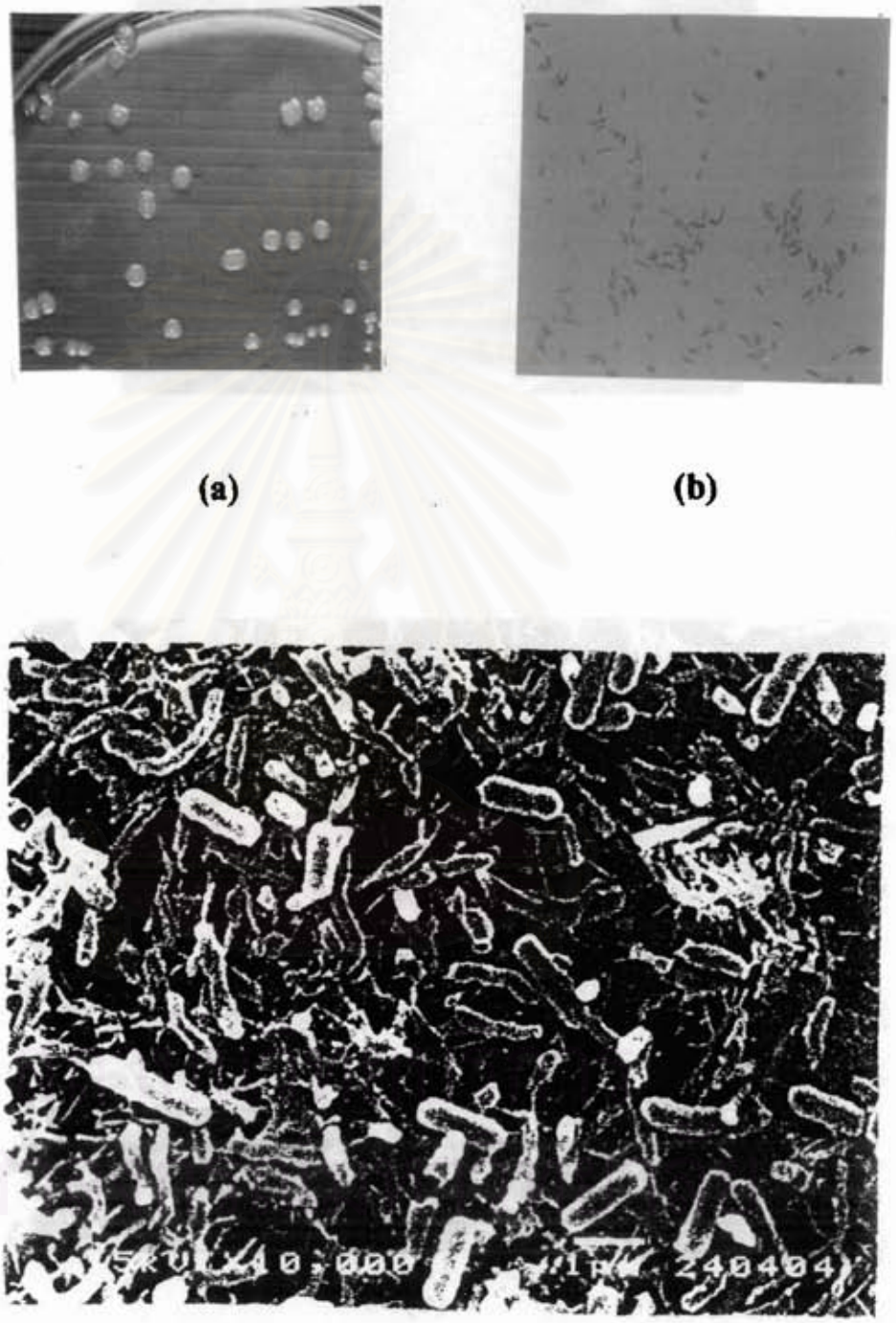
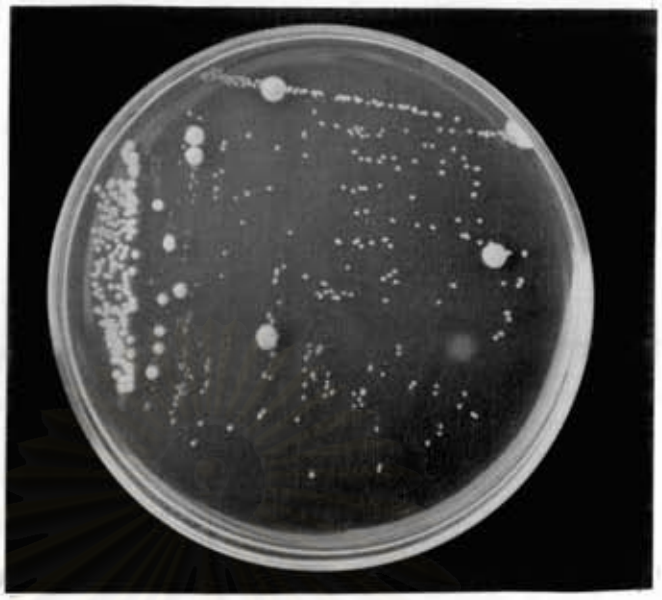
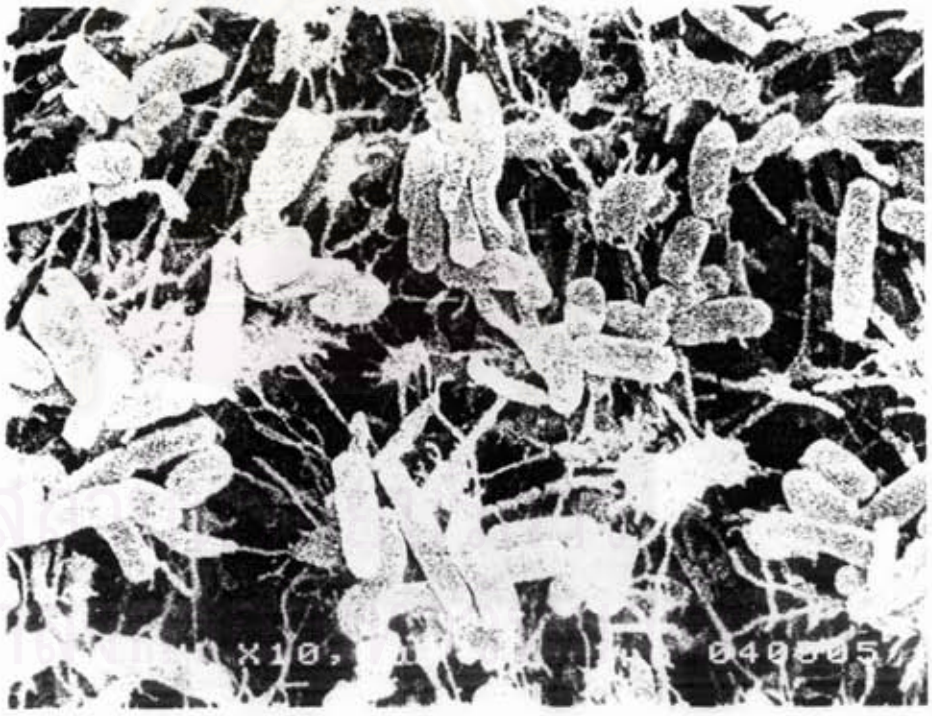


Figure 4.4 Colonial characteristic (a), gram staining (b) on freshwater minimal medium and high resolution scanning electron micorgraph of AsR-20 bacteria strain (x 10,000).



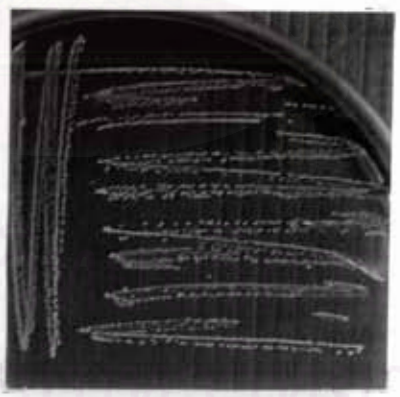
(a)



(b)

Figure 4.5 Colonial characteristic of AsR-19/AsR-20 coculture on freshwater minimal medium (a) and High resolution of AsR-19/AsR-20 bacterial strains (x 10,000) (b).

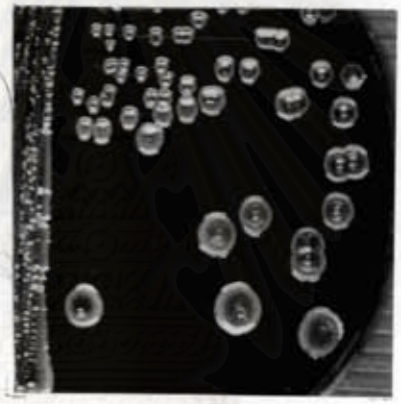
(a)



(b)

Figure 4.6 Colonial characteristic on MacConky agar (a) and Shigella-Salmonella agar (b) of AsR-19 bacteria strain.

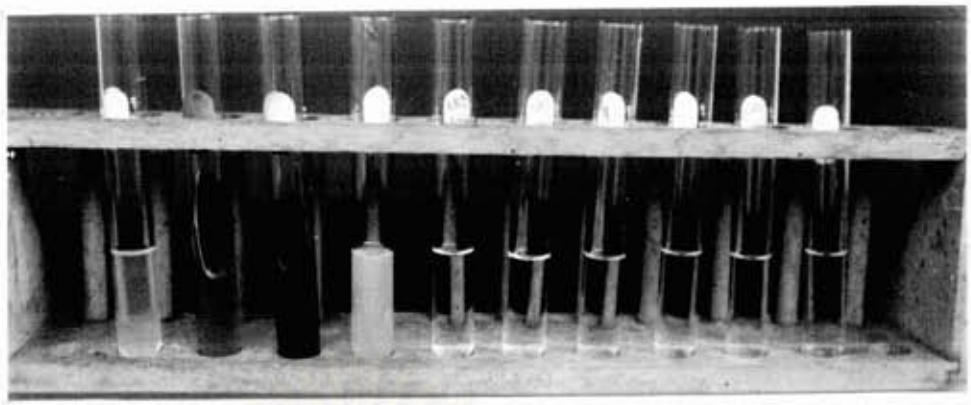
(a)



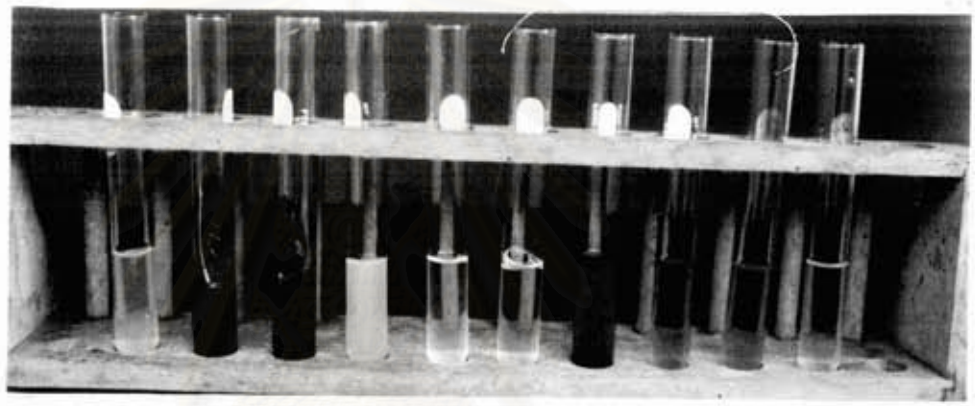
(b)

(c)

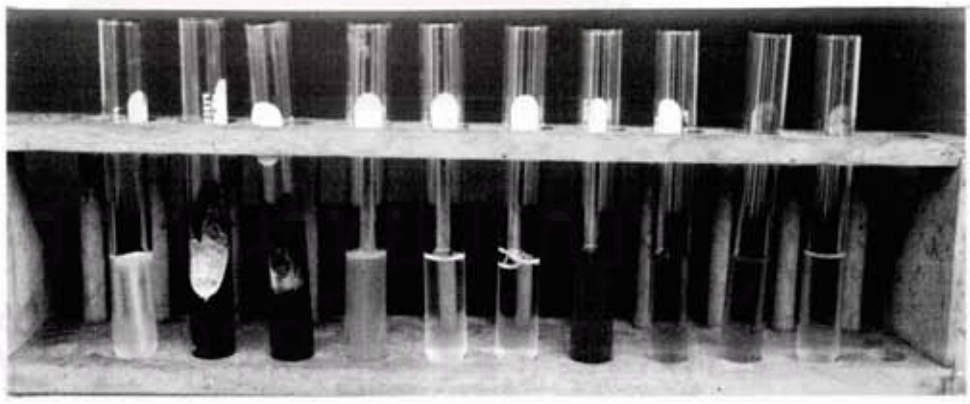
Figure 4.7 Colonial characteristic on MacConky agar (a), EMB agar (b) and Shigella-Salmonella agar (c) of AsR-20 bacteria strain.



(a)

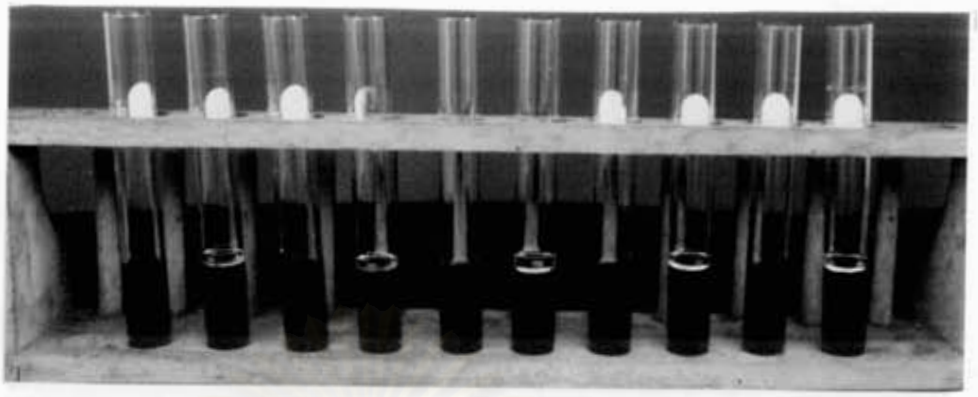


(b)

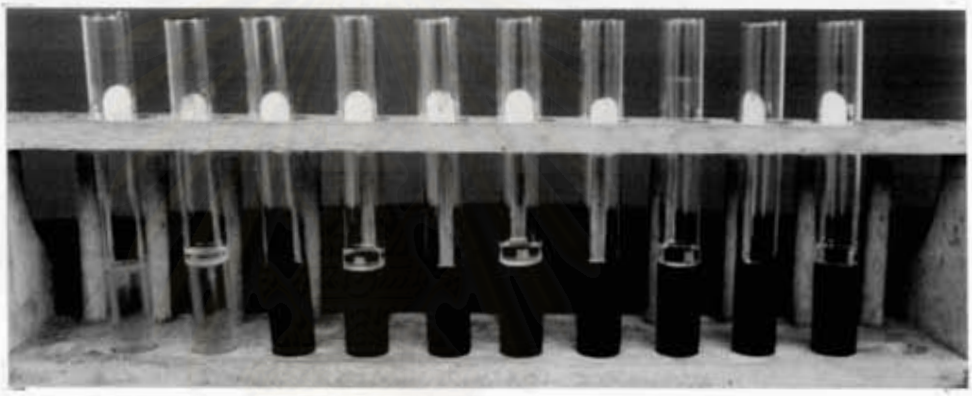


(c)

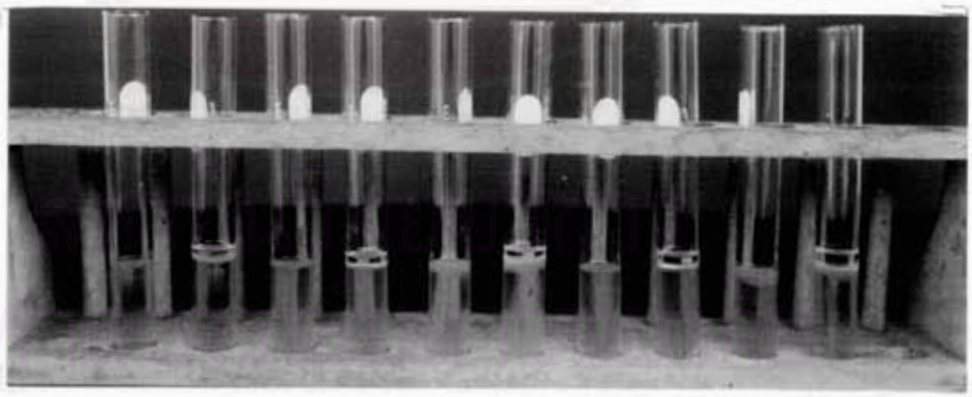
Figure 4.8 Characteristic of biochemical test (left to light; motility, TSI, citrate utilization, urease, KCN, no KCN, nitrate reduction, indole, MR and VP test) of control (a), AsR-19 (b) and AsR-20 (c) bacteria strain.



(a)



(b)



(c)

Figure 4.9 Characteristic of Oxidation-Fermentation test (left to night; glucose, dextrose, lactose, maltose and sucrose) of control (a), AsR-19 (b) and AsR-20 (c) bacteria strain.

Figure 4.10 Characteristic of arsenic precipitation : control (left), AsR-17 (middle) and AsR-19/AsR-20 (right) in freshwater minimal medium at concentration of arsenic 100 μg/ml, incubated at 35°C for 8 day

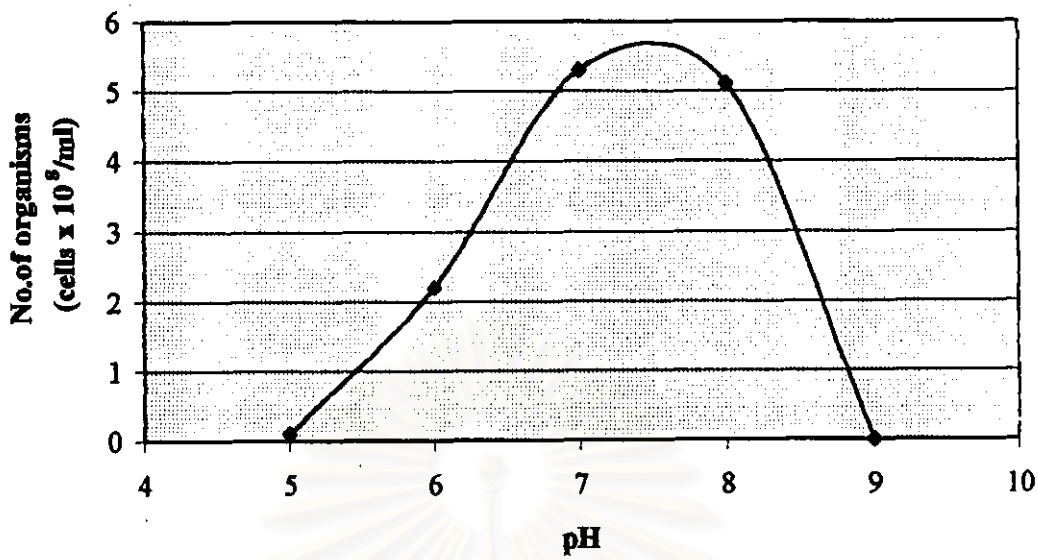


Figure 4.11a : Effect of pH on growth of the AsR-17 strain.

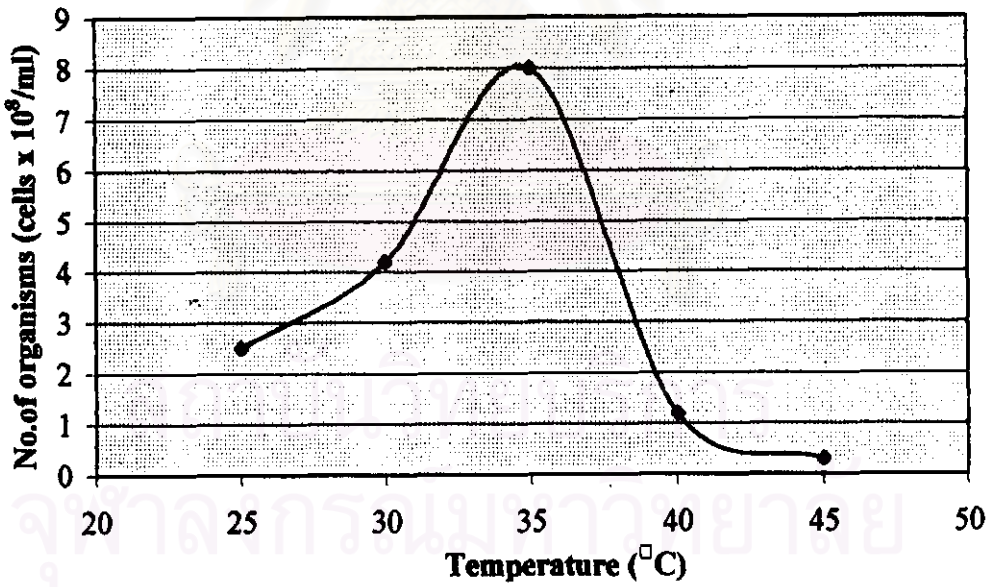


Figure 4.11b : Effect of temperature on growth of the AsR-17 strain.

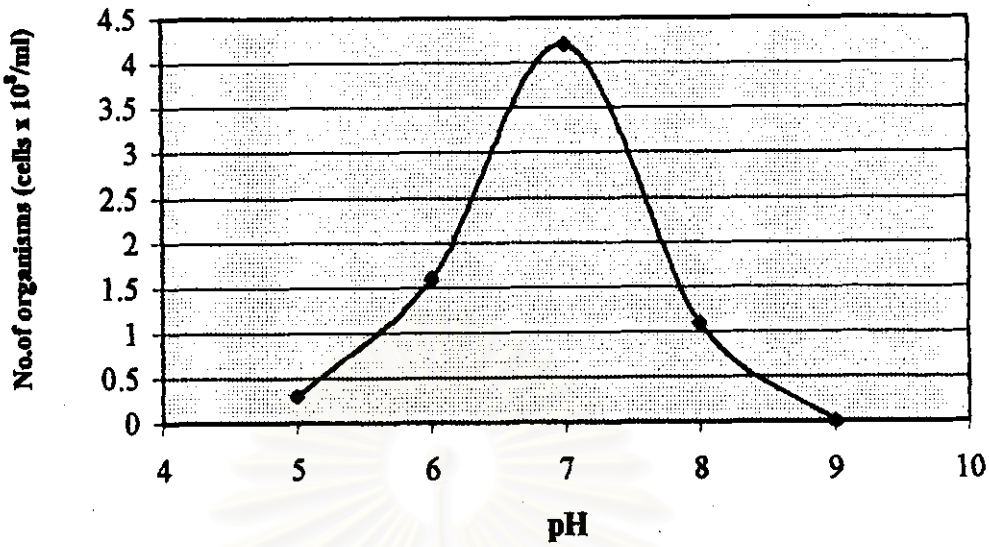


Figure 4.12a : Effect of pH on growth of the AsR-19 strain.

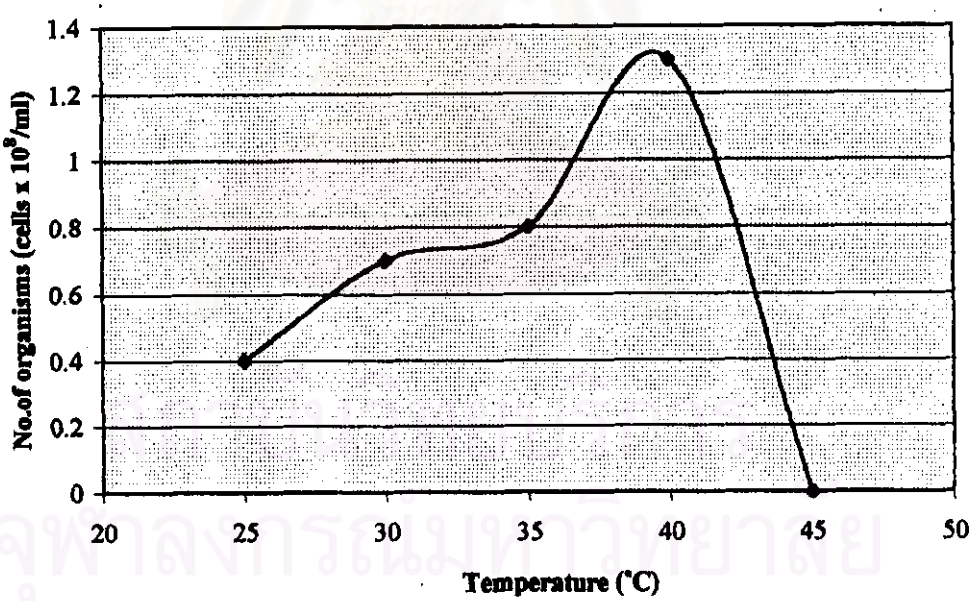


Figure 4.12b : Effect of temperature on growth of the AsR-19 strain.

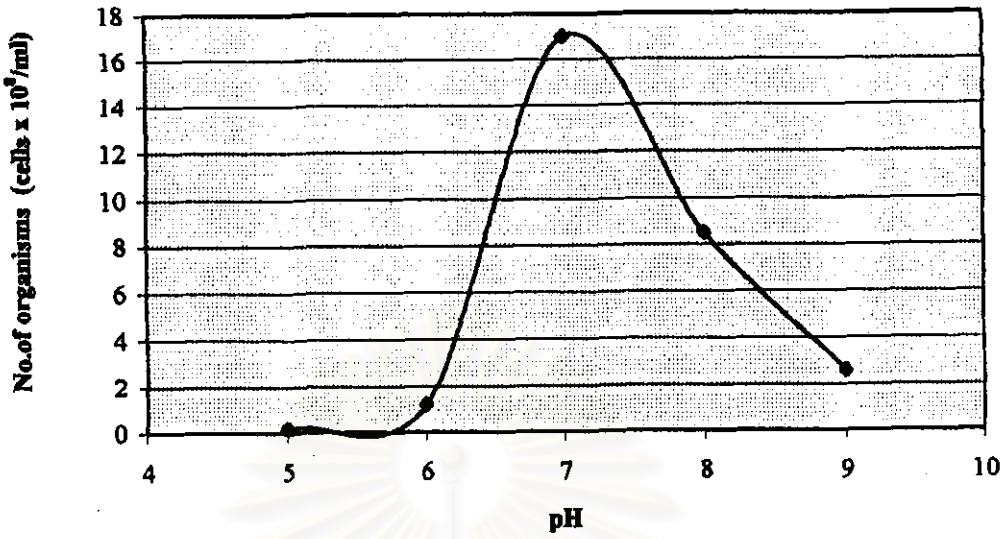


Figure 4.13a : Effect of pH on growth of the AsR-20 strain.

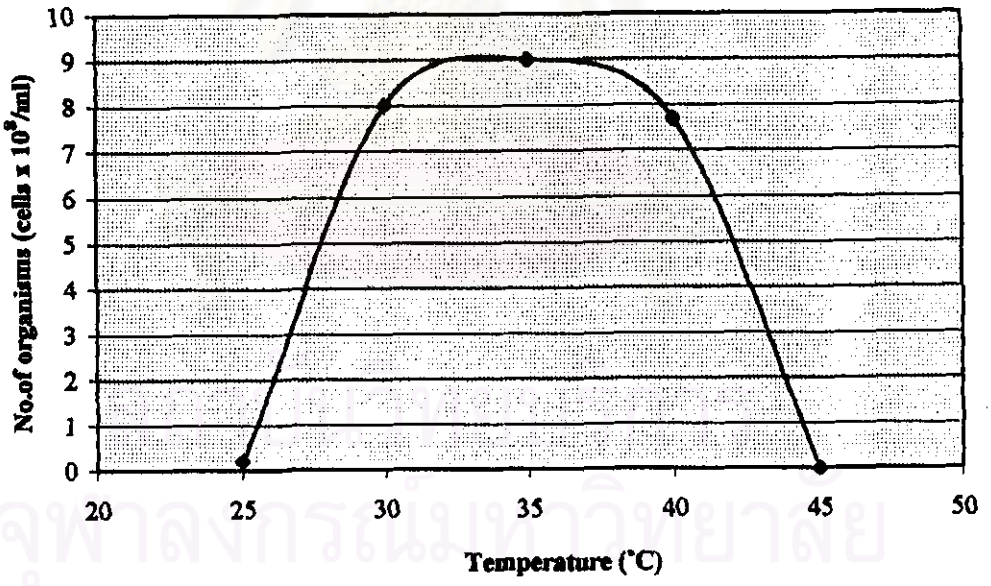


Figure 4.13b : Effect of temperature on growth of the AsR-20 strain.

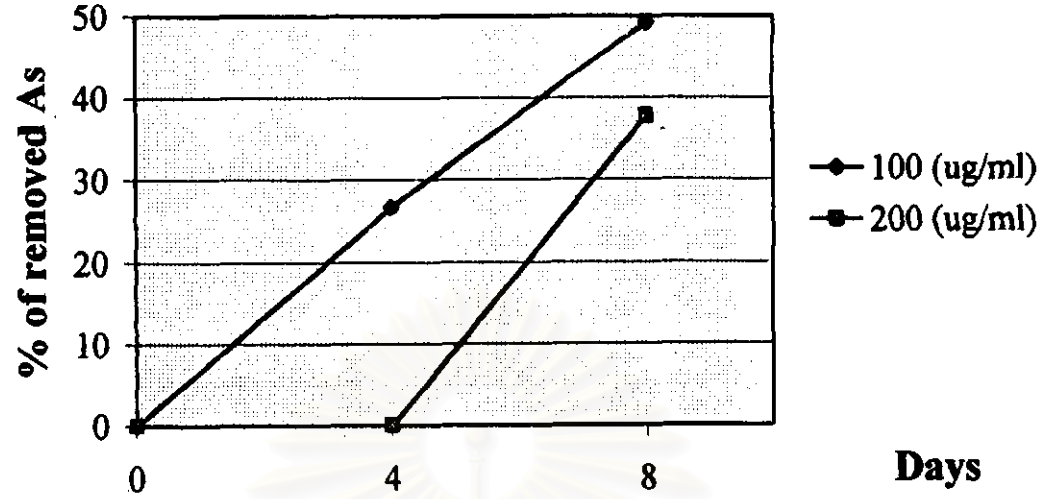


Figure 4.14: Percentage of removal arsenic in each arsenic concentration of the AsR-17

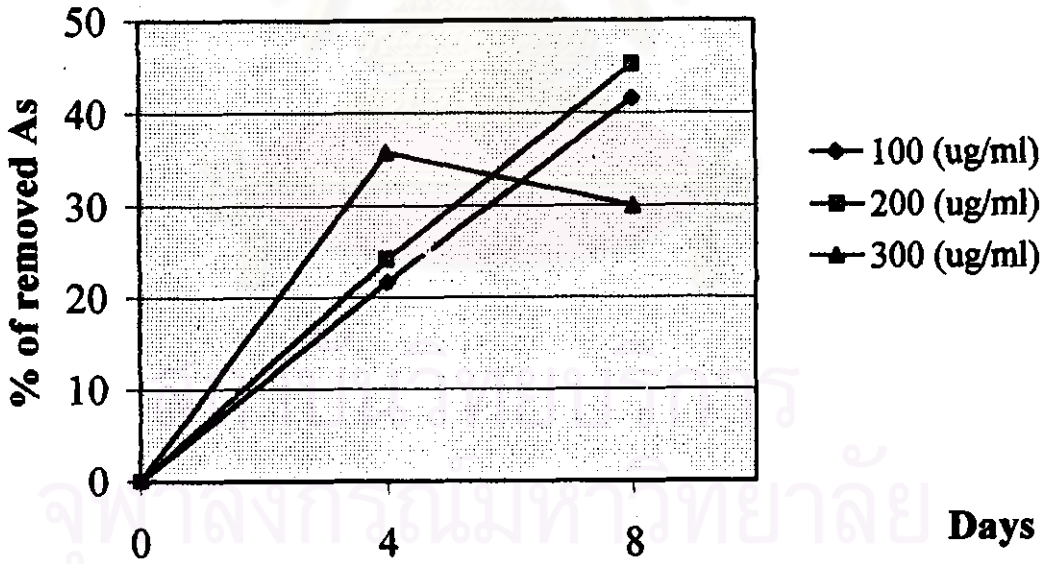


Figure 4.15: Percentage of removal arsenic in each arsenic concentration of the AsR-19/AsR-20

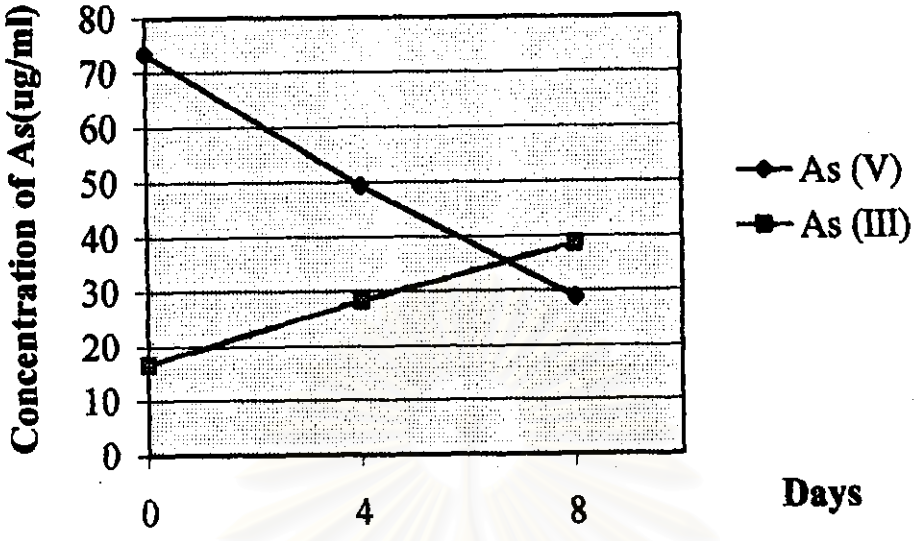


Figure 4.16a: Transformation of As(V) to As(III) in the AsR-17 at 100 ug/ml of As

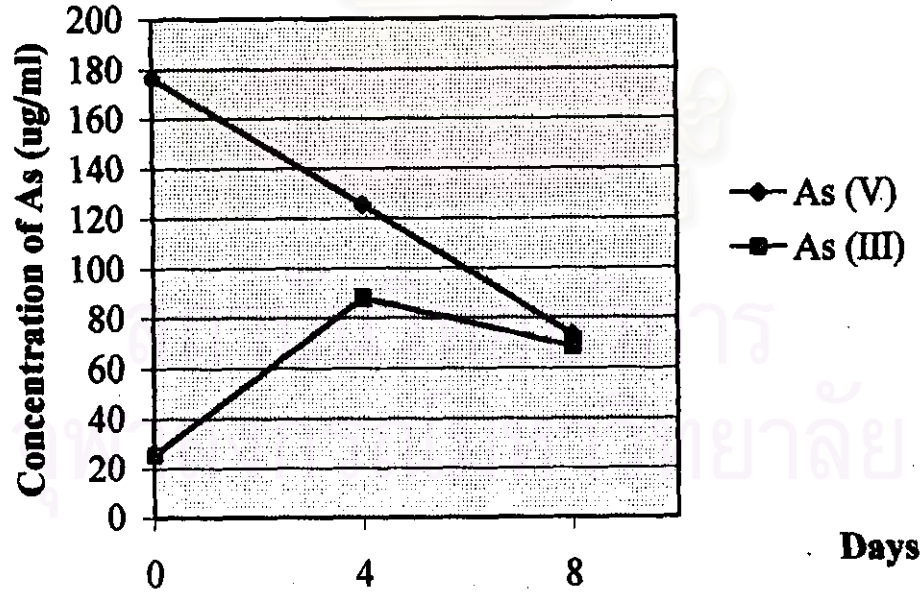


Figure 4.16b: Transformation of As(V) to As(III) in the AsR-17 at 200 ug/ml of As

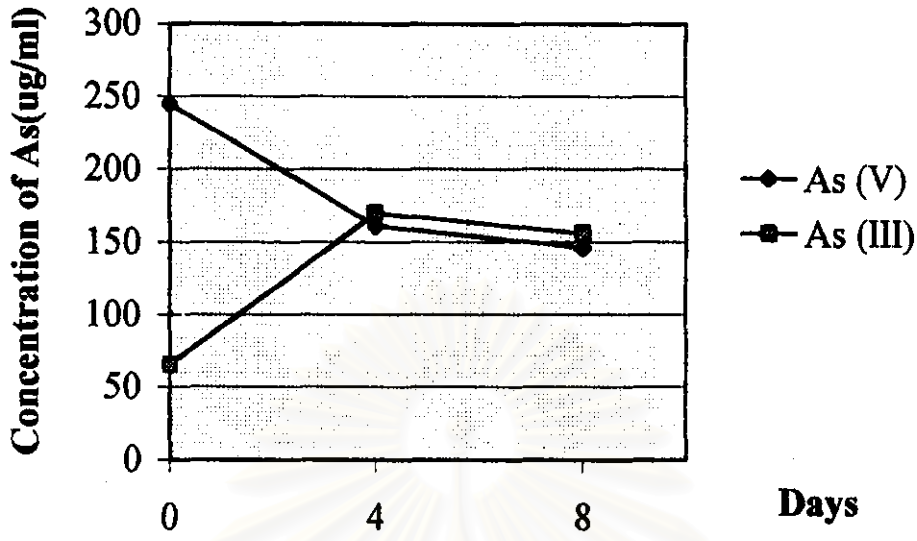


Figure 4.16c: Transformation of As(V) to As(III) in the AsR-17 at 300 ug/ml of As

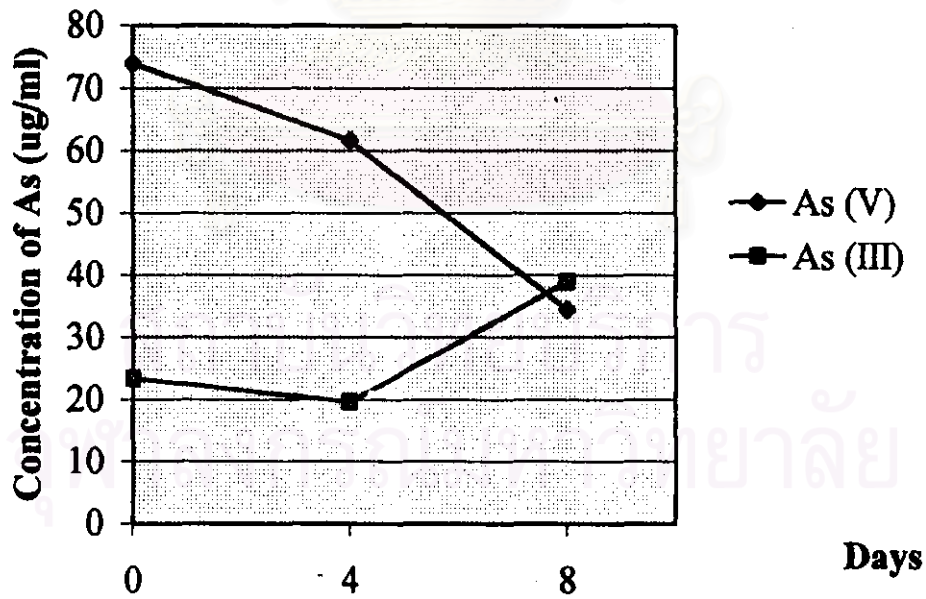


Figure 4.17a: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at 100 ug/ml of As

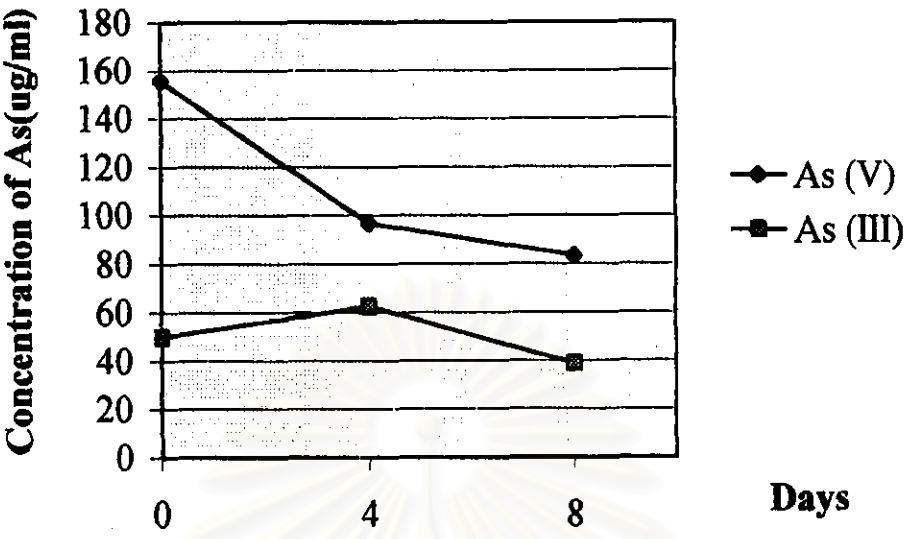


Figure 4.17b: Transformation of As(V) to As(III) in the AsR-19/As R-20 at 200 ug/ml of As

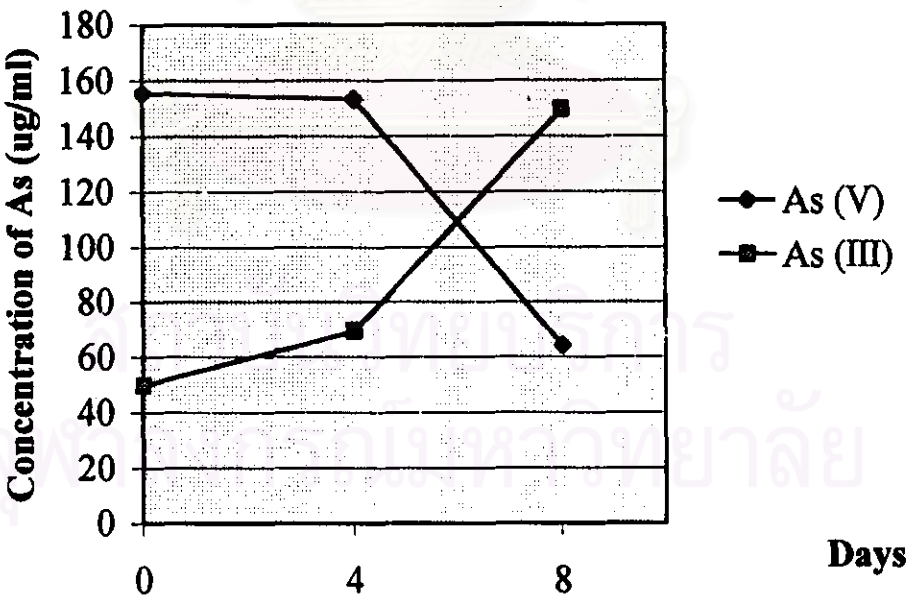


Figure 4.17c: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at 300 ug/ml of As

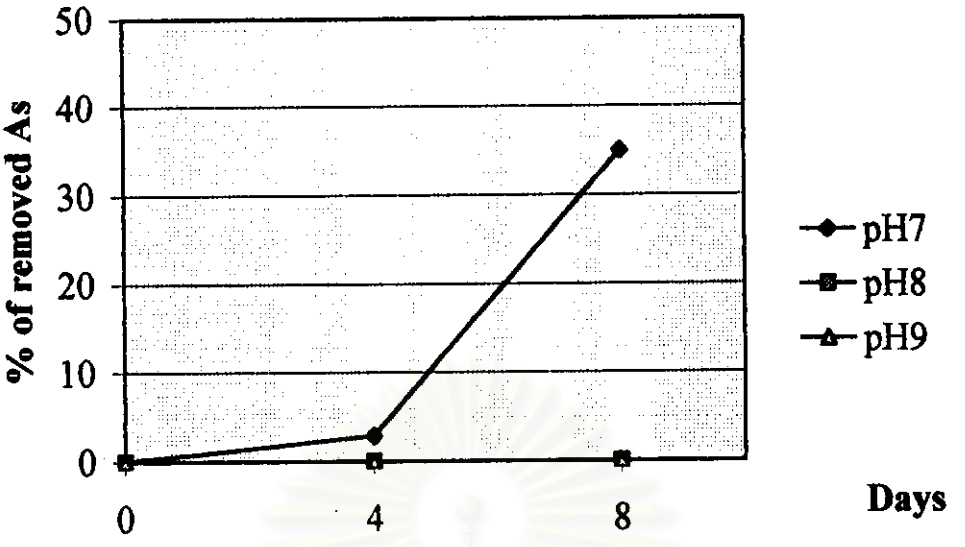


Figure 4.18: Percentage of removal arsenic at each pH of the AsR-17

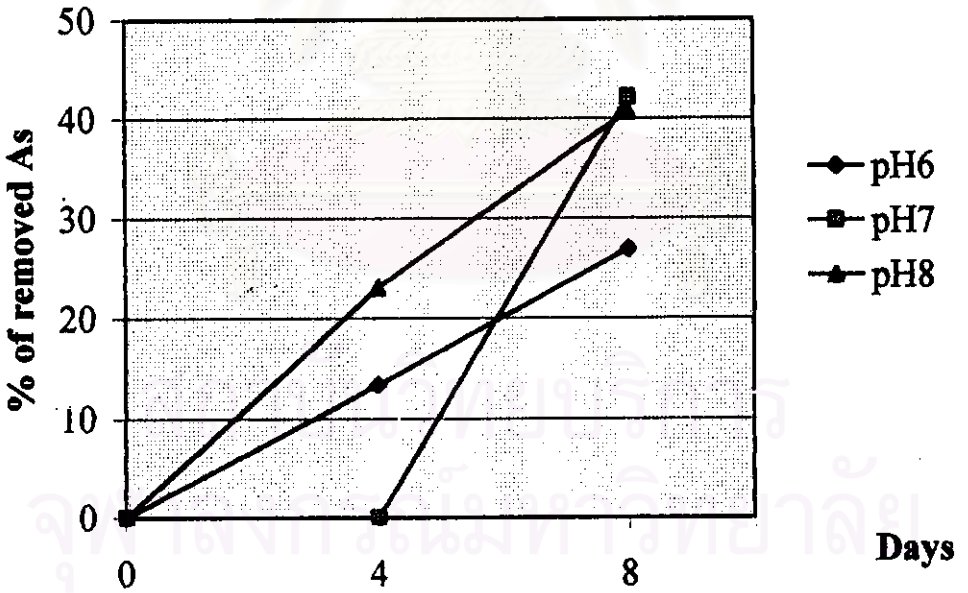


Figure 4.19: Percentage of removal arsenic at each pH of the AsR-19/AsR-20

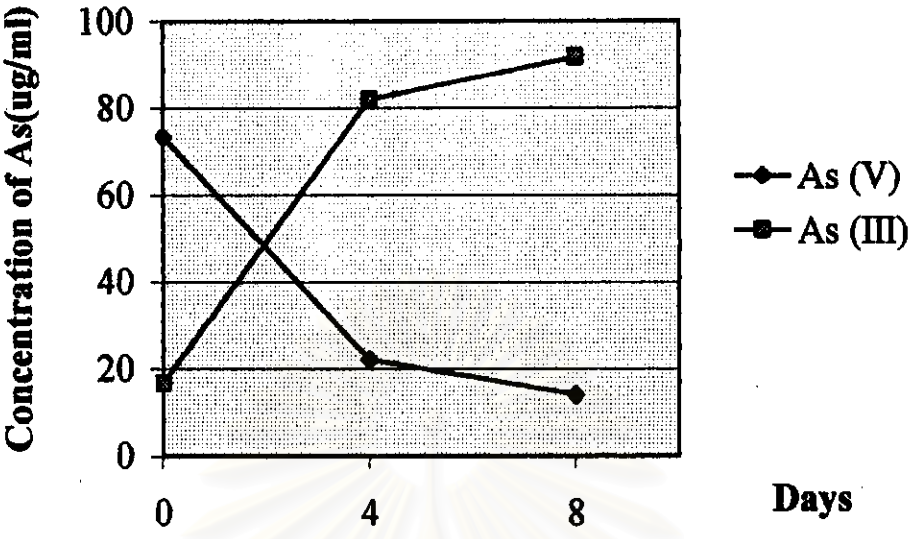


Figure 4.20a: Transformation of As(V) to As(III) in the AsR-17 at pH6

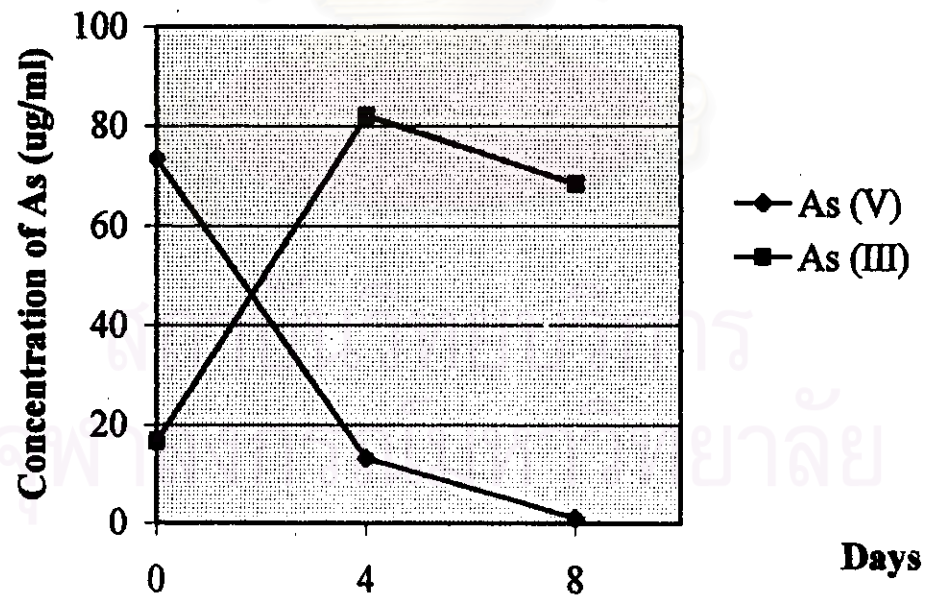


Figure 4.20b: Transformation of As(V) to As(III) in the AsR-17 at pH7

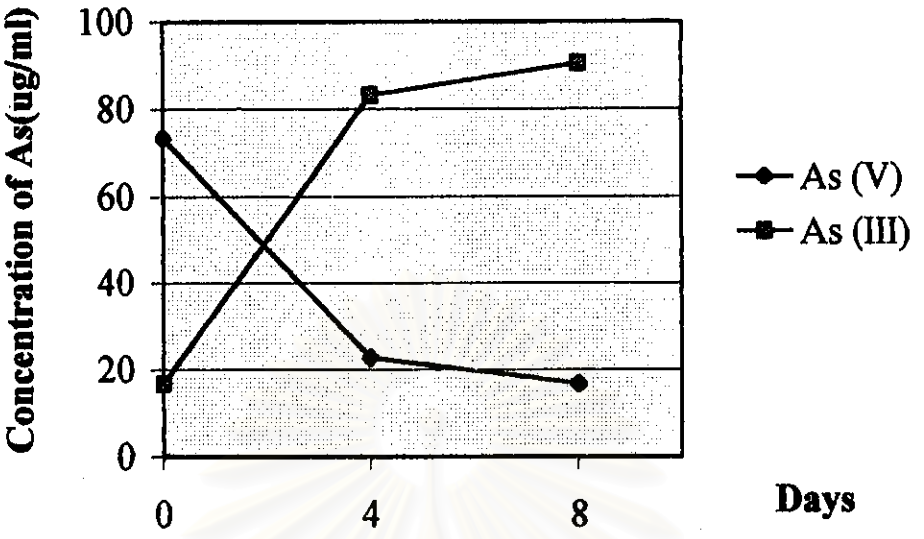


Figure 4.20c: Transformation of As(V) to As(III) in the AsR-17 at pH8

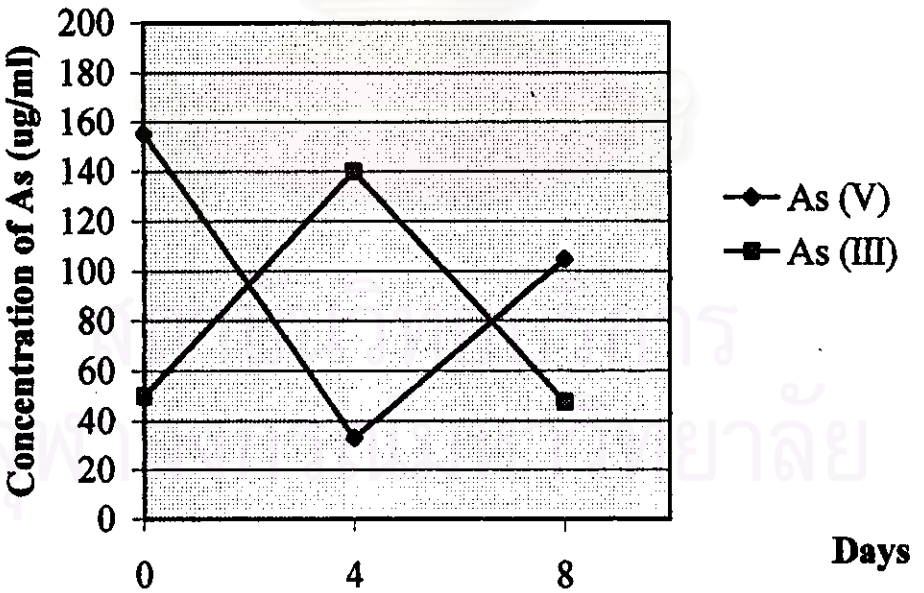


Figure 4.21a: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at pH6

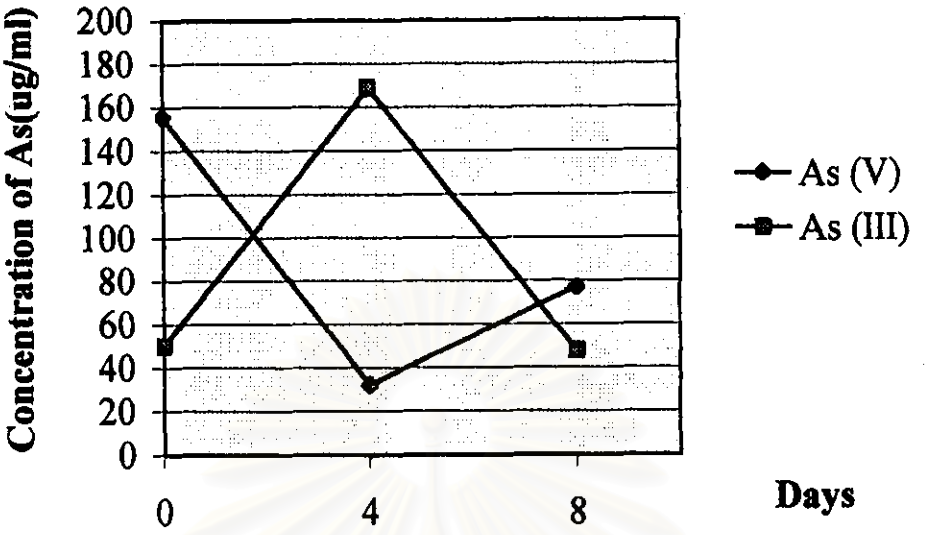


Figure 4.21b: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at pH7

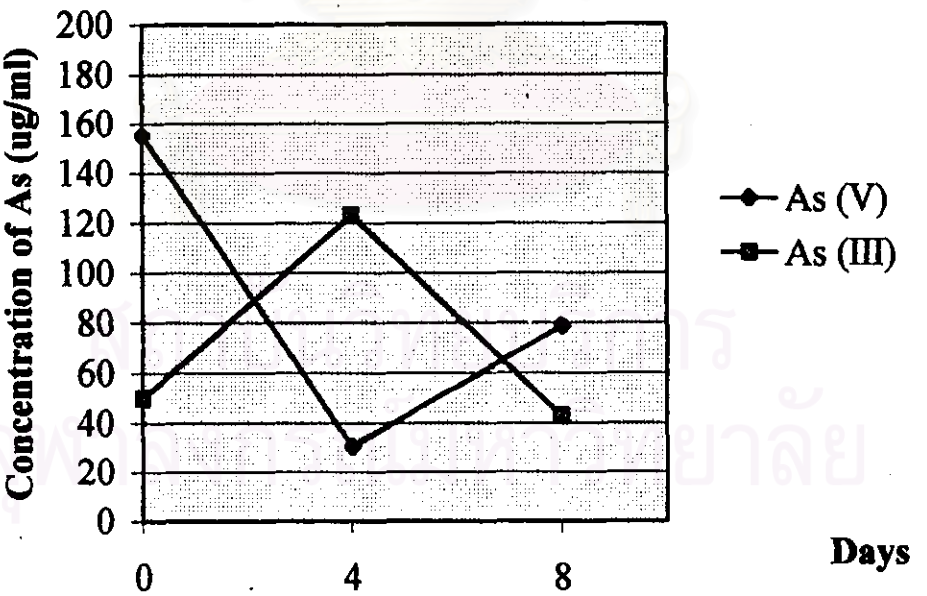


Figure 4.21c: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at pH8

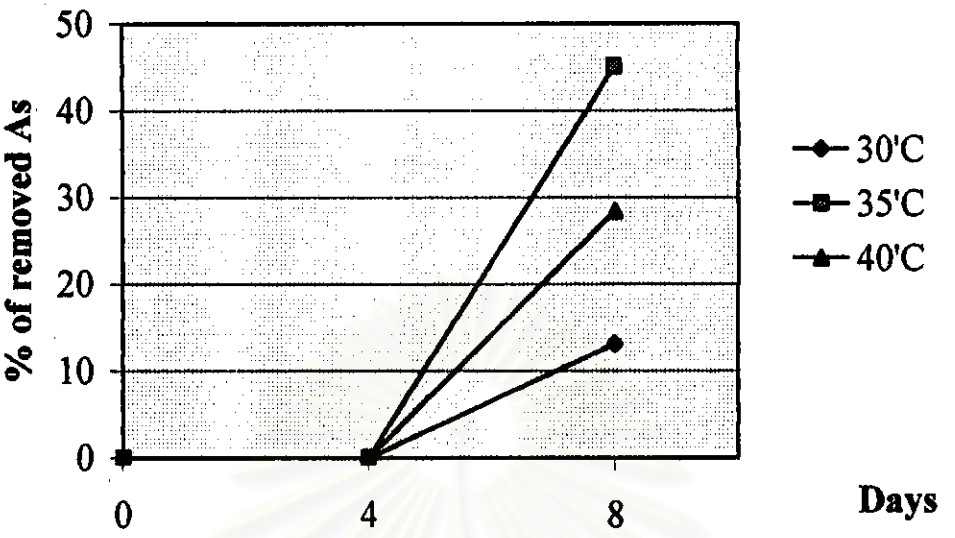


Figure 4.22: Percentage of removal arsenic in each temperature of the AsR-17

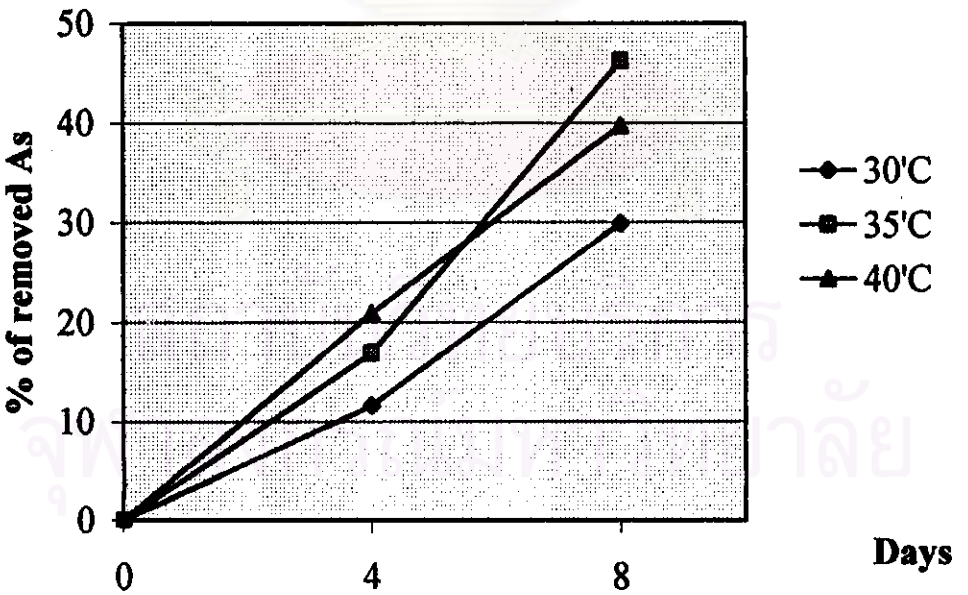


Figure 4.23: Percentage of removal arsenic in each temperature of the AsR-19/AsR-20

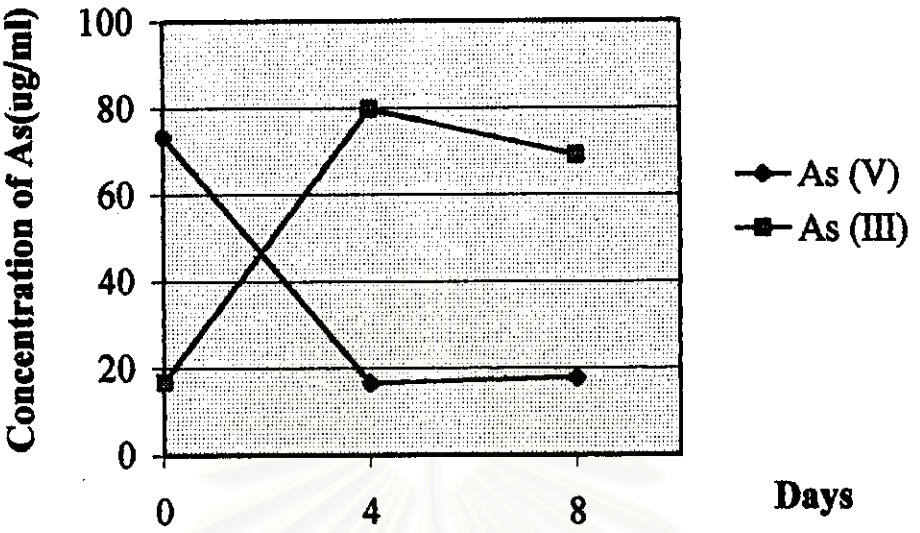


Figure 4.24a: Transformation of As(V) to As(III) in the AsR-17 at temperature 30'C

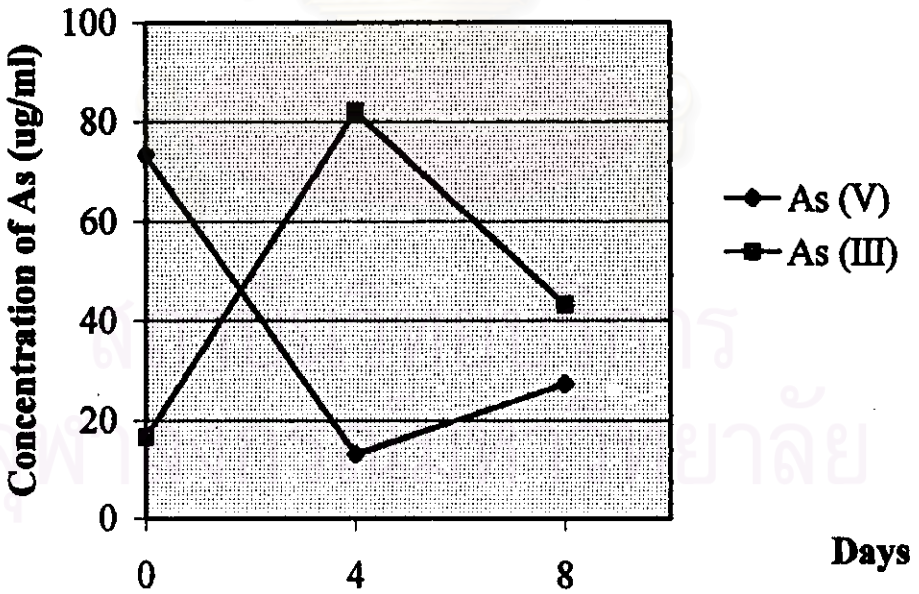


Figure 4.24b: Transformation of As(V) to As(III) in the AsR-17 at temperature 35'C

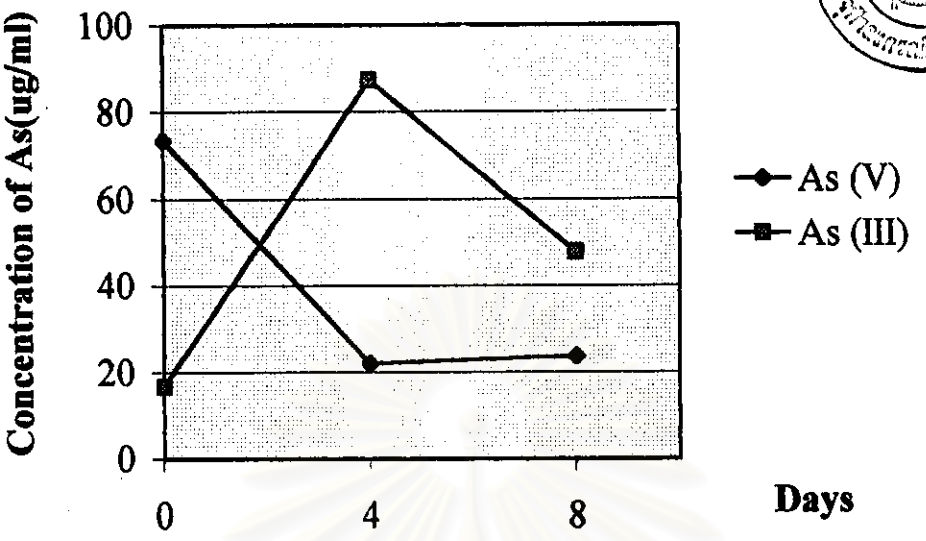


Figure 4.24c: Transformation of As(V) to As(III) in the AsR-17 at temperature 40'C

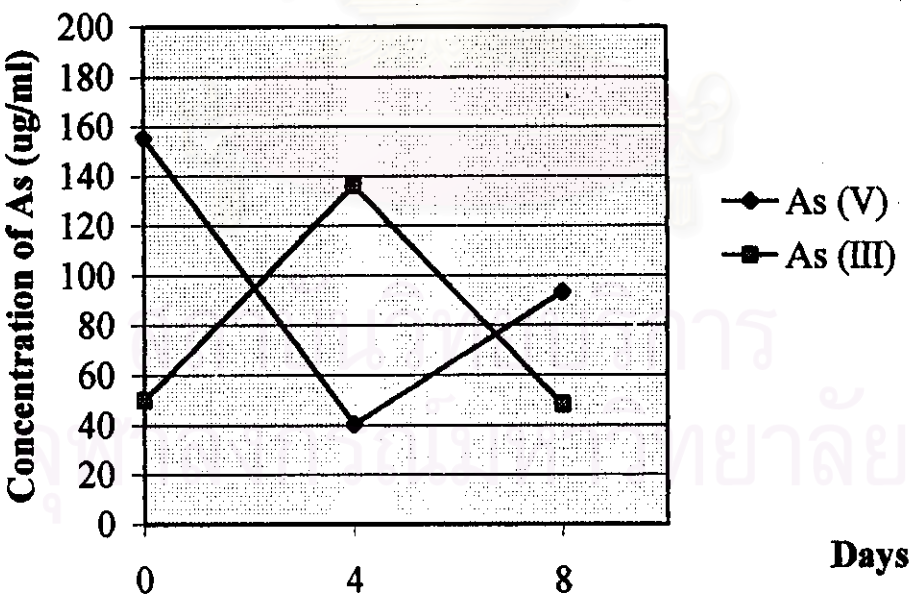


Figure 4.25a: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at temperature 30'C

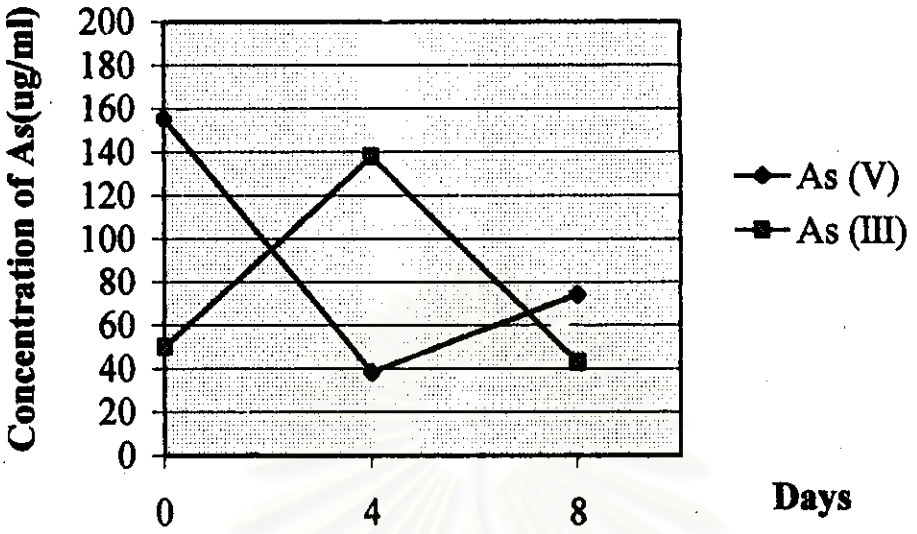


Figure 4.25b: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at temperature 35'C

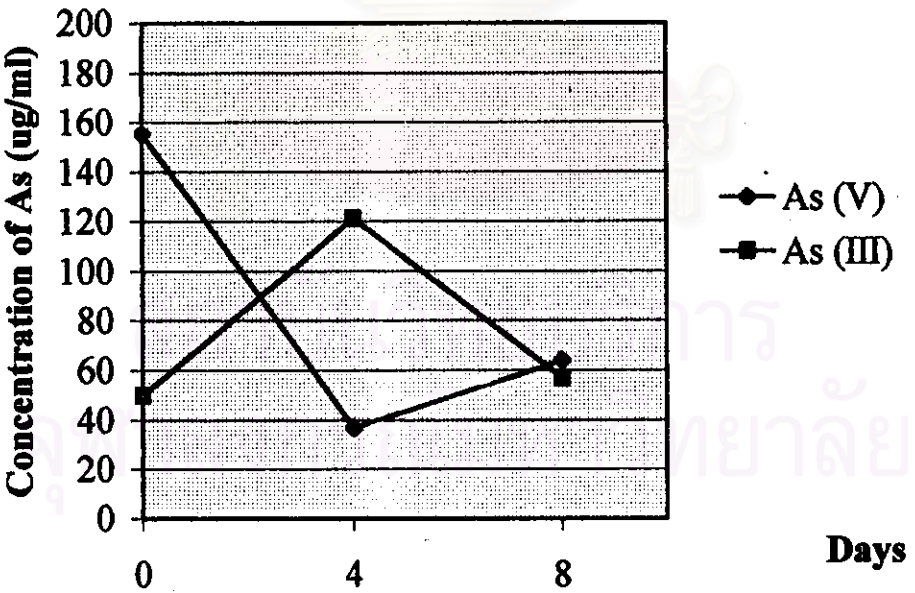


Figure 4.25c: Transformation of As(V) to As(III) in the AsR-19/AsR-20 at temperature 40'C