CHAPTER IV

RESULTS

4.1 SCREENING AND SELECTION OF CADMIUM RESISTANCE BACTERIA.

346 strains of bacteria resistant to 100 milligram/liter (mg/l) Cd were obtained. They were isolated from 34 samples. Five strains of cadmium-resistant bacterial isolates were selected, namely, CdR-87, CdR-98, CdR-205, CdR-207, and CdR-273. The result of characteristic and some of biochemical test were presented in **Table 4.1-4.2** and **Figure 4.1-4.6.**

Some sample of polluted areas were analyzed initial Cd concentration and other metals that were found a little amount of contaminated metals; Cd, Cu and Pb in unit of part per billion (ppb). The data was shown in **Table 4.3.**

346 strains of bacteria were tested the high concentration resistance of Cd and other metals by multi-innoculation method (**Figure 4.7**). The CdR-87, CdR-98, CdR-205, CdR-207, and CdR-273 can growth on the medium mixed Cd at 500, 400, 750, 400, and 750, respectively. The resistance of 5 strains for other metals followed the series Mn > Cu > Zn > Cr, Ni, Ag. The result was shown in **Table 4.4 .**

Table 4.1 The characteristics of cadmium-resistant bacterial isolated from different sources.

Selected Bacteria	Source (sampling sites)	Characteristic of colony	Characteristic of morphology	Proposed Genus
CdR-87	Activated sludge (sewage treatment plant)	2.5 mm in diameter, yellow, convex,entire	Rod shape, Gram negative, Produce capsule	***Enterobacter*** **sp.**
CdR-98	Sediment soil (Paint plant)	2.0 mm in diameter, yellow, convex, entire	Rod shape, Gram negative, Produce capsule	***Enterobacter*** **sp.**
CdR-205	Sediment soil (Canal in Bangkok)	4.5 mm in diameter, yellow, convex, entire	Rod shape, Gram negative, Produce capsule	***Enterobacter*** **sp.**
CdR-207	Sediment soil (Canal in Bangkok)	2.0 mm in diameter, yellow, convex, entire	Rod shape, Gram negative, Produce capsule	***Enterobacter*** **sp.**
CdR-273	Soil (Chemical plant)	2.5 mm in diameter, yellow, convex, entire	Rod shape, Gram negative, Produce capsule	***Enterobacter*** **sp.**

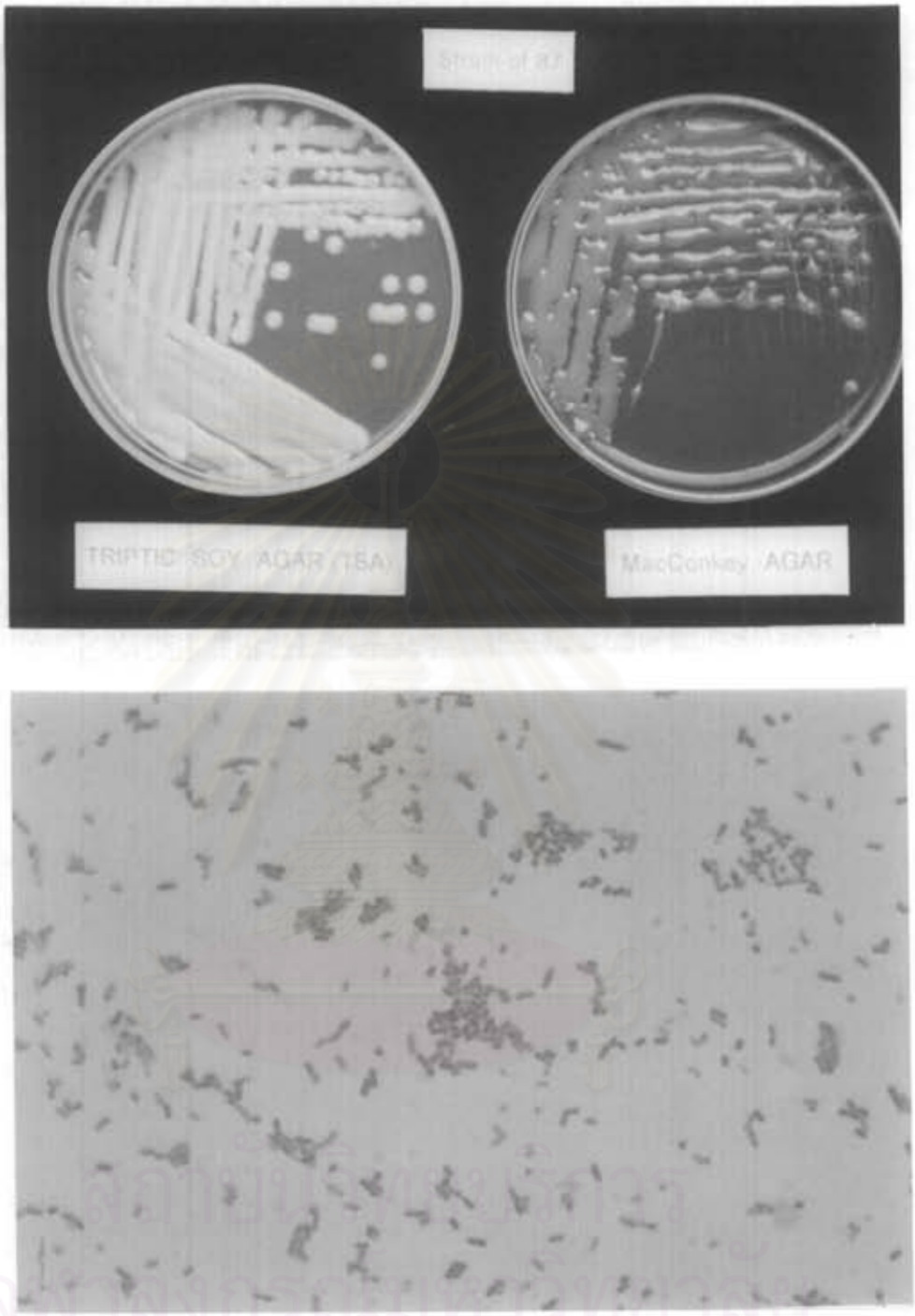


Figure 4.1 Colonial characteristic and gram staining (x1093; lower) of Cd-resistance bacterial strain CdR-87 on TSA (left) and on MacConkey agar (right).

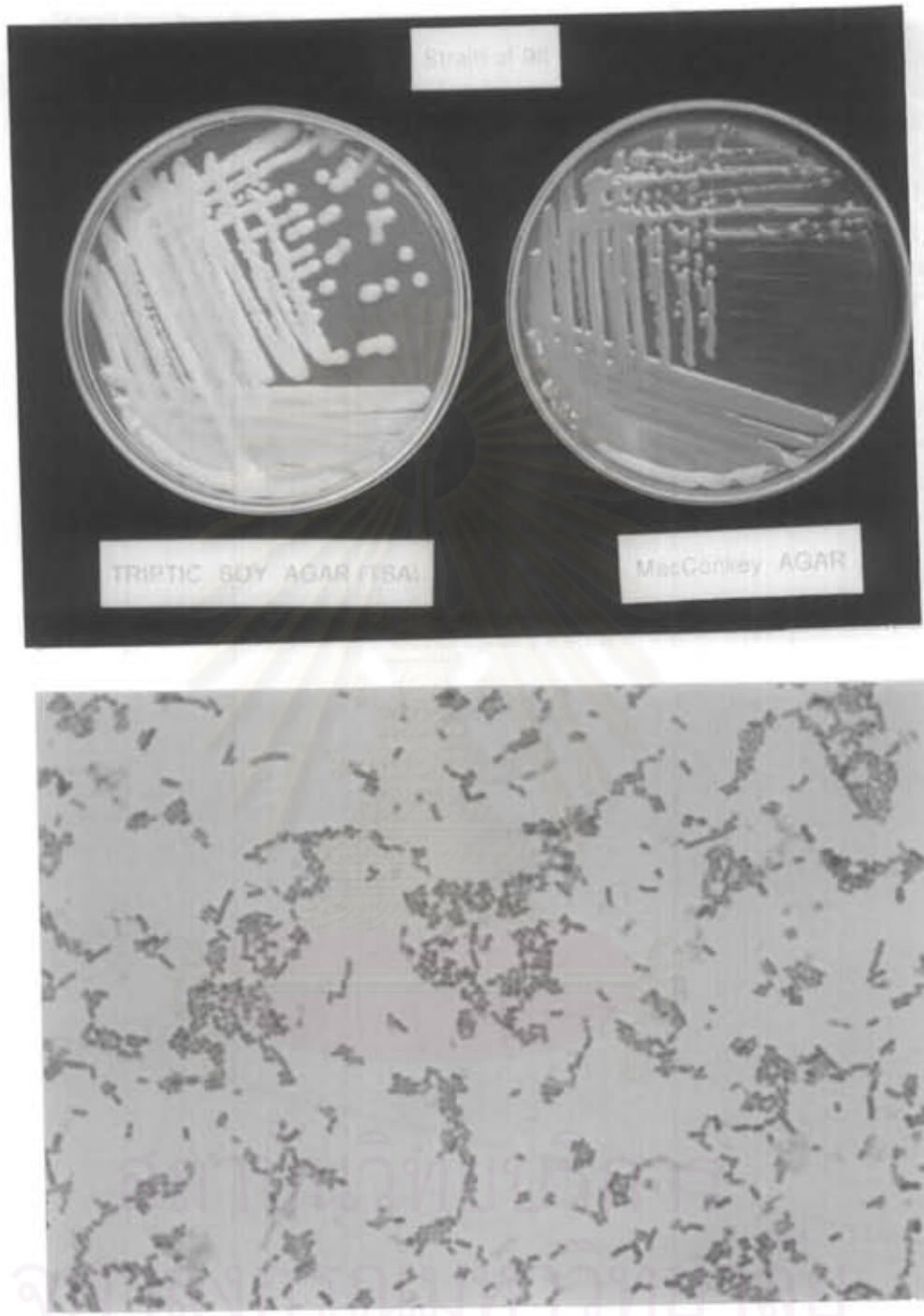


Figure 4.2 Colonial characteristic and gram staining (x1093; lower) of Cd-resistance bacterial strain CdR-98 on TSA (left) and on MacConkey agar (right).

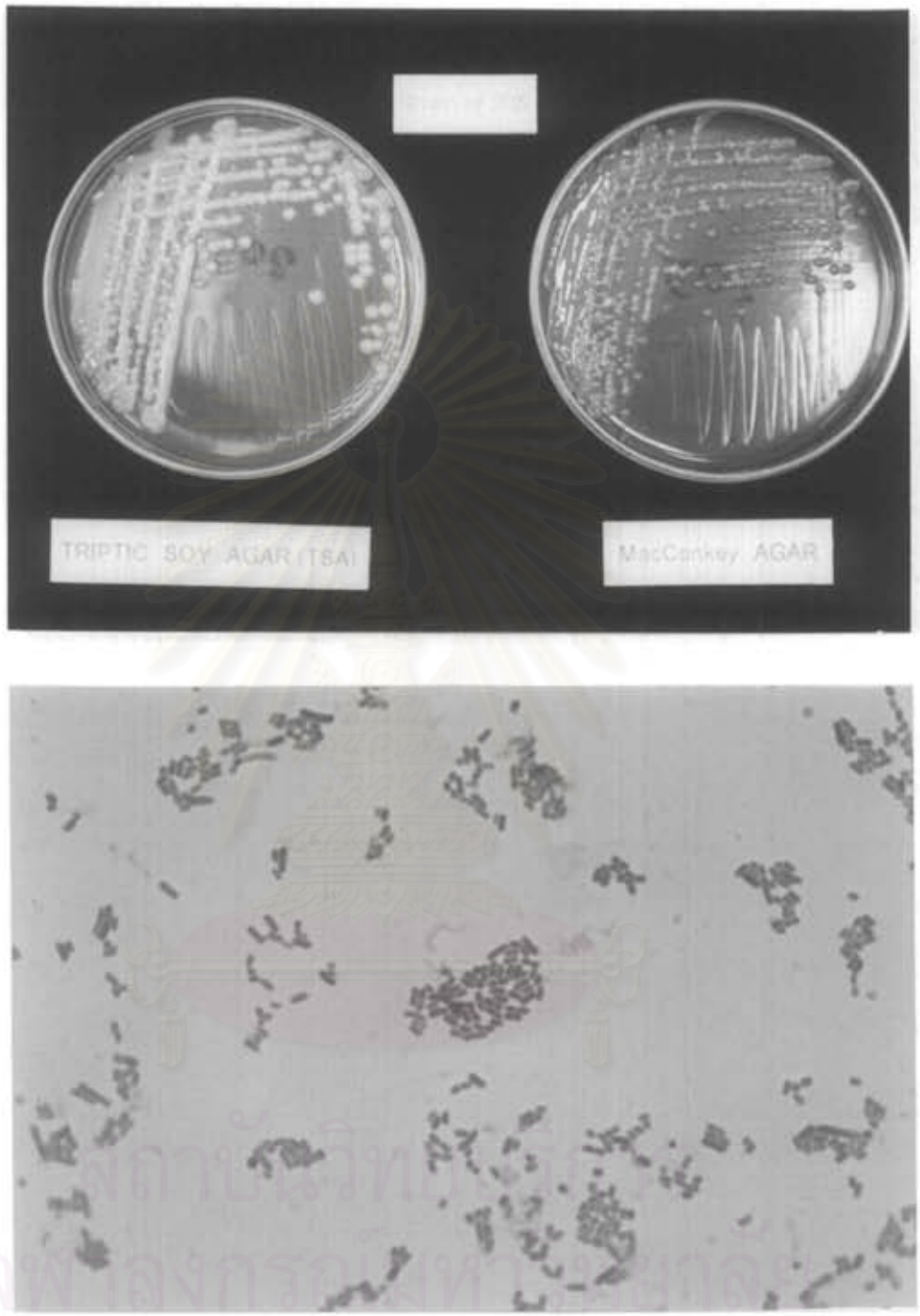


Figure 4.3 Colonial characteristic and gram staining (x1093; lower) of Cd-resistance bacterial strain CdR-205 on TSA (left) and on MacConkey agar (right).

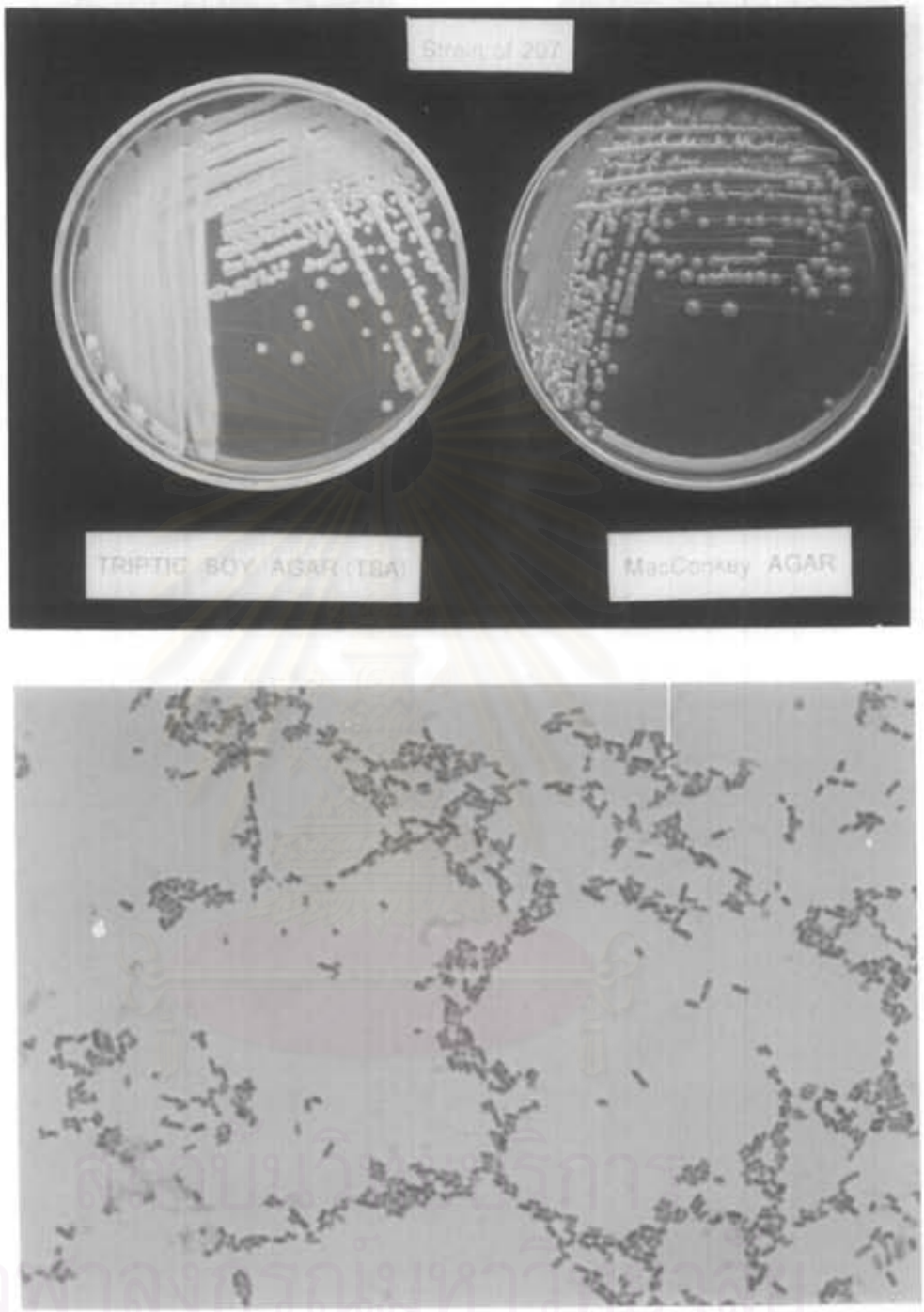


Figure 4.4 Colonial characteristic and gram staining (x1093; lower) of Cd-resistance bacterial strain CdR-207 on TSA (left) and on MacConkey agar (right).

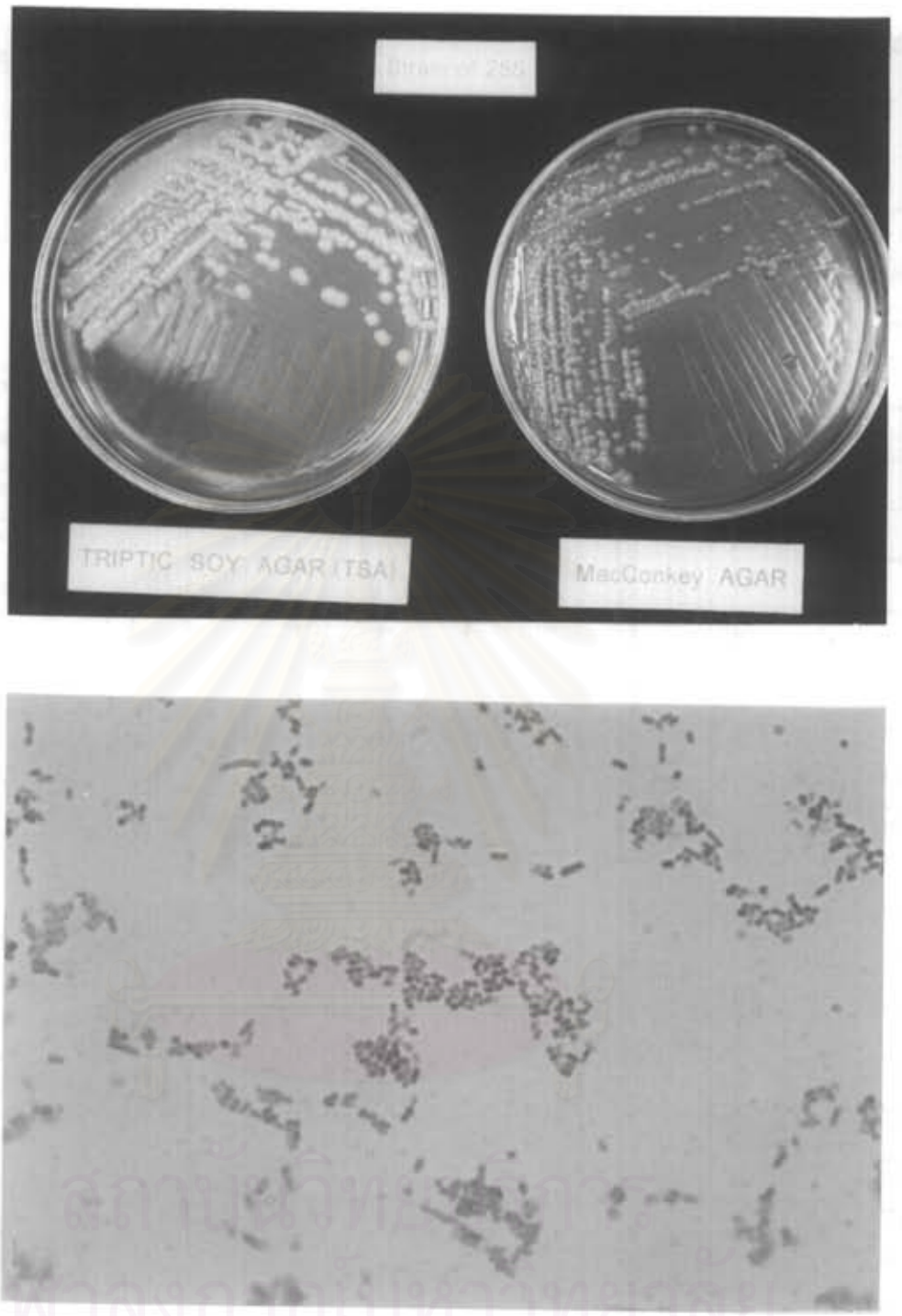


Figure 4.5 Colonial characteristic and gram staining (x1093; lower) of Cd-resistance bacterial strain CdR-273 on TSA (left) and on MacConkey agar (right).

Table 4.2 Some selective media and biochemical tests for identification characteristic of selected bacterial isolates.

Biochemical Test	Selected Bacterial Isolates				
	CdR-87	CdR-98	CdR-205	CdR-207	CdR-273
TSI	A/A	A/A	A/A	A/A	A/A
H_2S production	-	-	-	-	-
Gas production	+	+	+	+	+
Citrate	+	-	+	+	+
Urease	+	+	+	++	+
Oxidase	-	-	-	-	-
Catalase	+	+	+	+	+
Motility	+	+	+	+	+
MacConkey Agar	growth,pink, moist	growth,pink, moist	growth,red moist	growth,red, dry	growth,pink moist
SS agar	growth	growth	growth	growth	growth
PSIA	-	-	-	-	-
MCIK	growth,red	-	-	-	-
OF test					
Glucose	A/A	A/A	A/A	A/A	A/A
Dextrose	A/A	A/A	A/A	A/A	A/A
Lactose	A/A	A/A	A/A	A/A	A/A
Sucrose	A/A	A/A	A/A	A/A	A/A
Mannose	A/A	A/A	A/A	A/A	A/A
Moltose	A/A	A/A	A/A	A/A	A/A
MR	+	-	-	+	+
VP	+	+	+	+	+

A = acid

++ = more positive

- = negative

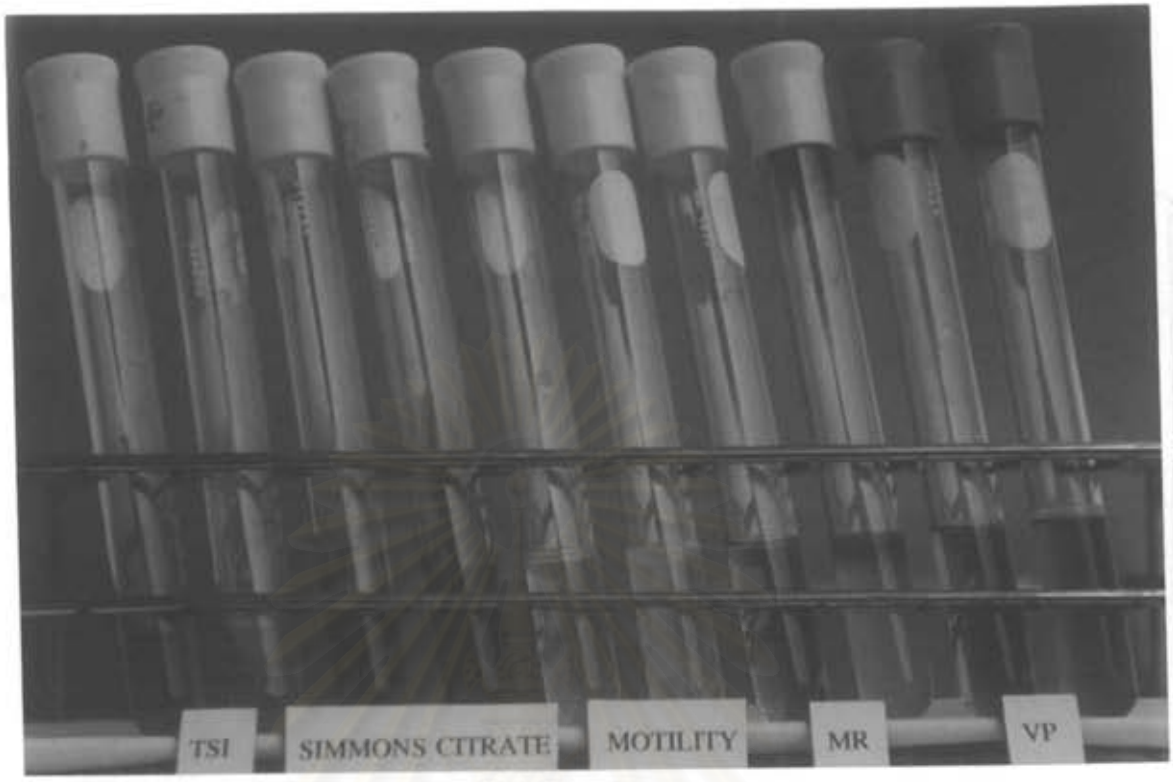


Figure 4.6 Some characteristic of biochemical test of bacterial strains.

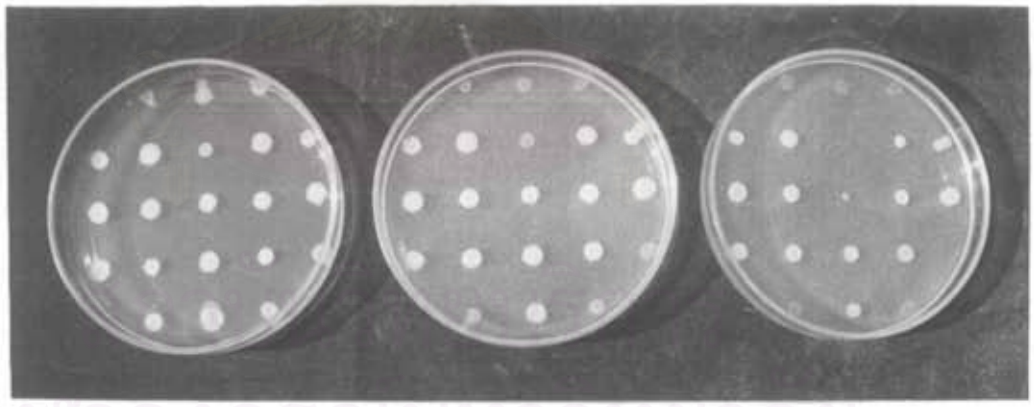


Figure 4.7 Multi-inoculation test plate, characteristic of bacterial growth on media, control plate (a), media+500mg/l Cd (b), and media +750mg/l Cd (c).

Table 4.3 Concentrations of metals ,e.g., Cd, Pb and Cu were detected in samples.

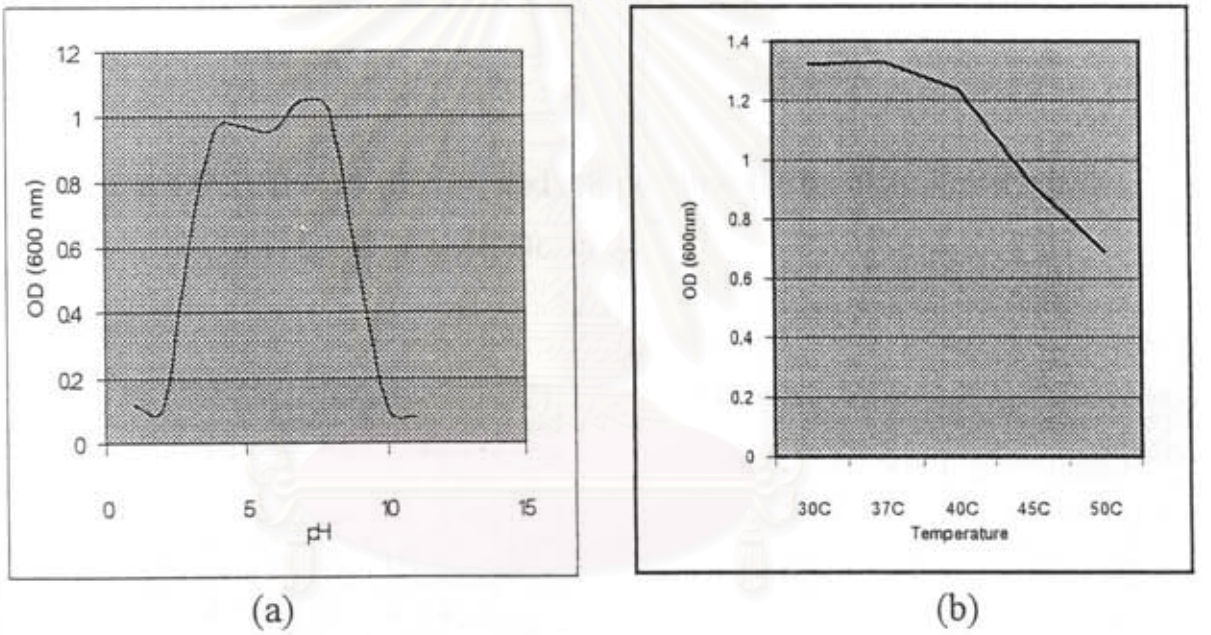
Sample no.	Type of sample	Amount of Cd (ppb)	Amount of Pb (ppb)	Amount of Cu (ppb)
9	Sediment soil	0.19	1.0	6.8
10	Waste water	0.74	2.0	21.24
15	Sediment soil	0.18	1.3	84.48
16	Sludge	0.29	10.5	85.52
17	Sediment soil	0.31	1.4	7.32

Table 4.4 Resistance of other metals by cadmium-resistant bacterial isolates.

Bacteria No.	Concentration of heavy metals (mg/l)						
	Cd	Cu	Mn	Zn	Ni	Cr	Ag
CdR-87	500	250	>500	150	0	0	0
CdR-98	400	350	>500	<100	0	0	0
CdR-205	750	250	>500	150	0	0	0
CdR-207	400	500	>500	150	0	0	0
CdR-273	750	500	>500	200	0	0	0

4.2 OPTIMUM CONDITION OF GROWTH RATE

The growth rate was found the optimum pH was alkaline (7-9). Growth at extreme pH 2-4 and 10-12 were rare. The temperature at 30-40 °C were more growth than high temperature (45-50 °C). CdR-87, CdR-98, CdR-205, CdR-207, and CdR-273 have the similar trend that pH 7-9 and temperature 30-40 °C were suitable for growth rate. The result of each strain was presented in **Figure 4.8-4.12**.



(a) (b)

Figure 4.8 Effect of pH (a), and temperature (b) on cadmium-resistant bacterial strain CdR-87.

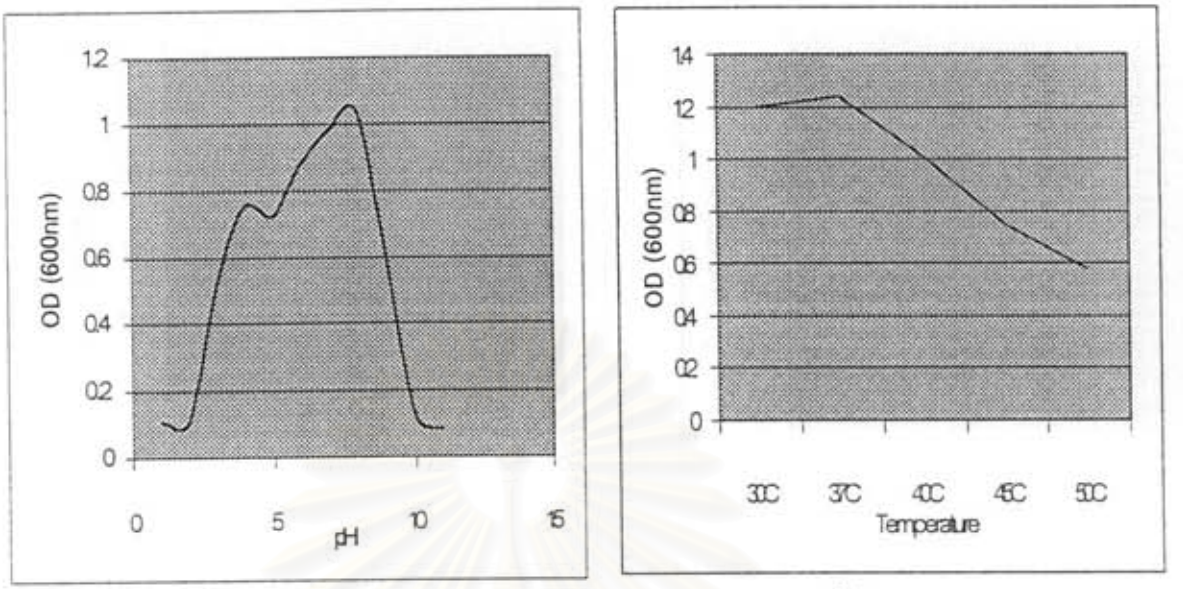


Figure 4.9 Effect of pH (a), and temperature (b) on cadmium-resistant bacterial strain CdR-98.

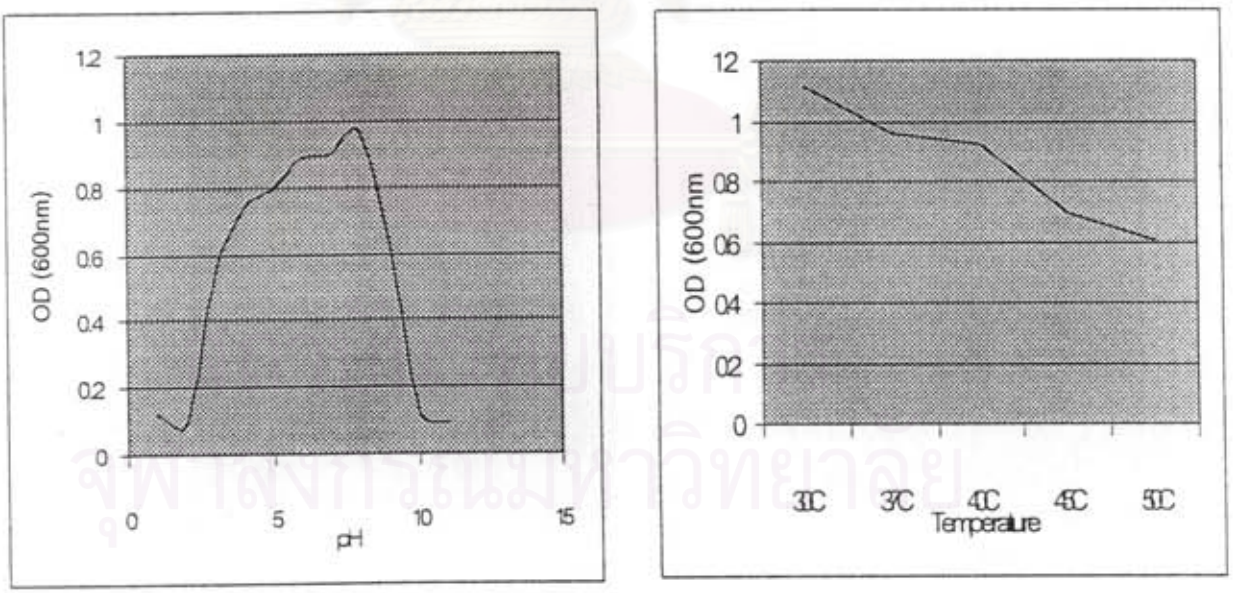


Figure 4.10 Effect of pH (a), and temperature (b) on cadmium-resistant bacterial strain CdR-205.

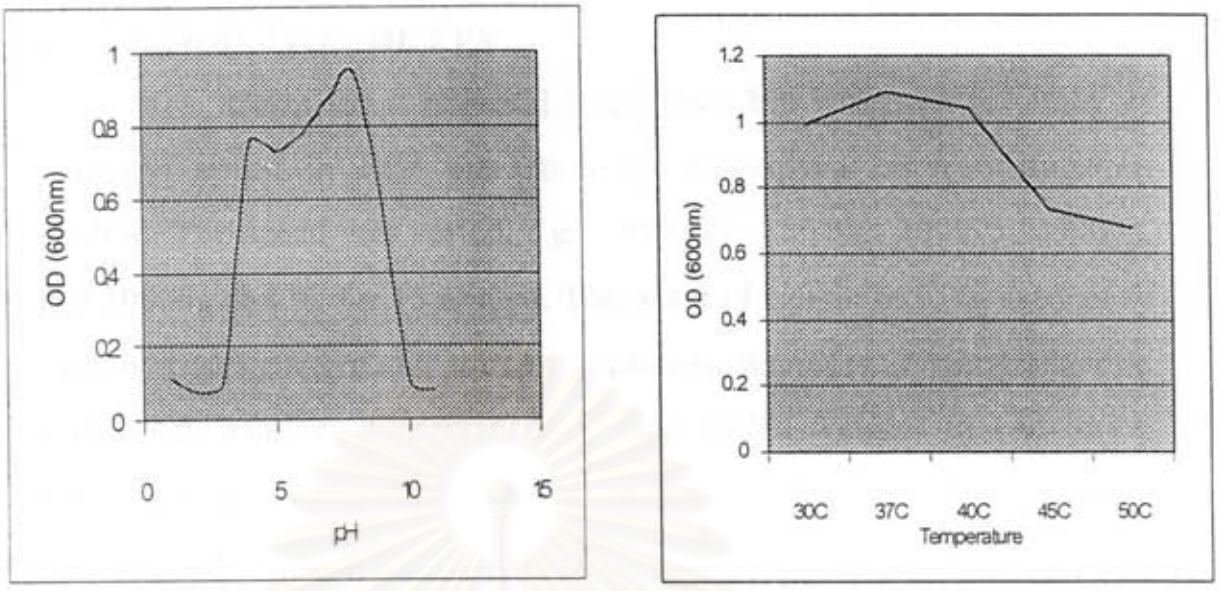


Figure 4.11 Effect of pH (a), and temperature (b) on cadmium-resistant bacterial strain CdR- 207.

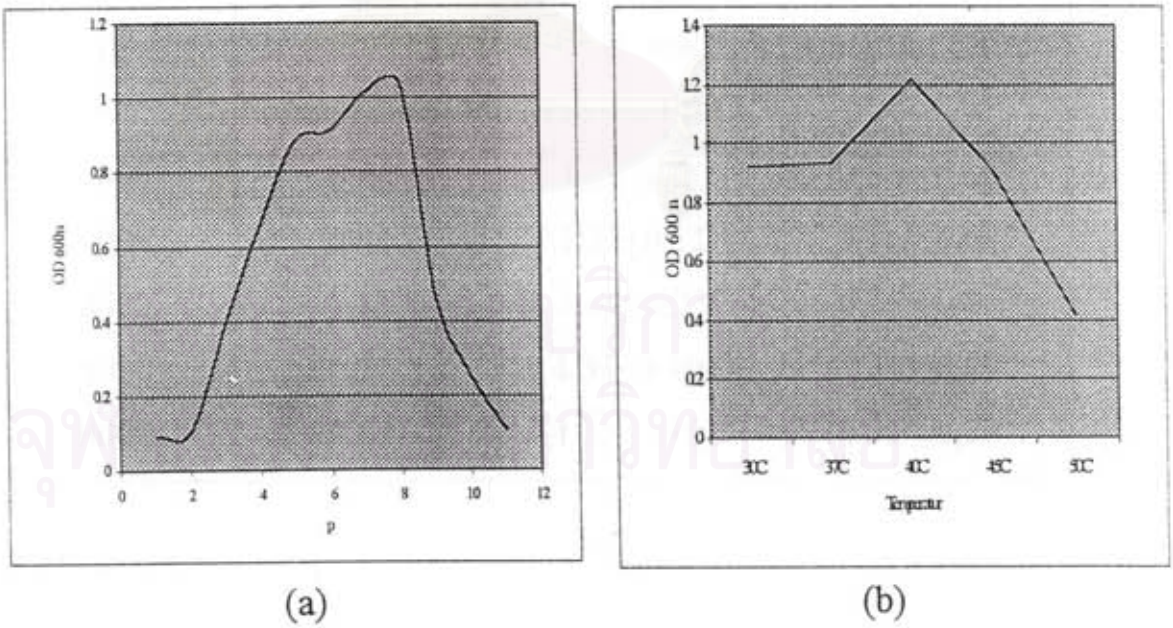


Figure 4.12 Effect of pH (a), and temperature (b) on cadmium-resistant bacterial strain CdR-273.

4.3 EXTRACTION OF EPS

Cd-resistant bacterial strain CdR-205 was used for study of optimum speed in EPS extraction by high-speed ultracentrifugation method. The speed was varied, e.g., 10000 g, 15000g, 20000g, 25000g, and 30000g at 4 °C for 15 minute. The result of weight of EPS, amount of carbohydrates content and amount of protein content were summarized in **Table 4.5**. Maximum proportion of EPS (dried weight) to cell (dried weight); to protein content was not accepted. Thereafter, this speed is future used of extraction of EPS.

Table 4.5 Effect of centrifugation speed on extractions of EPS and other EPS component of the selected Cd-resistant bacterial isolate, CdR-205 (***Enterobacter*** **sp.**).

Centrifugation (g)	Amount of EPS (mg/mg cell)	Carbohydrate content (mg/mg EPS) [a]	Protein content (mg/mg EPS) [b]
10000	0.68[c]	1.21±0.20[d]	0.30 ±0.03 [d]
15000	0.98 [c]	1.37±0.11 [d]	0.36 ±0.06 [d]
20000	1.12 [c]	1.49±0.25 [d]	0.35 ±0.06 [d]
25000	0.71 [c]	1.31±0.10 [d]	0.34 ±0.03 [d]
30000	0.89 [c]	1.35±0.19 [d]	0.35 ±0.03 [d]

a= Phenol-sulfuric acid method

b = Lowry's method

c = of duplicates

d = of six replicates

4.4 OPTIMUM CONDITIONS FOR EPS PRODUCTION

The influence of pH, temperature, incubation period, and Cd concentration in medium EPS production of each strain culture was shown in **Figure 4.13-4.16**. EPS production were measured and collected dried EPS and dried cells (without EPS) were prepared for further studies.

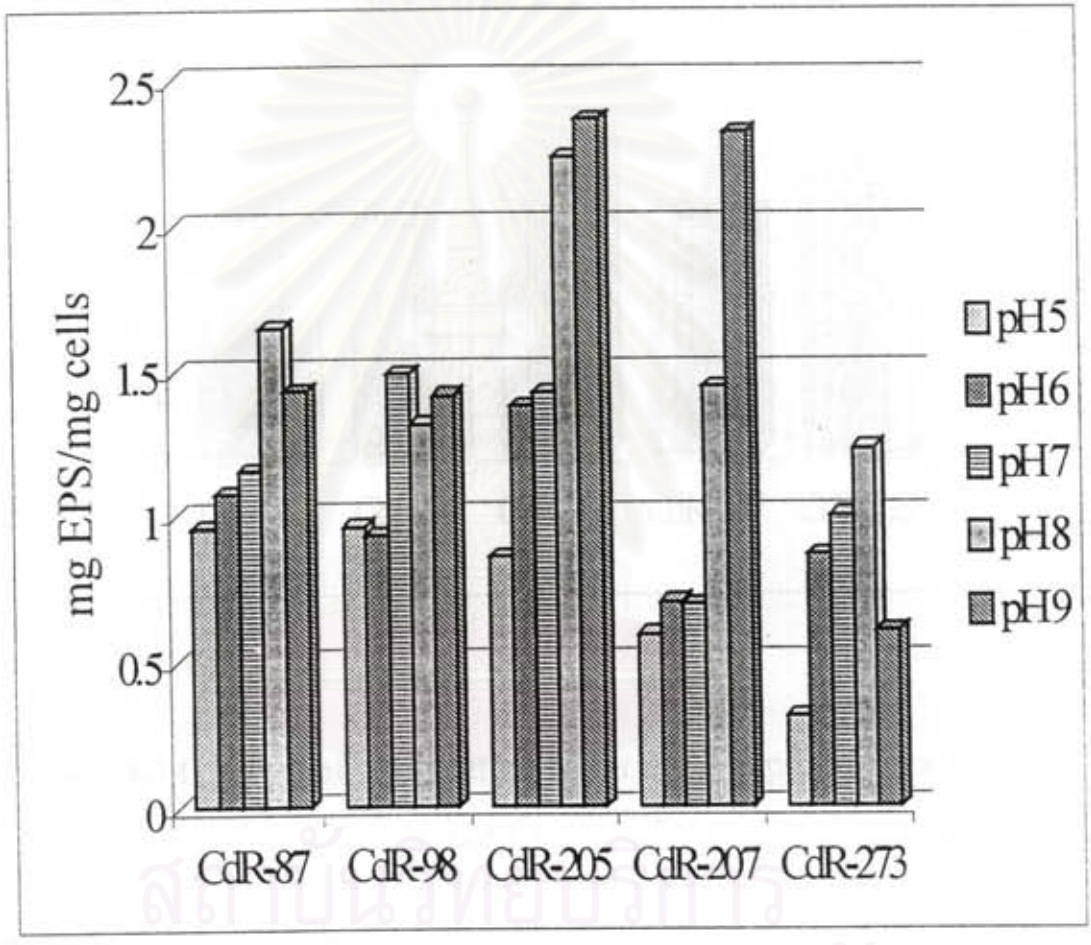


Figure 4.13 Effect of pH on EPS production of the selected Cd-resistant bacterial strains, e.g., CdR-87, CdR-98, CdR-205, CdR-207 and CdR-273.

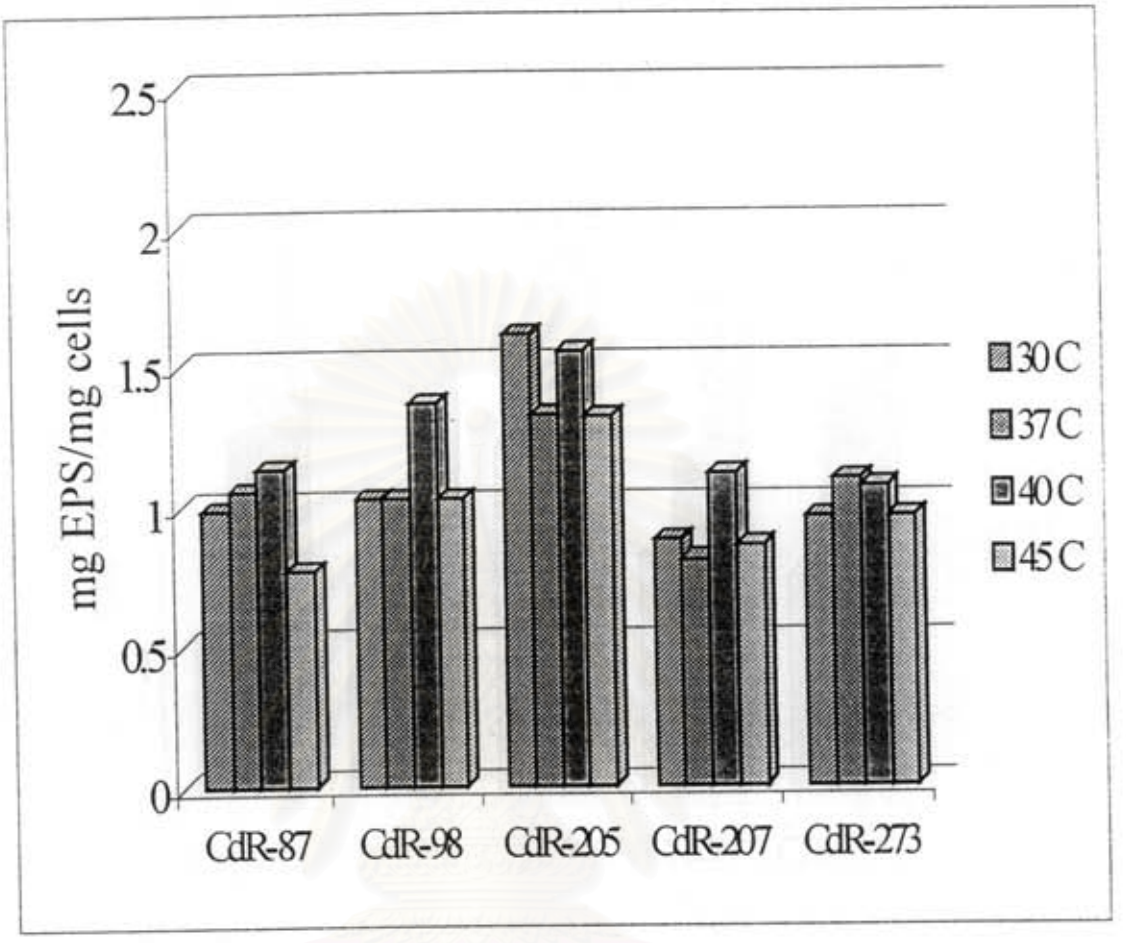


Figure 4.14 Effect of temperature factor on EPS production of the selected Cd-resistant bacterial strains, e.g., CdR-87, CdR-98, CdR-205, CdR-207 and CdR-273.

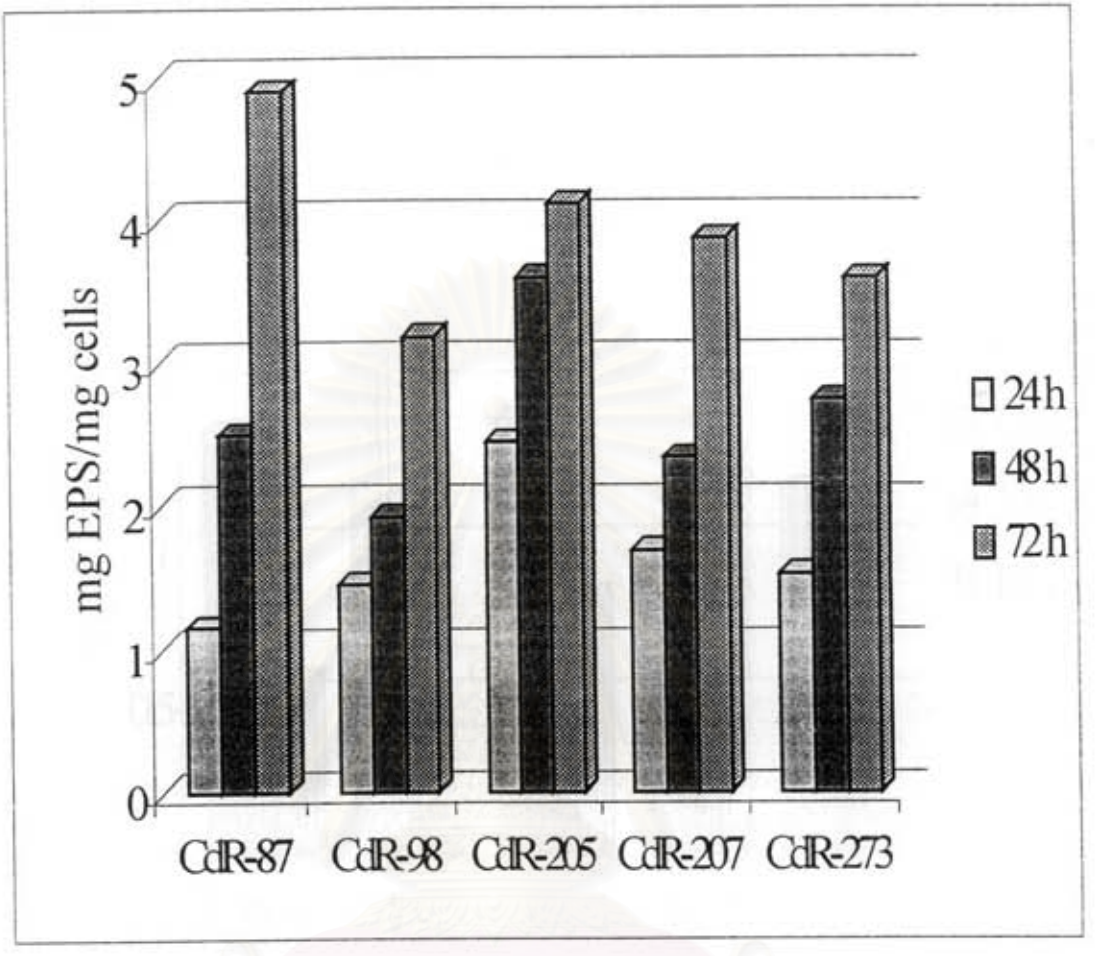


Figure 4.15 Effect of incubation period on EPS production of the Selected Cd-resistant bacterial strains, e.g., CdR-87, CdR-98, CdR-205, CdR-207 and CdR-273.

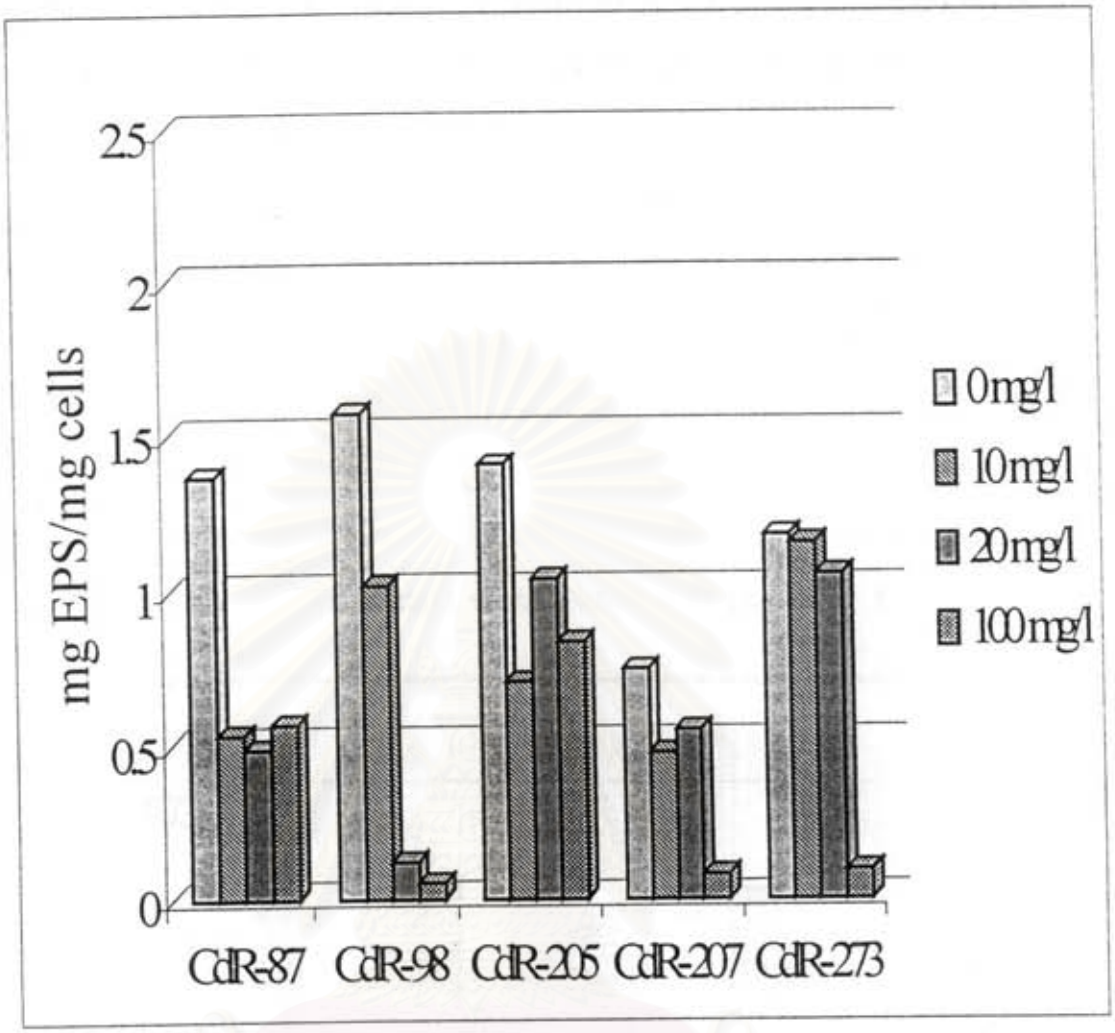


Figure 4.16 Effect of cadmium concentration in medium on EPS production of the selected Cd-resistant bacterial strains, e.g., CdR-87, CdR-98, CdR-205, CdR-207 and CdR-273.

4.5 ACCUMULATION OF CADMIUM AND OTHER METALS

4.5.1 Effect of Initial Cd Concentration on Cd Adsorption by Viable Cells.

Percentage of Cd adsorption and amount of Cd uptake by whole cells of those selected bacterial strains was shown in **Figure 4.17-4.18**. In medium containing low Cd concentration, percentage of Cd adsorption by whole cells of the test organisms were quite high, e.g., 87, 70 and 28 % but the amount of Cd uptake (nM per mg dry cells) were found to be increased, when the medium containing 10, 50 and 100 μg/ml Cd, respectively. The raw data were shown in **Appendix F**.

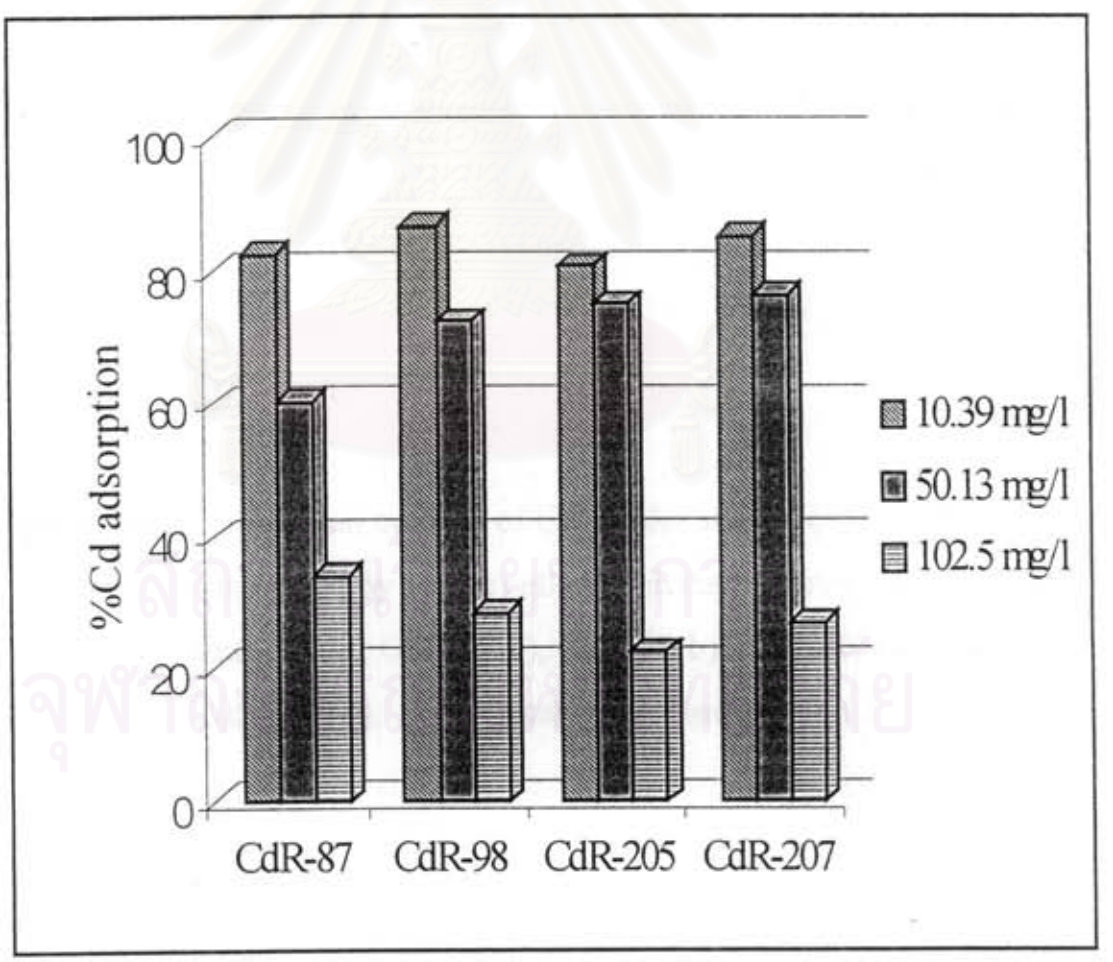


Figure 4.17 Effect of initial Cd concentration on Cd adsorption by viable cells of the selected Cd-resistant bacterial strains, e.g., CdR-87, CdR-98, CdR-205, CdR-207 and CdR-273.

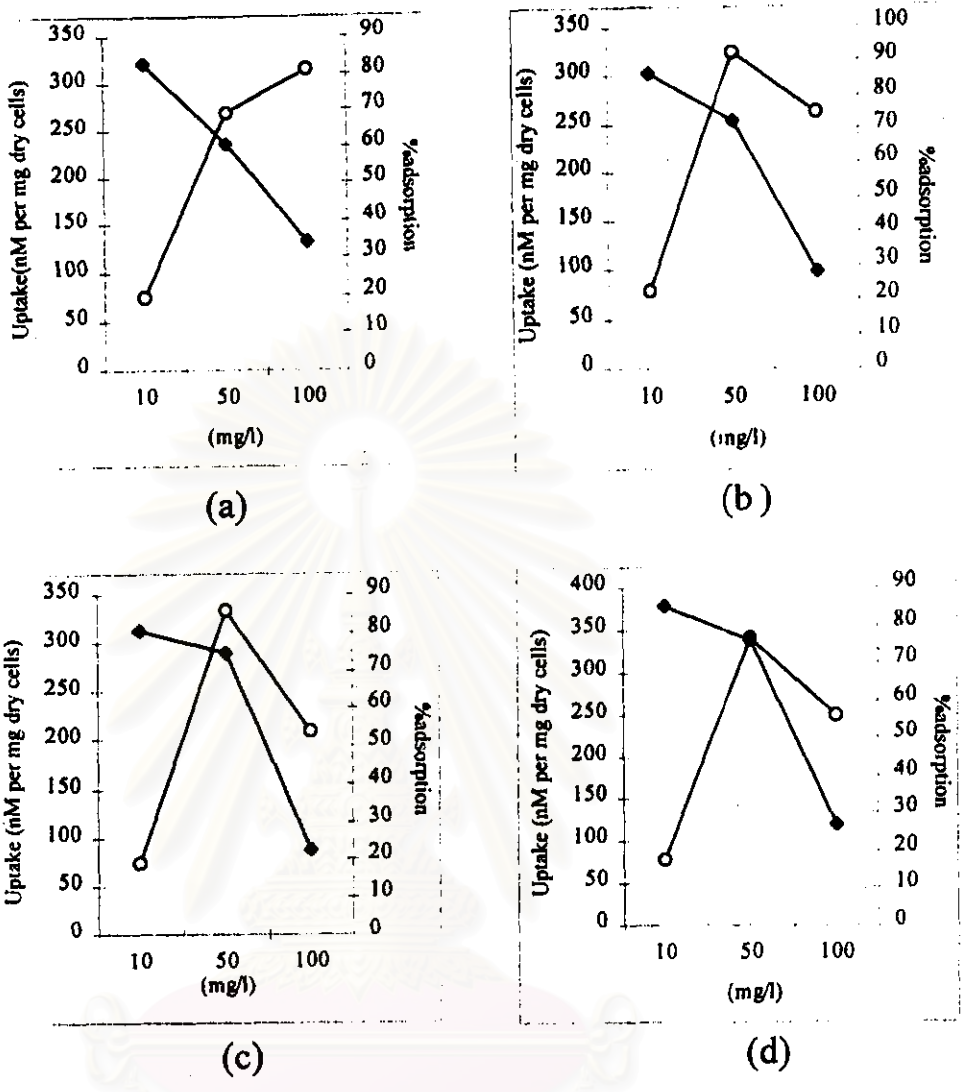


Figure 4.18 Comparison amount of Cd uptake and percentage of Cd adsorption by viable cells of selected Cd-resistant bacterial strains, e.g., CdR-87(a), CdR-98(b), CdR-205(c) and CdR-207(d). (O, Uptake; ■, %adsorption)

4.5.2 Cadmium Adsorption by Viable Cells, Dead Cells and EPS

From **Figure 4.19** Cd adsorption by viable cells, dead cells, and EPS of all isolates was found to be similar. It may imply that no energy or any metabolic process involved in Cd adsorption. Strain CdR-98 was able to adsorb Cd little higher than other isolates. Being high or low resistance to Cd in each of Cd-resistant bacterial strains, the adsorption capability seems to be equal in low concentration of Cd (10.39 mg/l). The raw data was shown in **Appendix G, Table G-1**.

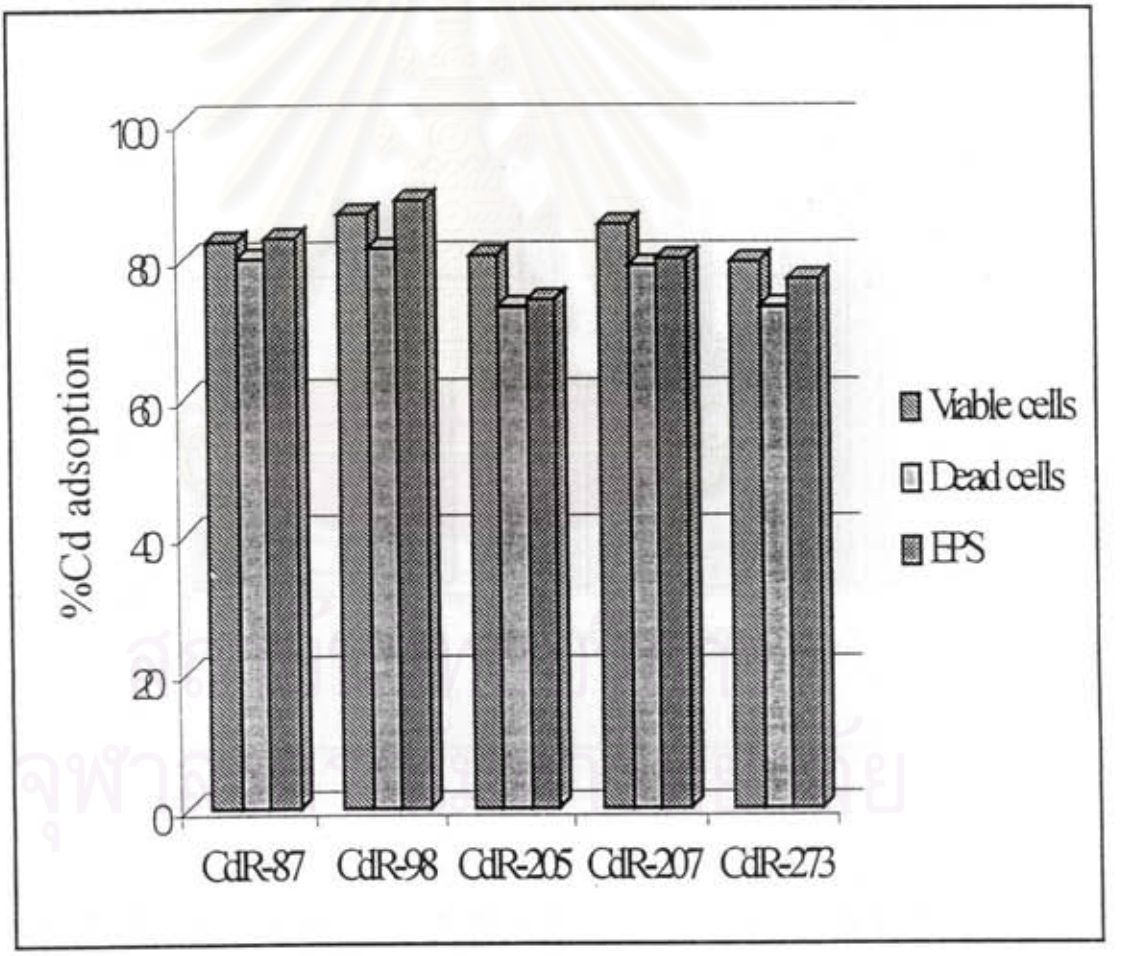


Figure 4.19 The Cd adsorption by viable cells, dead cells and EPS at initial 10.39 mg/l Cd.

4.4.3 Copper Adsorption by Viable Cells, Dead Cells and EPS

Higher Cu adsorption was found in dead cells of all test isolates, especially in CdR-273. The result was shown in **Figure 4.20** The raw data was shown in **Appendix G, Table G-2.**

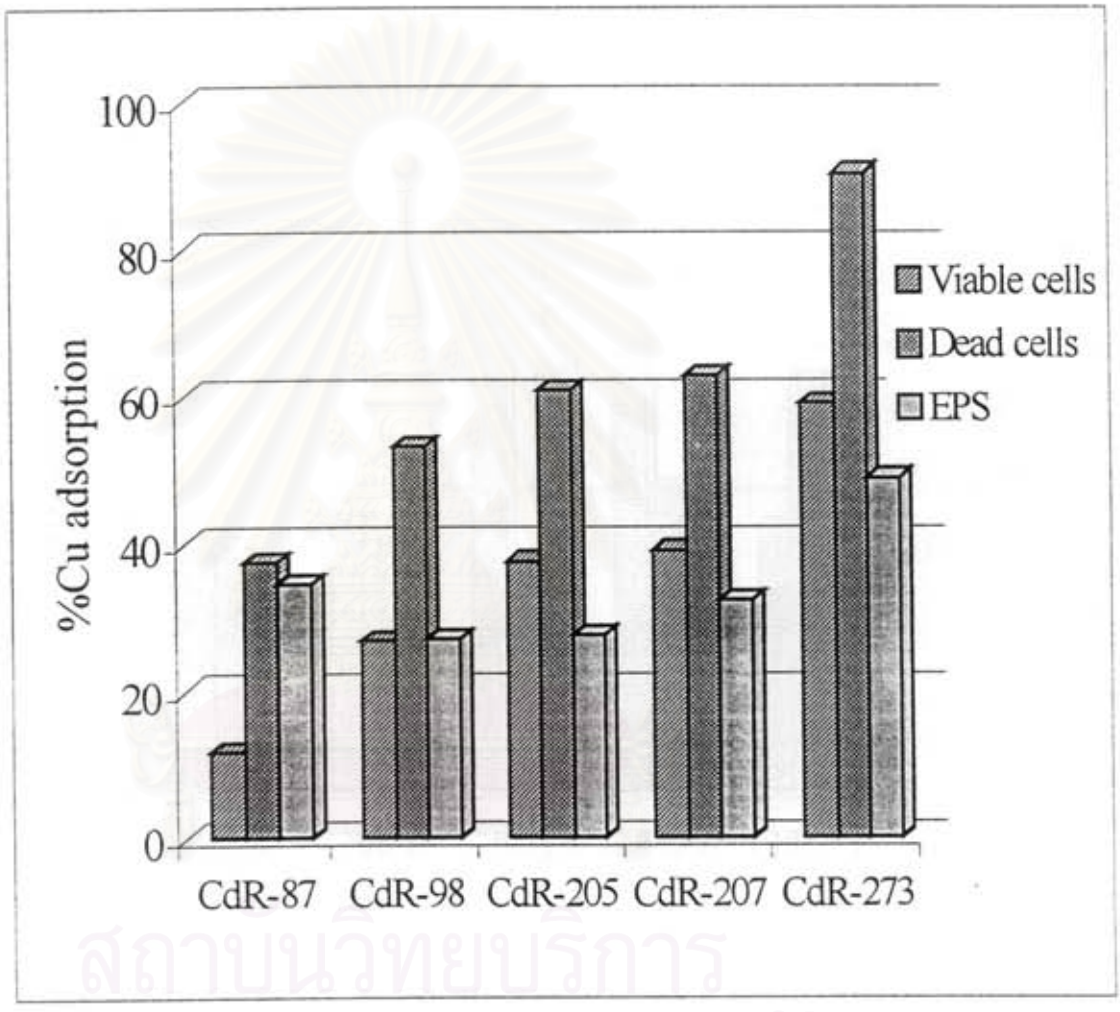


Figure 4.20 The Cu adsorption by viable cell, dead cell and EPS of the selected Cd-resistant bacterial strains, e.g.,CdR-87,CdR-98, CdR-205, CdR-207and CdR-273.

4.5.4 Manganese Adsorption by Viable Cells, Dead Cells and EPS

Higher Mn adsorption was found in viable cells of 4 test isolates, e.g., CdR-87, CdR-98, CdR-205 and CdR-207; but dead cells of CdR-273 was able to adsorb Mn in high concentration (**Figure 4.21**) The raw data were shown in **Appendix G, Table G-3**.

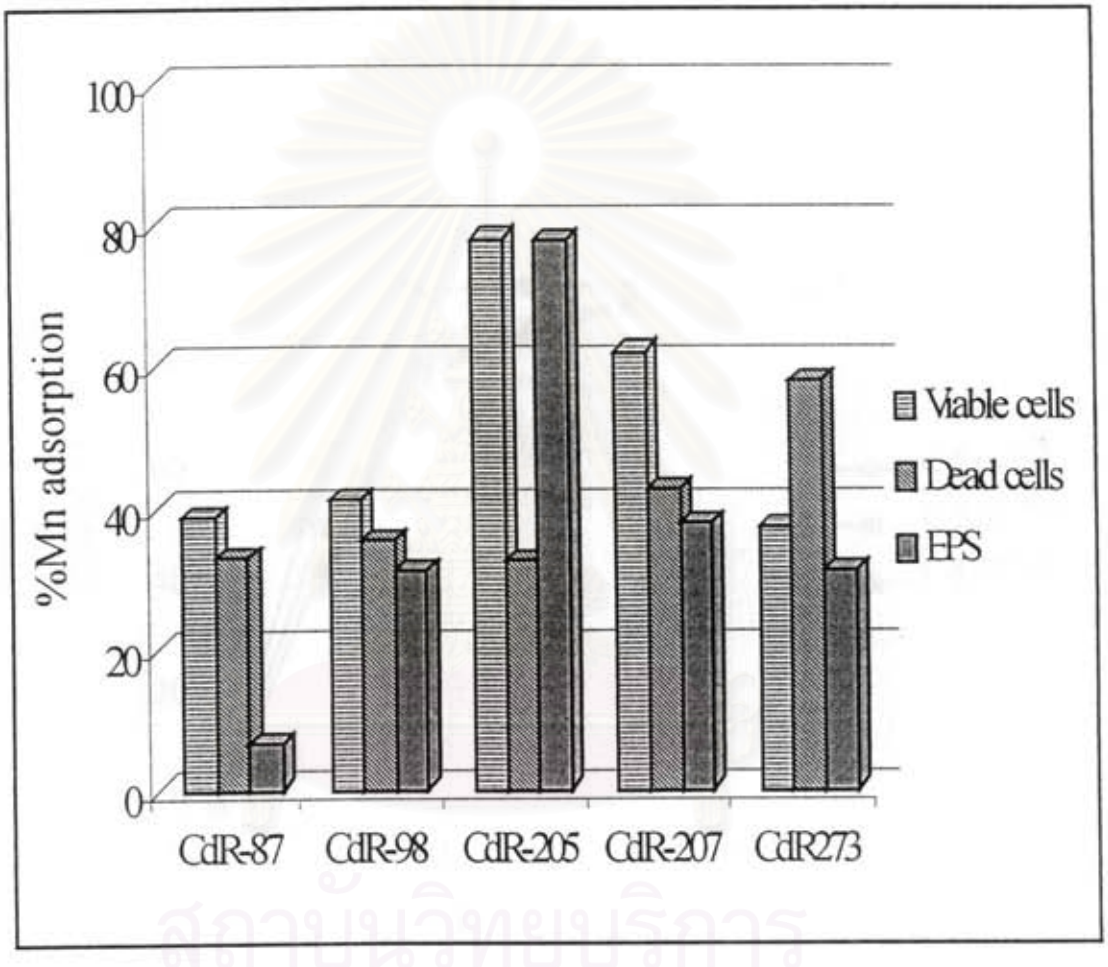


Figure 4.21 The Mn adsorption by viable cell, dead cell and EPS of the Selected Cd-resistant bacterial strains,e.g.,CdR-87, CdR-98, CdR-205, CdR-207and CdR-273.

4.6 IMMOBILIZATION OF SELECTED BACTERIAL STRAINS

4.6.1 Rate of Cd adsorption

Optimum contact time for Cd adsorption by CdR-205 immobilized cells in different Cd concentration were 10 minute in solution contained low Cd concentration (6.86 mg/L)and 20 minutes in higher concentration of Cd (51.95 mg/L) see **Figure 4.22.**

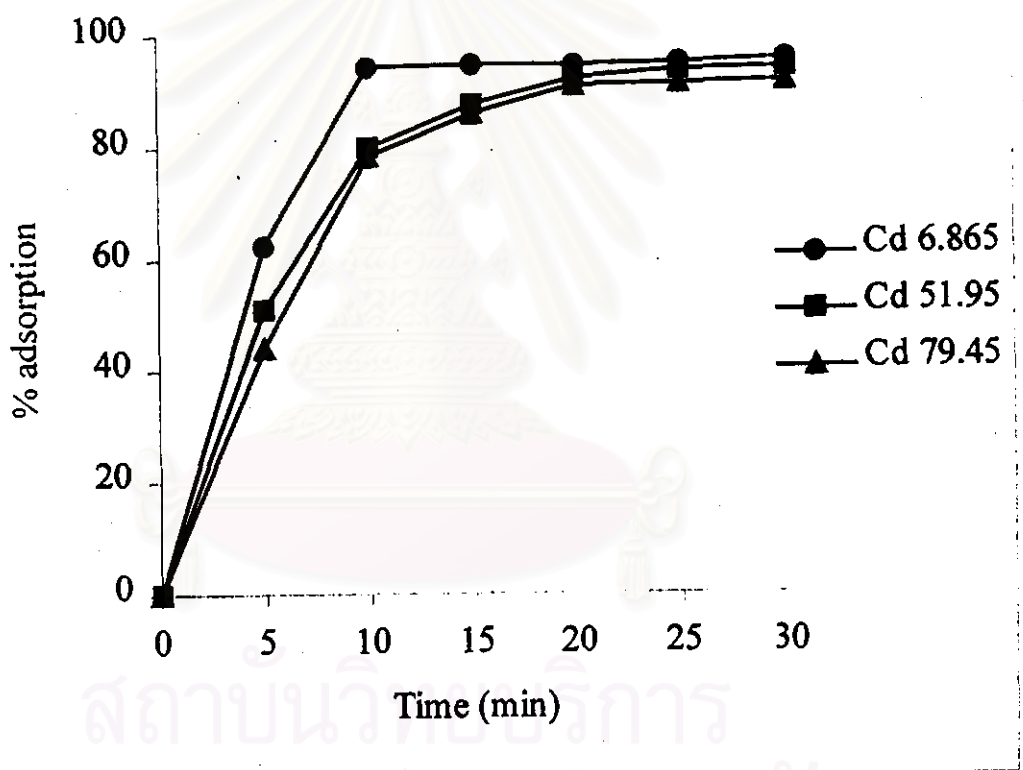


Figure 4.22 Rate of Cd uptake from solution by CdR-205 immobilize cells (●, 10; ■,50; ▲ ,100 mg/l).

4.6.2 Effect of Ca-alginate Beads to Cd Adsorption.

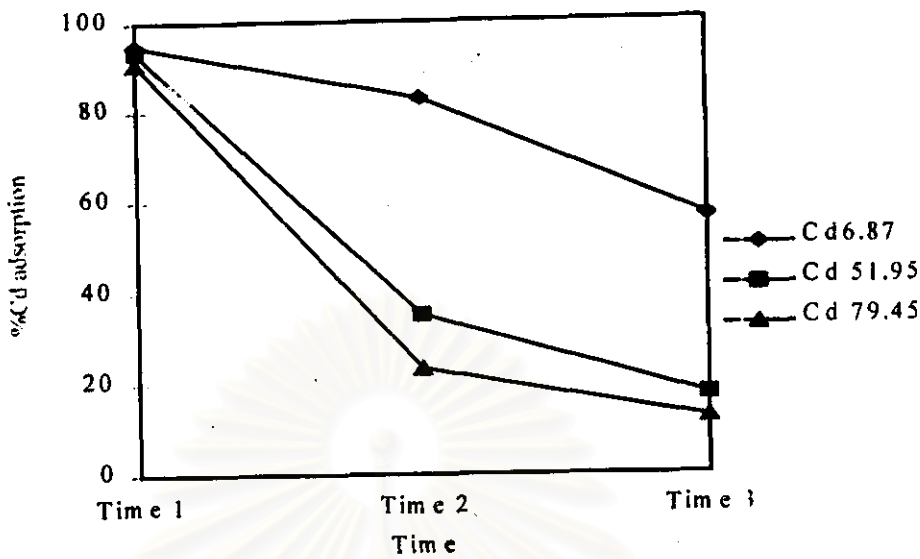
Cd adsorption by calcium alginate bead was detected, however, the capability of adsorption was lower than immobilized cell (**Table 4.6**). Also, higher in Cd concentration, lower in percentage of Cd adsorption by alginate or immobilized cell.

Table 4.6 Percentage of Cd adsorbed by calcium-alginate beads and immobilized cells (calcium-alginate) beads exposed to different concentration.

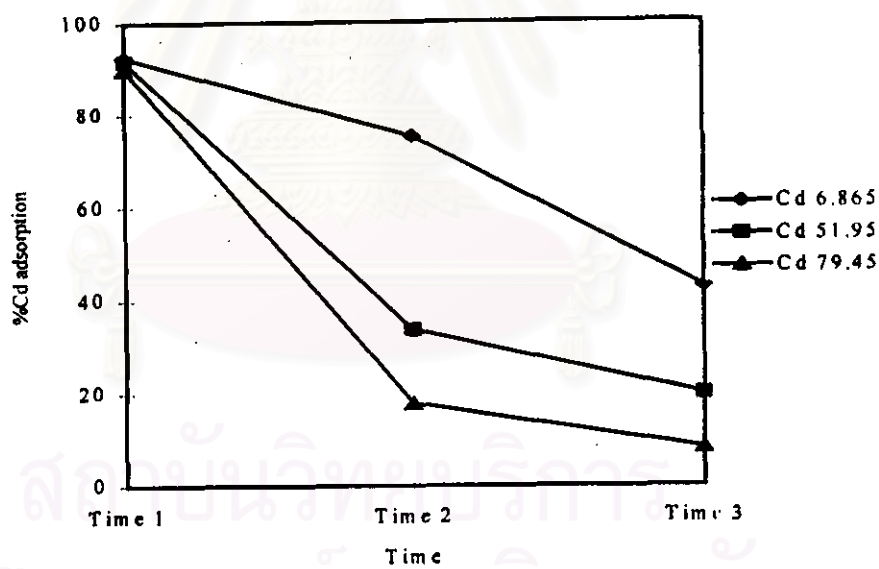
Cd Conc.	Percentage Cd adsorption		
	Alginate without cells	Alginate with CdR-205 cells	Alginate with CdR-273 cells
6.87	48.37	94.80	92.54
51.95	50.64	93.18	91.73
79.45	37.11	90.75	89.96

4.6.3 Regeneration of cells beads for Cd adsorption

The beads were used to recover adsorbed Cd at 6.86, 51.95 and 79.45 mg/l $CdCl_2$ by repeat 3 trials. Percentage of Cd adsorption was more decreased when used 2 and 3 trial, see **Figure 2.23**.



(a)



(b)

Figure 4.23 Cd adsorption by regenerate CdR-205 immobilized bead (a) and CdR-273 immobilized bead (b) (3 times of regeneration).