CHAPTER 4

RESULTS

4.1 ISOLATION, SCREENING AND SELECTION OF CHROMIUM-RESISTANT BACTERIA, PHENOL-RESISTANT BACTERIA AND CHROMIUM/PHENOL-RESISTANT BACTERIA

4.1.1 CHROMIUM, PHENOL AND CHROMIUM/PHENOL-RESISTANT BACTERIAL ISOLATES

In four hundreds and ninety-five strains bacterial isolate, one hundred and fifty bacterial strains resisted to 600 μg/ml Cr in ½ strength NA medium were isolated. Resistances to 800, 1200, 1600, 2000 and 2400 μg/ml Cr were found in 58, 24, 11.3, 4.7 and 2 percent; %, respectively. For phenol-resistant bacterial isolates, two hundred and twenty-five bacterial strains resisted to 600 μg/ml phenol; Ph, in ½ strength NA medium were isolated. Resistances to 800, 1200, 1600 and 2000 μg/ml Ph were found in 48.5, 32.9, 17.3 and 1.3 %, respectively. None of them was resistant to 2400 μg/ml Ph. And chromium/phenol-resistant bacterial isolates, one hundred and twenty bacterial strains resisted to 300 μg/ml Cr and 300 μg/ml Ph; CP in ½ strength NA medium were isolated. Resistances to 400, 600, 800, 1000 and 1200 μg/ml CP were found in 37.5, 30, 19.2, 10.8 and 2.5 %, respectively, all of these resistant bacterial isolates as shown in **Table 4.1**, on page 65. Three Cr-resistant isolates, three Ph-resistant isolates and three CP-resistant isolates were selected, namely: CrR-2; CrR-14; CrR-15, PhR-26; PhR-33; PhR-64 and CPR-4; CPR-16; CPR-17. From **Table 4.2** on page 66-67, the first

three bacterial strains were rod shape, gram-negative and identified as ***Escherichia* sp.**, ***Pseudomonas* sp.** and ***Enterobacter* sp.**, respectively, and they were isolated from soil collected from oil refinery and chromium plating. The second three bacterial strains were rod shape, gram-negative and identified as ***Klebsiella* sp.**, ***Pseudomonas* sp.** and ***Escherichia* sp.**, respectively, and they were isolated from soil collected from wood preserving, painting and chemical production. The last three bacterial strains were also rod shape, gram-negative and identified as ***Pseudomonas* sp.**, ***Proteus* sp.** and ***Escherichia* sp.**, respectively, and they were isolated from soil collected from oil refinery and metal plating.

The colonial and cell characteristics of the nine selected bacterial isolates are shown in **Figure 4.1-4.9**, on page 68-76.

4.1.2 STABILITY OF BACTERIAL RESISTANCE

The results of the stability of Cr resistance, Ph resistance and CP resistance of the nine selected bacterial isolates are briefly summarized in **Table 4.3**, on page 77. Cr-resistant and CP-resistant bacterial isolates were able to maintain the highest Cr and CP resistance, 2400 and 1200 μg/ml, respectively, after, at least 18 times of subculturing in NA containing small amount of Cr and CP. But Ph–resistant bacterial isolates were able to maintain the highest Ph resistance (2000 μg/ml) after, at least 15 times of subculturing in NA containing small amount of Ph.

Table 4.1 Three sets of experiment about resistance in 495 strains bacterial isolates

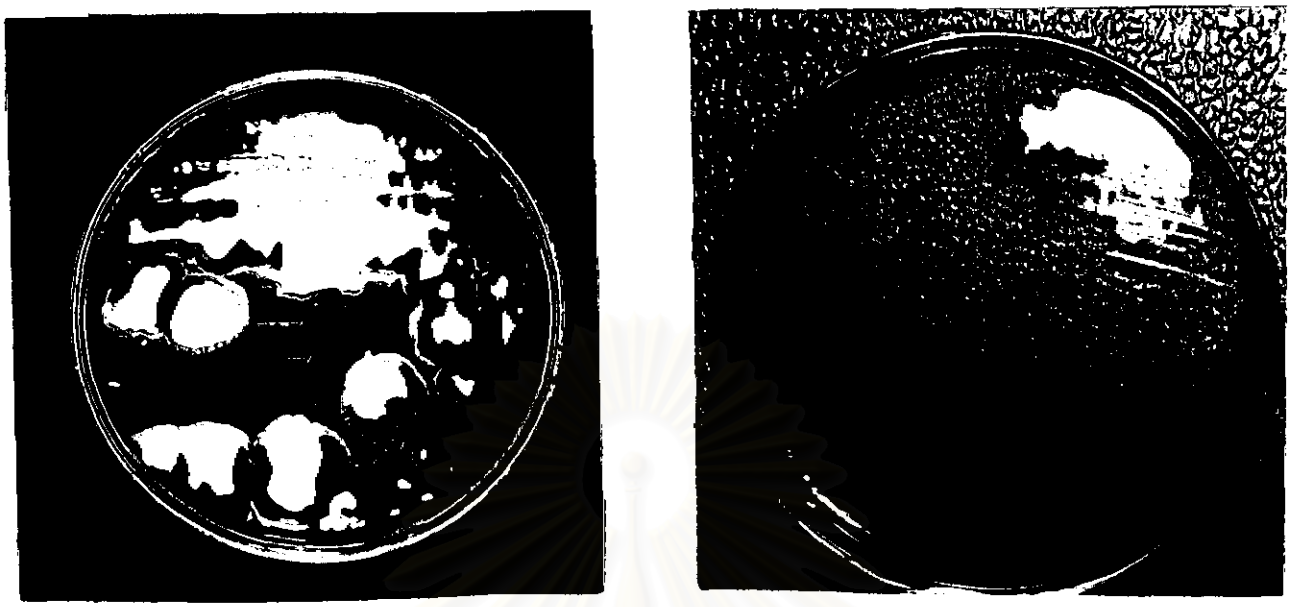
Sets of Experiment	Concentration (μg/ml)	No. of Strains	%
Chromium	800	87	58.0
	1200	36	24.0
	1600	17	11.3
	2000	7	4.7
	2400	3	2.0
Sub Total		150	100
Phenol	800	109	48.5
	1200	74	32.9
	1600	39	17.3
	2000	3	1.3
	2400	0	0
Sub Total		225	100
Chromium/Phenol	400	45	37.5
	600	36	30.0
	800	23	19.2
	1000	13	10.8
	1200	3	2.5
Sub Total		120	100
Grand Total		495	100

Table 4.2 Some characteristics and identification on nine strains of the chromium-resistant, phenol-resistant and chromium/phenol-resistant bacterial isolates

Bacterial Isolates	Sources (Sampling Site)	Characteristic of		Identified as
		Colony	Morphology	
CrR-2	Soil, (Oil refinery, area VI, Samutprakarn)	~3 mm in diameter, white	Rod-shape, gram-negative,	***Escherichia* sp.**
CrR-14	Soil, (Chromium plating, area I, Bangkok)	~2 mm in diameter, white	Rod-shape, gram-negative,	***Pseudomonas* sp.**
CrR-15	Soil, (Chromium plating, area I, Bangkok)	~3 mm in diameter, white	Rod-shape, gram-negative,	***Enterobacter* sp.**
PhR-26	Soil, (Wood, area I, Bangkok)	~2 mm in diameter, white	Rod-shape, gram-negative,	***Klebsiella* sp.**
PhR-33	Soil, (Painting, area II, Bangkok)	~3 mm in diameter, white	Rod-shape, gram-negative,	***Pseudomonas* sp.**
PhR-64	Soil, (Chemical Production Industry, area V, Bangkok)	~3 mm in diameter, white	Rod-shape, gram-negative,	***Escherichia* sp.**

Table 4.2 (Cont.)

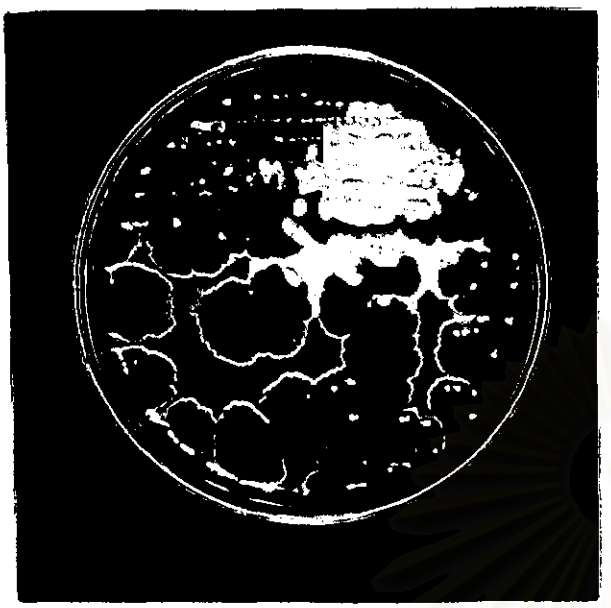
Bacterial Isolates	Sources (Sampling Site)	Characteristic of		Identified as
		Colony	Morphology	
CPR-4	Soil, (Oil refinery, area VI, Samutprakarn)	~3 mm in diameter, white	Rod-shape, gram-negative,	***Pseudomonas* sp.**
CPR-16	Soil, (Metal Plating, area V, Bangkok)	~2 mm in diameter, white	Rod-shape, gram-negative,	***Proteus* sp.**
CPR-17	Soil, (Metal Plating, area V, Bangkok)	~2 mm in diameter, white	Rod-shape, gram-negative,	***Escherichia* sp.**



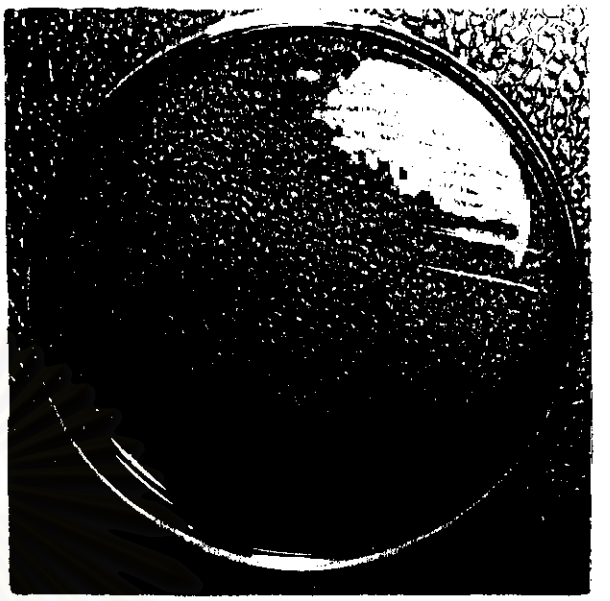
(a) (b)

(c)

Figure 4.1 Colonial characteristics of chromium-resistant bacterial strains CrR-2 (***Escherichia* sp.**) grown on NA (a) and NA containing 2400 μg/ml chromium (b), incubated at 37°C for 24 hr., and gram staining (c)

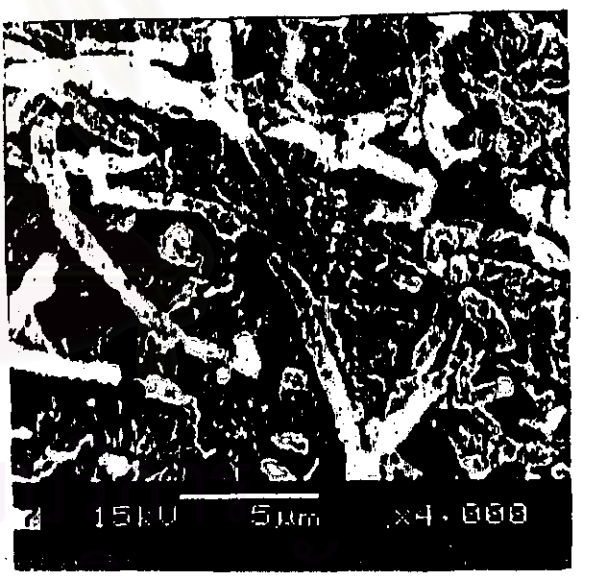


(a)



(b)

(c)



(d)

Figure 4.2 Colonial characteristics of chromium-resistant bacterial strains CrR-14 (***Pseudomonas*** **sp.**) grown on NA (a) and NA containing 2400 µg/ml chromium (b), incubated at 37°C for 24 hr., gram staining (c), and Electron Microscope (d)

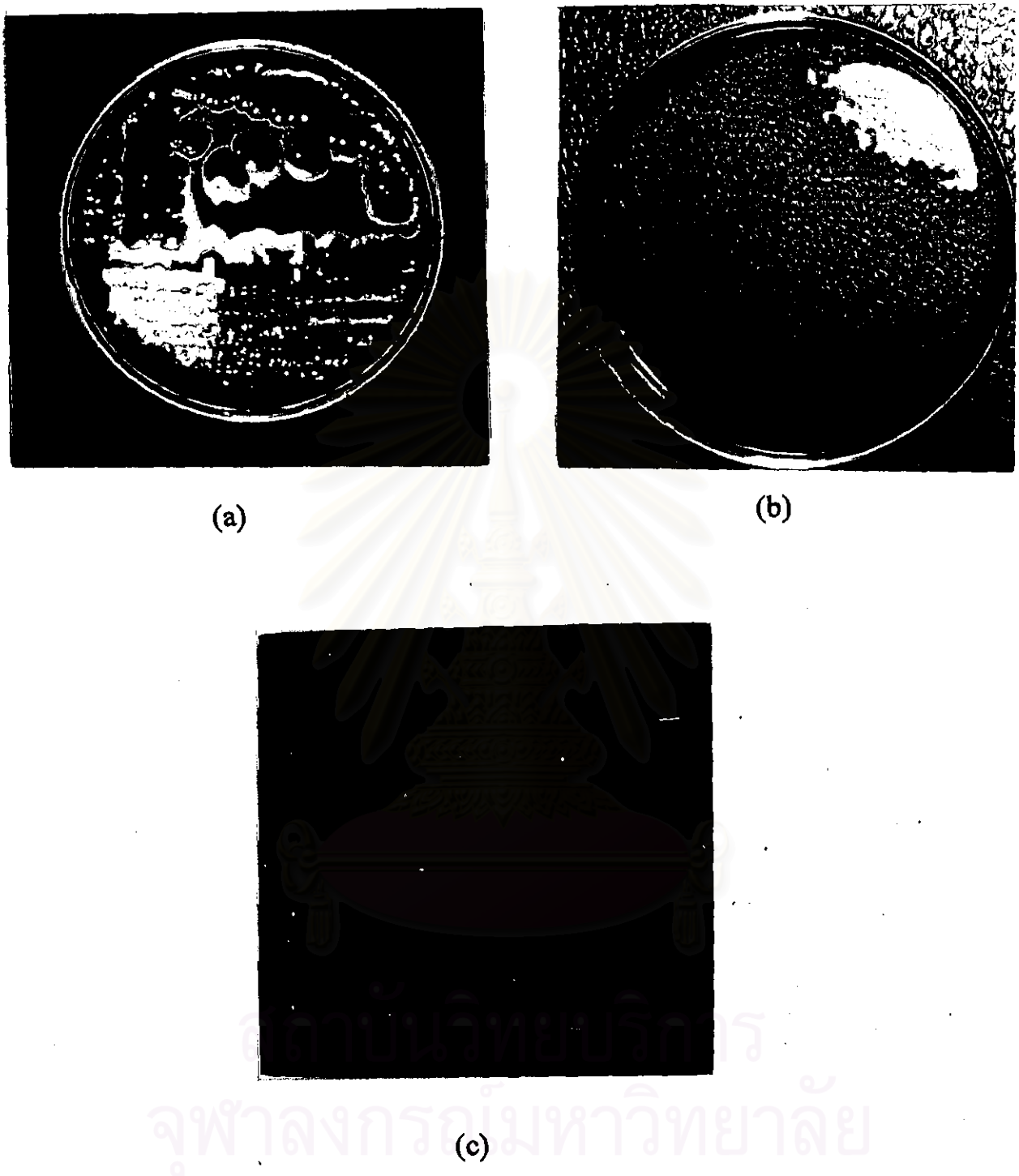
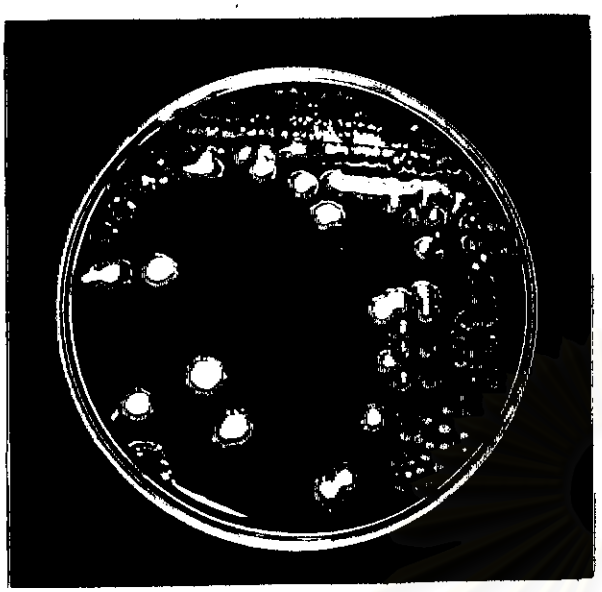
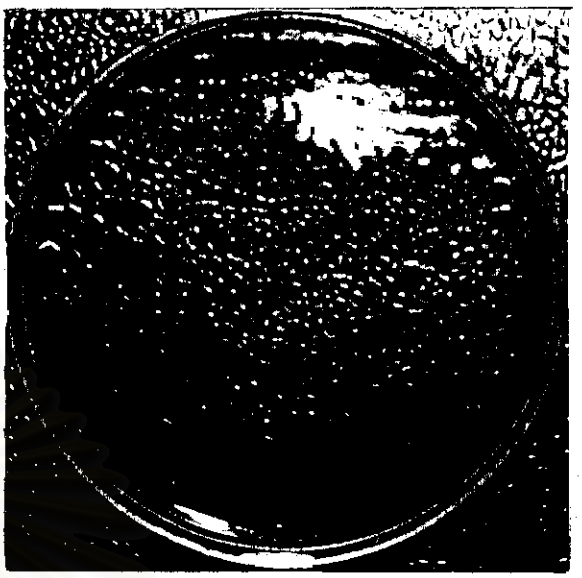


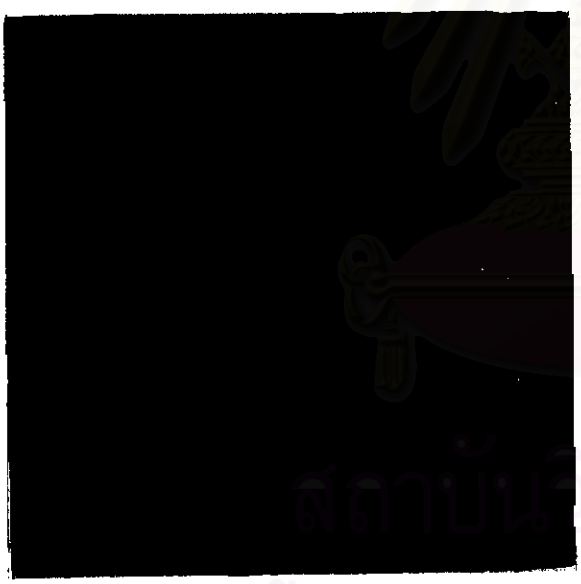
Figure 4.3 Colonial characteristics of chromium-resistant bacterial strains CrR-15 (***Enterobacter*** **sp.**) grown on NA (a) and NA containing 2400 μg/ml chromium (b), incubated at 37°C for 24 hr., and gram staining (c)



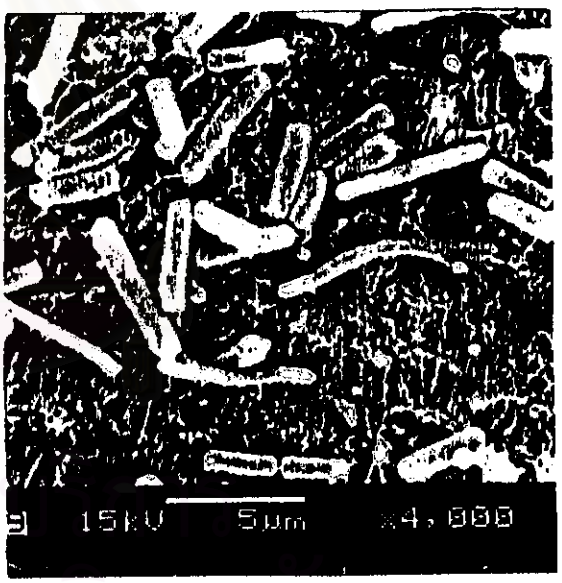
(a)



(b)

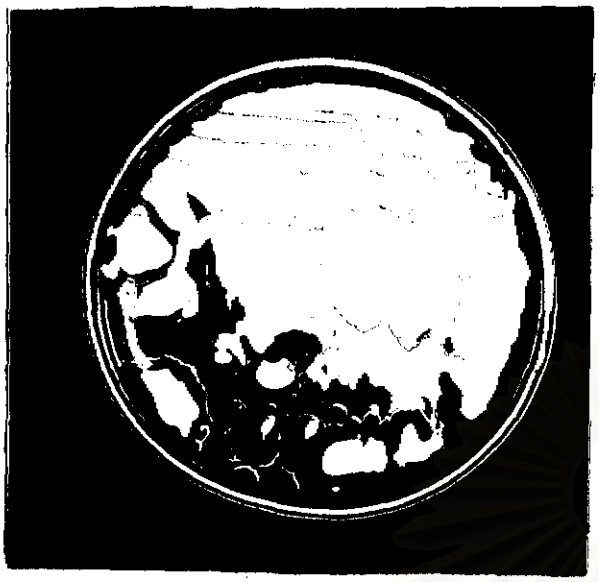


(c)



(d)

Figure 4.4 Colonial characteristics of phenol-resistant bacterial strains PhR-26 (***Klebsiella* sp.**) grown on NA (a) and NA containing 2000 μg/ml phenol (b), incubated at 37°C for 24 hr., gram staining (c), and Electron Microscope (d)



(a)

(b)

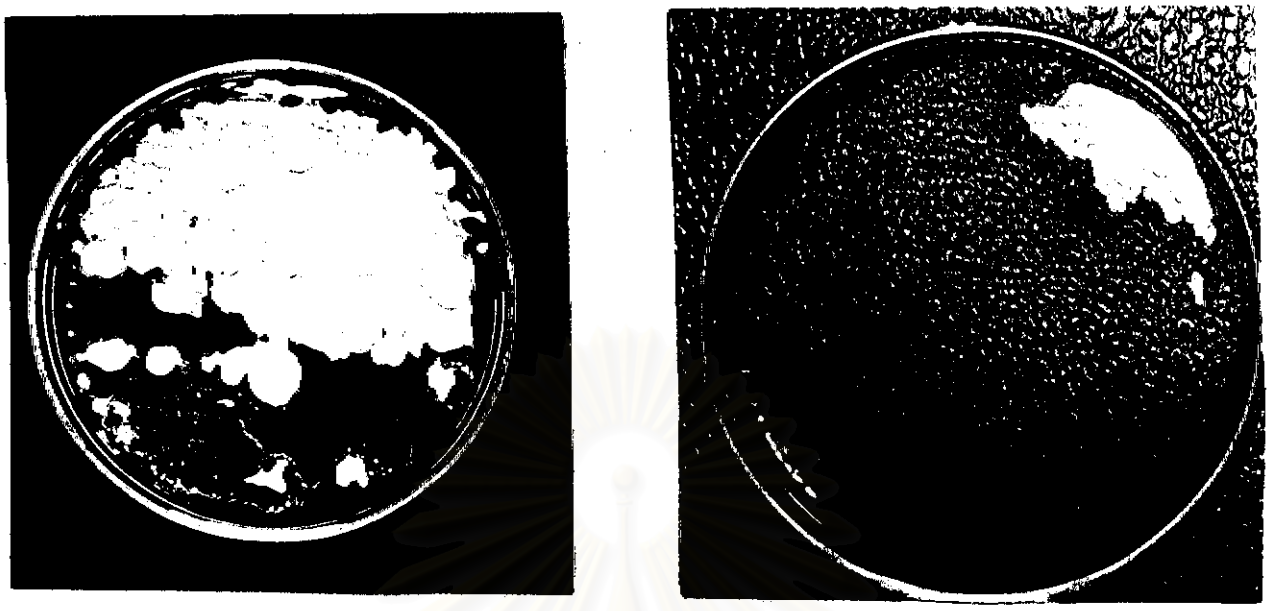
(c)

Figure 4.5 Colonial characteristics of phenol-resistant bacterial strains PhR-33 (***Pseudomonas*** **sp.**) grown on NA (a) and NA containing 2000 μg/ml phenol (b), incubated at 37°C for 24 hr., and gram staining (c)

(a) (b)

(c)

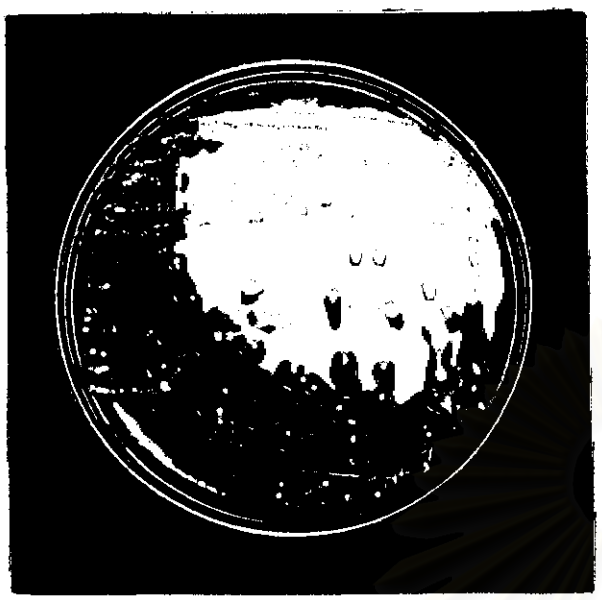
Figure 4.6 Colonial characteristics of phenol-resistant bacterial strains PhR-64 (***Escherichia* sp.**) grown on NA (a) and NA containing 2000 µg/ml phenol (b), incubated at 37°C for 24 hr., and gram staining (c)



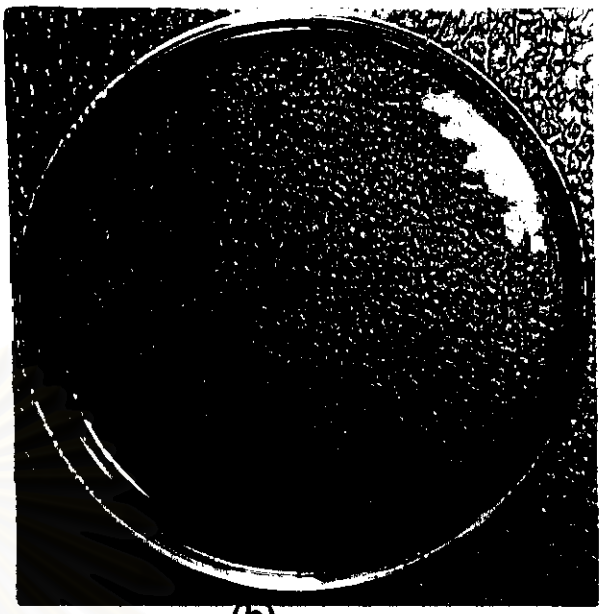
(a) (b)

(c)

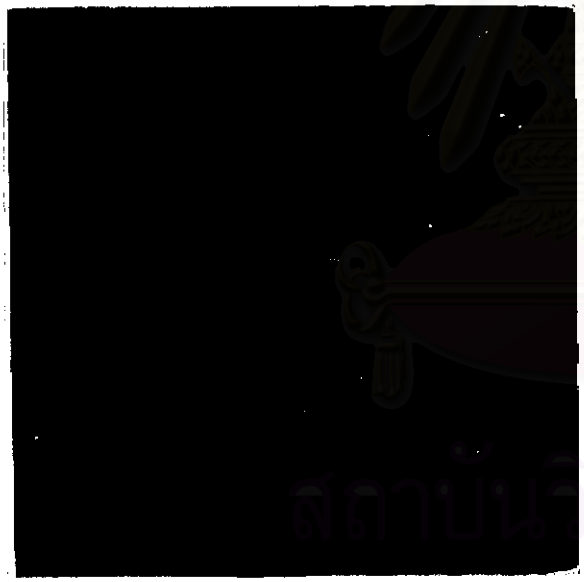
Figure 4.7 Colonial characteristics of chromium/phenol-resistant bacterial strains CPR-4 (***Pseudomonas*** **sp.**) grown on NA (a) and NA containing 1200 μg/ml chromium-phenol (b), incubated at 37°C for 24 hr., and gram staining (c)



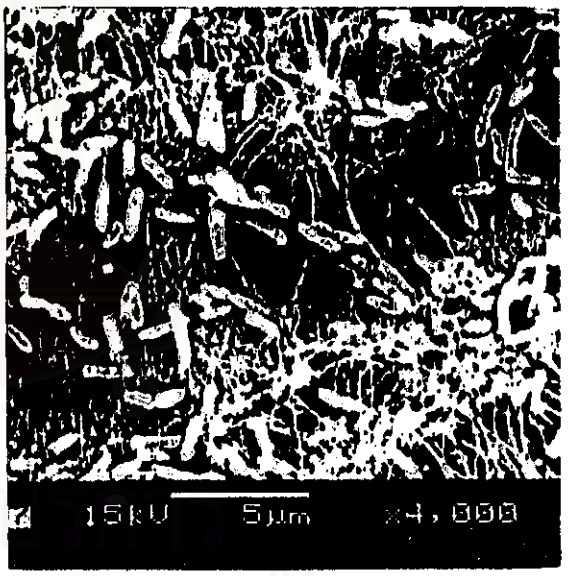
(a)



(b)



(c)



(d)

Figure 4.8 Colonial characteristics of chromium/phenol-resistant bacterial strains CPR-16 (***Proteus* sp.**) grown on NA (a) and NA containing 1200 µg/ml chromium-phenol (b), incubated at 37°C for 24 hr., gram staining (c), and Electron Microscope (d)

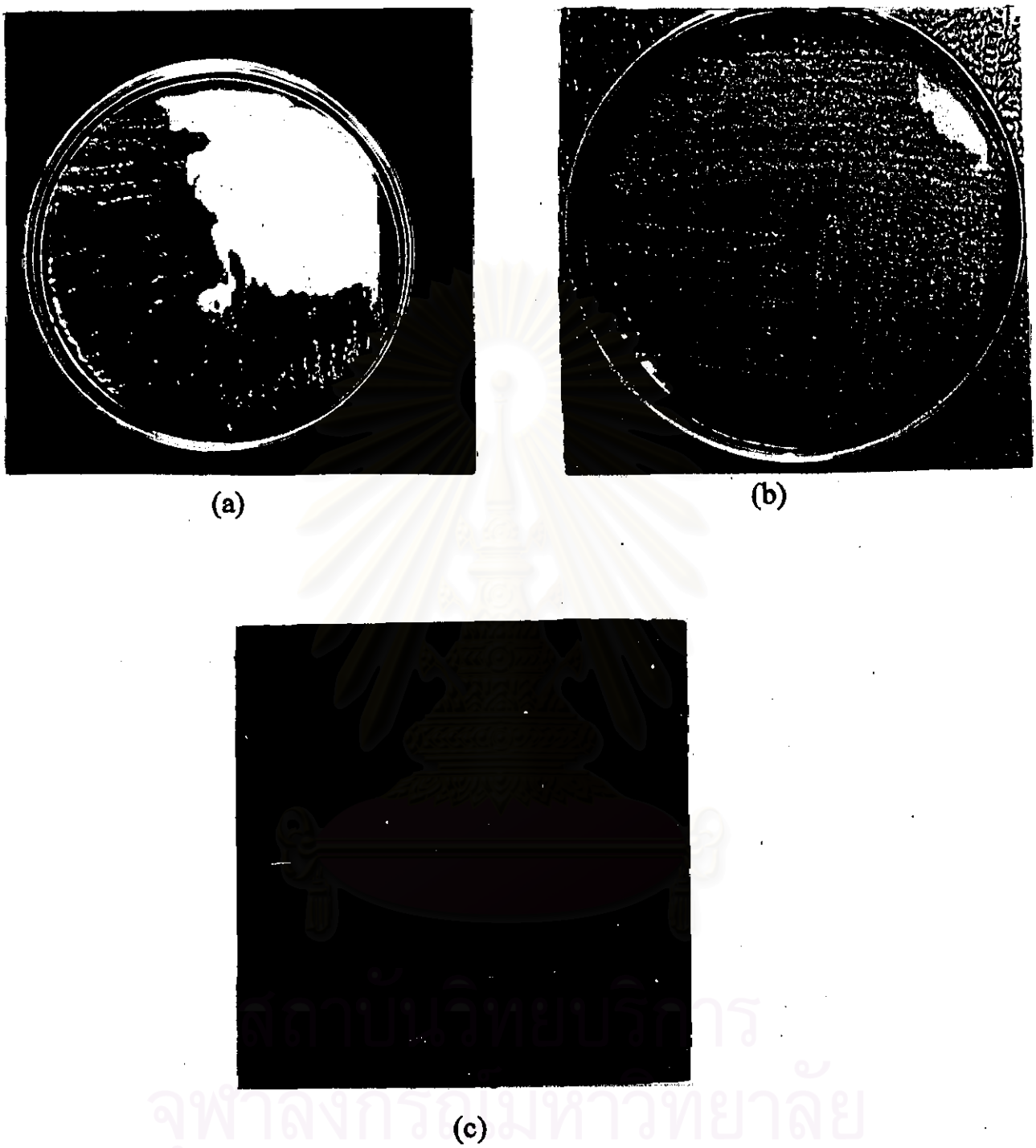


Figure 4.9 Colonial characteristics of chromium/phenol-resistant bacterial strains CPR-17 (***Escherichia* sp.**) grown on NA (a) and NA containing 1200 μg/ml chromium-phenol (b), incubated at 37°C for 24 hr., and gram staining (c)

Table 4.3 Stability of the resistance to chromium or phenol or chromium/phenol of nine selected bacterial strains after repeated culturing

Bacterial Isolates		Resistant to	Stability of
Strains	Identified as	Concentration (μg/ml)	Resistance*
CrR-2	***Escherichia* sp.**	2400	18
CrR-14	***Pseudomonas* sp.**	2400	18
CrR-15	***Enterobacter* sp.**	2400	18
PhR-26	***Klebsiella* sp.**	2000	15
PhR-33	***Pseudomonas* sp.**	2000	15
PhR-64	***Escherichia* sp.**	2000	15
CPR-4	***Pseudomonas* sp.**	1200	18
CPR-16	***Proteus* sp.**	1200	18
CPR-17	***Escherichia* sp.**	1200	18

* After at least 20 times of repeated subculturing

4.1.3 RESISTANCE OF THE SELECTED BACTERIAL STRAINS TO OTHER HEAVY METALS

All of nine strains of the Cr-resistant, Ph-resistant and CP-resistant bacterial isolates were found to be resistant to a number of other heavy metals, i.e., As and Zn, but none of them were able to resist to Ag, Cd, Cu, Ni and Mn; detailed results were summarized in **Table 4.4**, on page 79. The bacterial strains CrR-2, CrR-14 and CrR-15 were resistant to the same resistance levels of Cr, Ph, As and Zn, i. e., 2400, 1000, 200 and 100 μg/ml, respectively; strains, PhR-26, PhR-33 and PhR-64 resisted Ph, Cr, As and Zn at the same resistance levels, i. e., 2000, 500, 200 and 100 μg/ml, respectively. The bacterial strains, CPR-4, CPR-16 and CPR-17 were resisted Cr, Ph, As and Zn, i. e., 1200, 1200, 200 and 100 μg/ml.

4.1.4 EFFECTS OF pH AND TEMPERATURE ON VIABLE COUNTS OF THE SELECTED BACTERIAL STRAINS

The optimum pH and temperature of those selected bacterial isolates were found to be 7 and 37°C, respectively (summarized in **Table 4.5**, on page 80). Most pH level 10, the reduction on number of viable cells was higher than at the pH level 4. It may imply that the effect of alkaline condition on growth was stronger than acidic condition, but the effect of temperature at higher (40°C) or lower (30°C) levels was not as strong as pH levels.

In bacterial isolates, the effects of pH and temperature were shown in **Figure 4.10 and Figure 4.11**, on page 81 and 82.

Table 4.4 Resistance of the selected bacterial strains to other heavy metals

Strains	Resistant Concentration of Heavy Metal (μg/ml)								
	Cr	Ph	As	Zn	Ag	Cd	Cu	Ni	Mn
CrR-2	2400	1000	200	100	-	-	-	-	-
CrR-14	2400	1000	200	100	-	-	-	-	-
CrR-15	2400	1000	200	100	-	-	-	-	-
PhR-26	500	2000	200	100	-	-	-	-	-
PhR-33	500	2000	200	100	-	-	-	-	-
PhR-64	500	2000	200	100	-	-	-	-	-
CPR-4	1200	1200	200	100	-	-	-	-	-
CPR-16	1200	1200	200	100	-	-	-	-	-
CPR-17	1200	1200	200	100	-	-	-	-	-

Table 4.5 Effect of pH and temperature on growth of the selected bacterial strains

Strains	Initial no. of Organism ($x10^8$ cells/ml)	Viable Count ($x10^8$ cells/ml)									
		pH							Temp (°C)		
		4	5	6	7	8	9	10	30	37	40
CrR-2	1.12	1.09	1.12	1.14	1.15	1.13	1.09	1.08	1.13	1.17	1.15
CrR-14	1.16	1.02	1.16	1.21	1.24	1.22	1.18	1.17	1.17	1.25	1.20
CrR-15	1.15	1.12	1.16	1.17	1.20	1.17	1.14	1.09	1.15	1.18	1.17
PhR-26	1.14	1.02	1.09	1.21	1.25	1.24	1.15	1.14	1.15	1.17	1.16
PhR-33	1.14	1.01	1.15	1.20	1.25	1.22	1.18	1.17	1.16	1.19	1.18
PhR-64	1.14	1.02	1.13	1.20	1.26	1.25	1.19	1.16	1.17	1.20	1.18
CPR-4	1.15	1.03	1.14	1.21	1.23	1.22	1.20	1.15	1.16	1.21	1.17
CPR-16	1.12	1.02	1.13	1.15	1.22	1.21	1.20	1.18	1.18	1.21	1.20
CPR-17	1.13	1.02	1.07	1.17	1.21	1.19	1.17	1.15	1.15	1.24	1.18

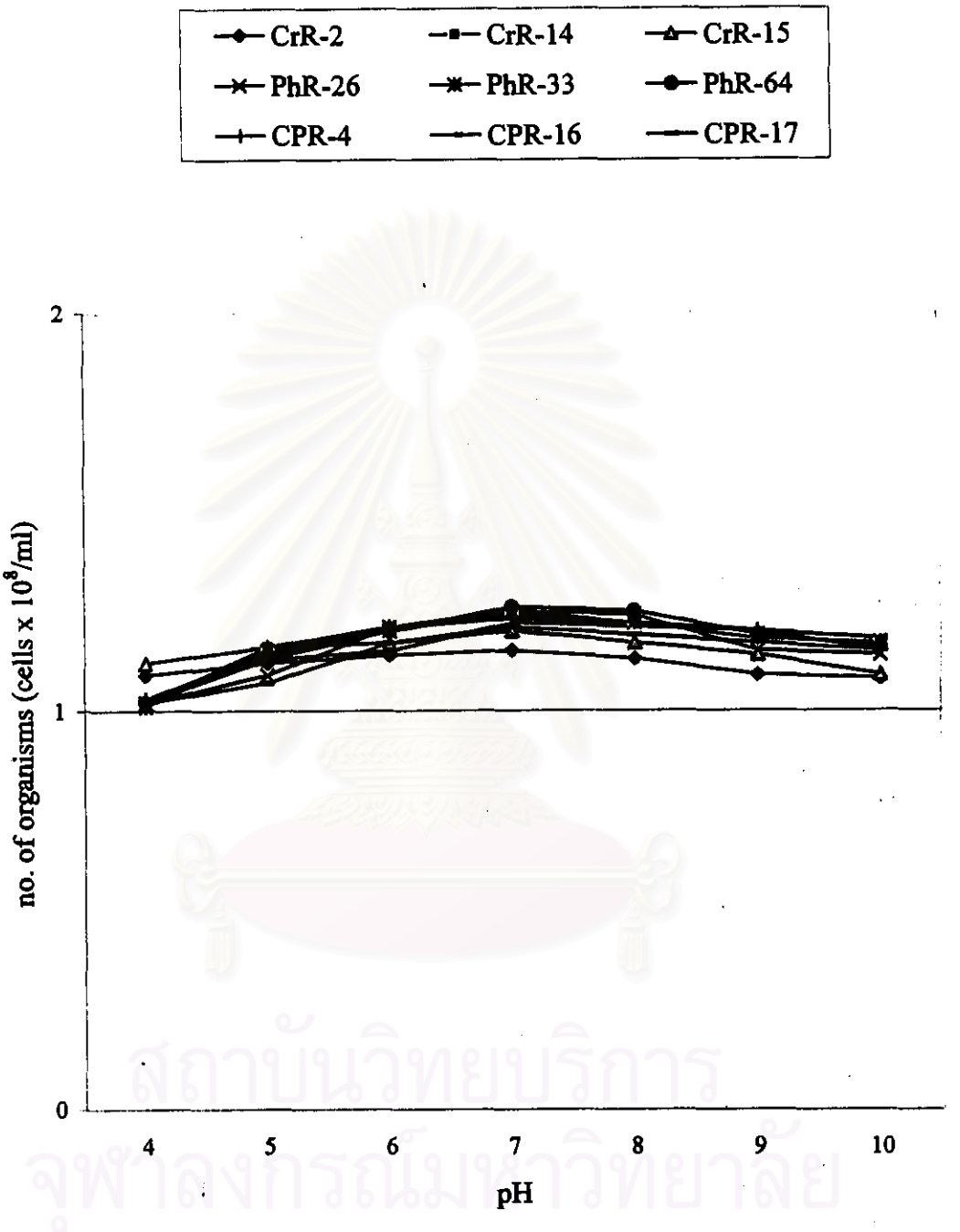


Figure 4.10 Effect of pH on growth of the bacterial strains

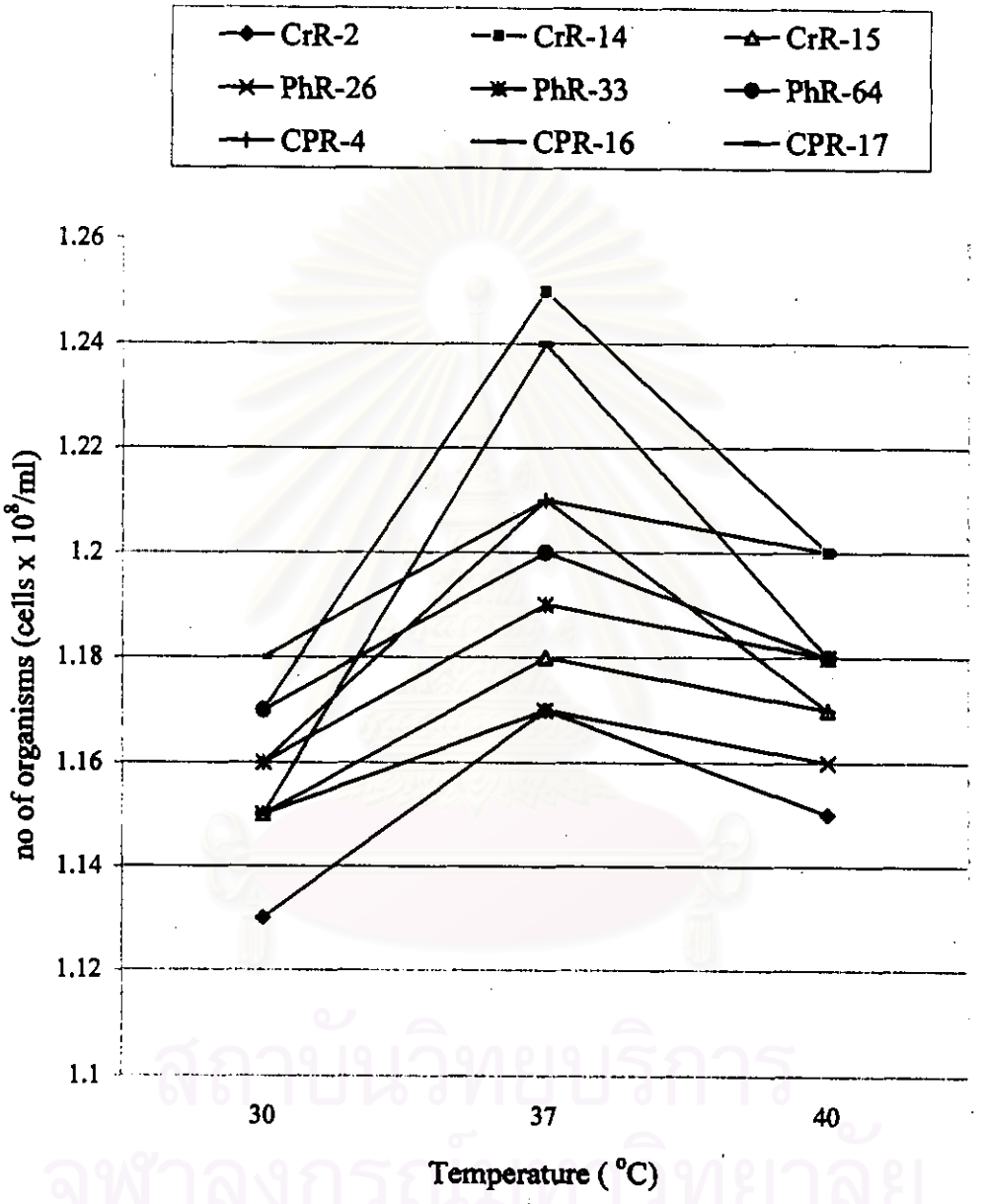


Figure 4.11 Effect of temperature on growth of the bacterial strains

4.2 EFFECTS OF PHENOL ON GROWTH RATE OF THE SELECTED BACTERIAL STRAINS

The results on growth of the Cr-resistant, Ph-resistant and CP-resistant bacterial strains; CrR-15, PhR-26, PhR-33, PhR-64 and CPR-16, respectively, were shown in **Table 4.6**, on page 84. Comparing between growth on 0.85% normal saline and phosphate buffer, as control, with 0.85% normal saline and phosphate buffer containing 300 μg/ml, as testing. It was found that growth of all test bacterial isolates in the medium containing 300 μg/ml (testing) was better than medium (control), was shown in **Figure 4.12**, on page 85. It is possible to say that, in the condition containing phenol, the selected bacterial strains in this study seems to use phenol as carbon and energy source.

4.3 EFFICIENCY OF CHROMIUM DETOXIFICATION AND PHENOL DEGRADATION BY THE SELECTED BACTERIAL STRAINS

4.3.1 INCUBATION PERIODS AND CONTACT TIME

Incubation periods and contact time for growth on chromium detoxification and phenol degradation of the bacterial isolate; CPR-16 were found to be 6-hr. and 15 min.; summarized briefly in **Table 4.7**, on page 86. The results indicated that the equilibrium was taken within 6-hr., 15 min. and prolonged exposure time did not increase Cr(VI) detoxification, Cr(III) production and phenol degradation from the solution. Efficiency of Cr(VI) detoxification, Cr(III) production and phenol degradation were 75.3, 0.3 and 92.7%, respectively., as shown in **Figure 4.13** and **Figure 4.14**, on page 87 and 88.

Table 4.6 Effects of phenol on growth rate of the selected bacterial strains

Strains	Viable Count ($x10^8$ cells/ml)			
	0.85% Normal Saline		Phosphate Buffer	
	Control	NS + 300 µg/ml Phenol	Control	PB + 300 µg/ml Phenol
CrR-15	0.15	3.05	0.35	9.33
PhR-26	0.13	3.00	0.06	2.73
PhR-33	0.08	2.67	0.13	4.83
PhR-64	0.33	7.00	0.11	4.33
CPR-16	0.13	3.67	0.11	2.53

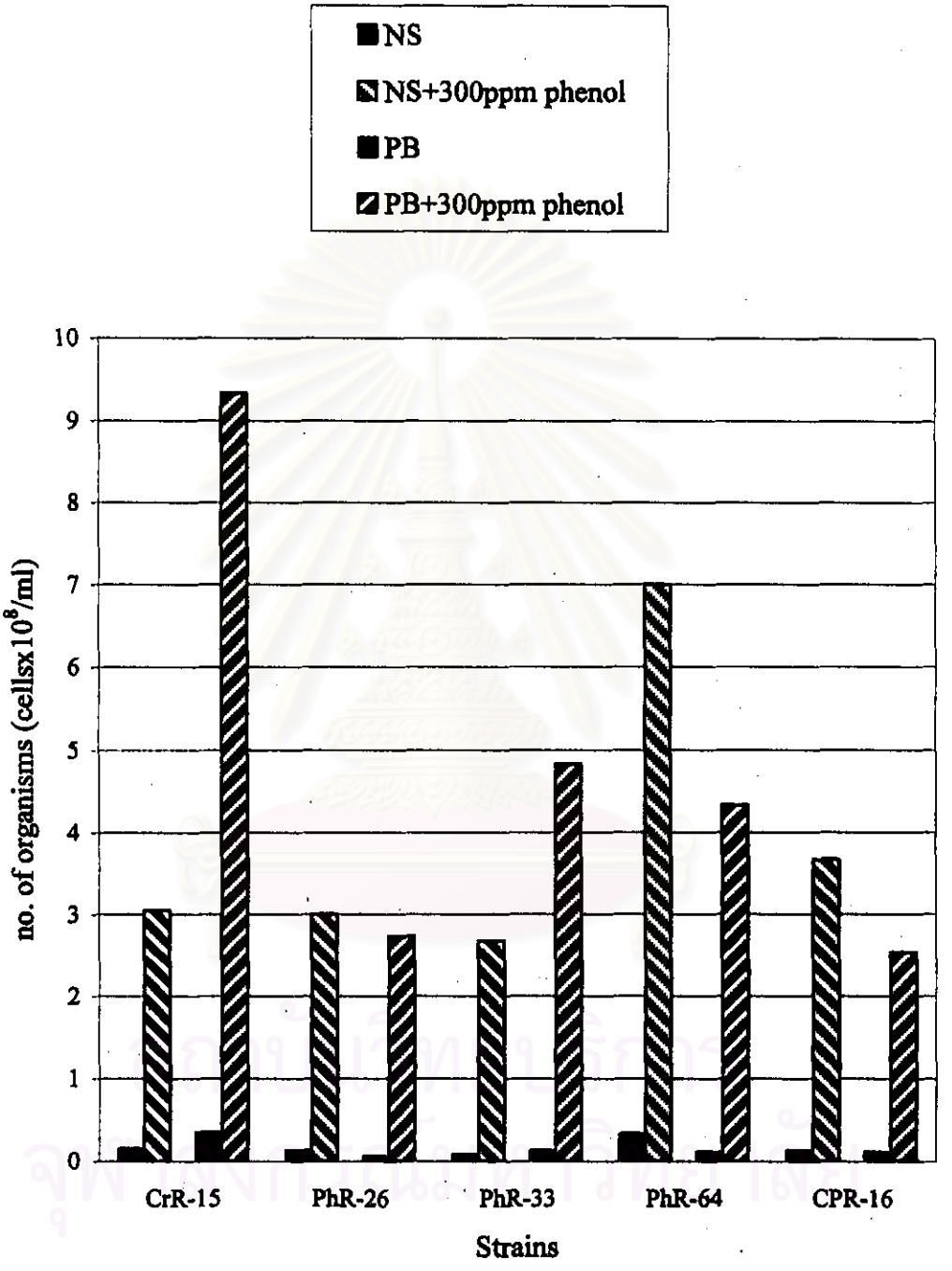


Figure 4.12 Effects of phenol on growth rate of the selected bacterial strains

Table 4.7 Effect of incubation periods of CPR-16 on contact times

Incubation Period (hr.)	Contact Time (min.)											
	15			30			45			60		
	Cr(VI)[1] (%)	Cr(III)[2] (%)	Ph[3] (%)	Cr(VI)[1] (%)	Cr(III)[2] (%)	Ph[3] (%)	Cr(VI)[1] (%)	Cr(III)[2] (%)	Ph[3] (%)	Cr(VI)[1] (%)	Cr(III)[2] (%)	Ph[3] (%)
6	226±0.7 (75.3)	1±0.0 (0.3)	278±0.8 (92.7)	227±0.8 (75.7)	1±0.0 (0.3)	277±0.8 (92.3)	230±0.7 (76.7)	2±0.1 (0.7)	274±0.8 (91.3)	226±0.7 (75.3)	3±0.1 (1.0)	282±0.8 (94.0)
12	231±0.6 (77.0)	5±0.1 (1.7)	252±0.8 (84.0)	230±0.7 (76.7)	3±0.1 (1.0)	258±0.8 (86.0)	228±0.7 (76.0)	3±0.1 (1.0)	277±0.8 (92.3)	227±0.8 (75.7)	5±0.1 (1.7)	276±0.8 (92.0)
24	246±0.8 (82.0)	11±0.3 (3.7)	262±0.8 (87.3)	240±0.7 (80.0)	8±0.2 (2.7)	265±0.8 (88.3)	239±0.7 (81.0)	6±0.2 (2.0)	224±0.7 (74.7)	239±0.7 (79.7)	6±0.2 (2.0)	223±0.7 (74.3)
48	242±0.8 (80.7)	12±0.4 (4.0)	226±0.7 (75.3)	245±0.8 (81.7)	18±0.5 (6.0)	261±0.8 (87.0)	246±0.8 (81.3)	12±0.4 (4.0)	251±0.8 (83.7)	246±0.8 (82.0)	12±0.4 (4.0)	248±0.7 (82.7)

[1] Loss of Cr(VI) Concentration, µg/ml (%)

[2] Found of Cr(III) Concentration, µg/ml (%)

[3] Loss of Phenol Concentration, µg/ml (%)

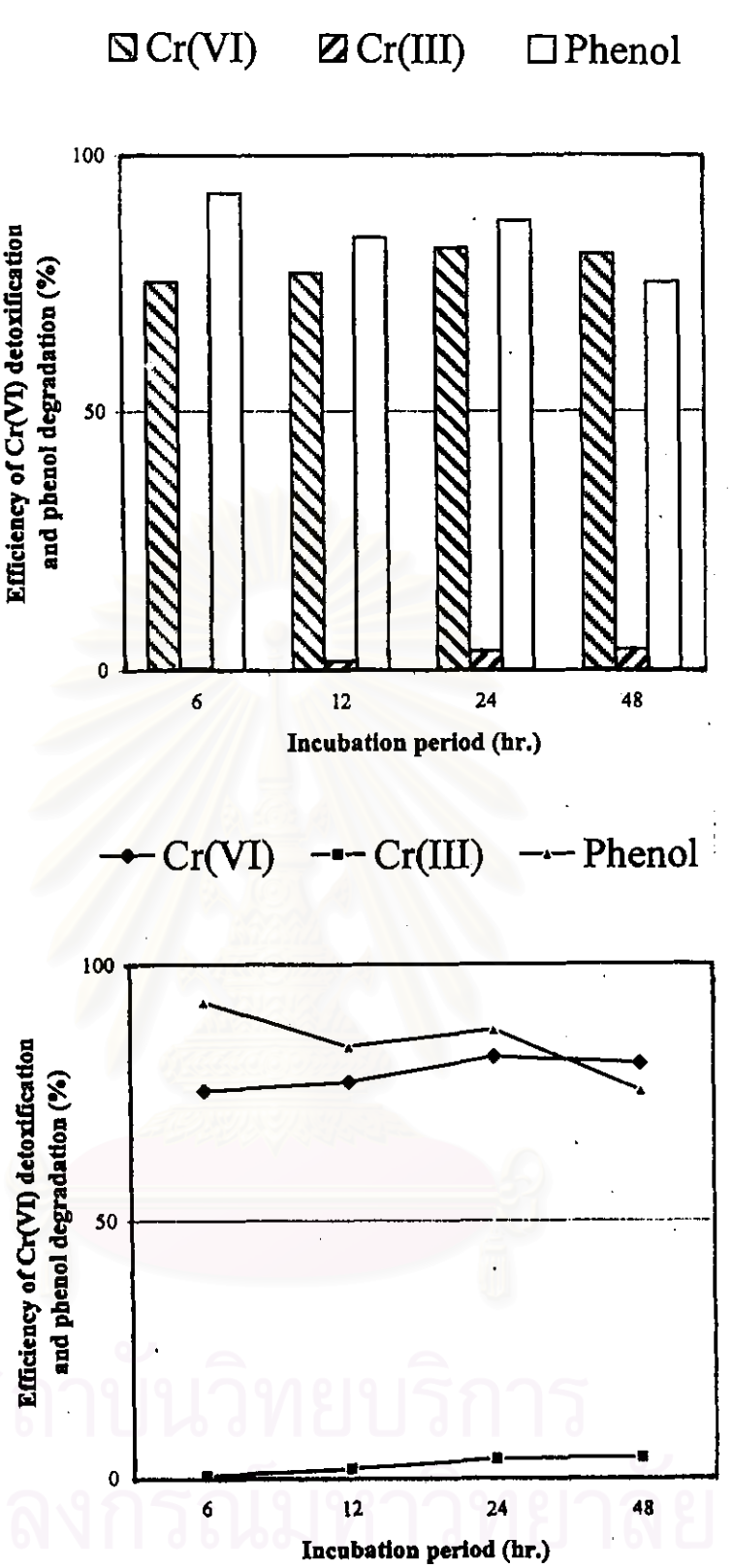


Figure 4.13 Efficiency of Cr(VI) detoxification and phenol degradation at contact time 15 min. varied from incubation period; 6, 12, 24 and 48 hr. by CPR-16

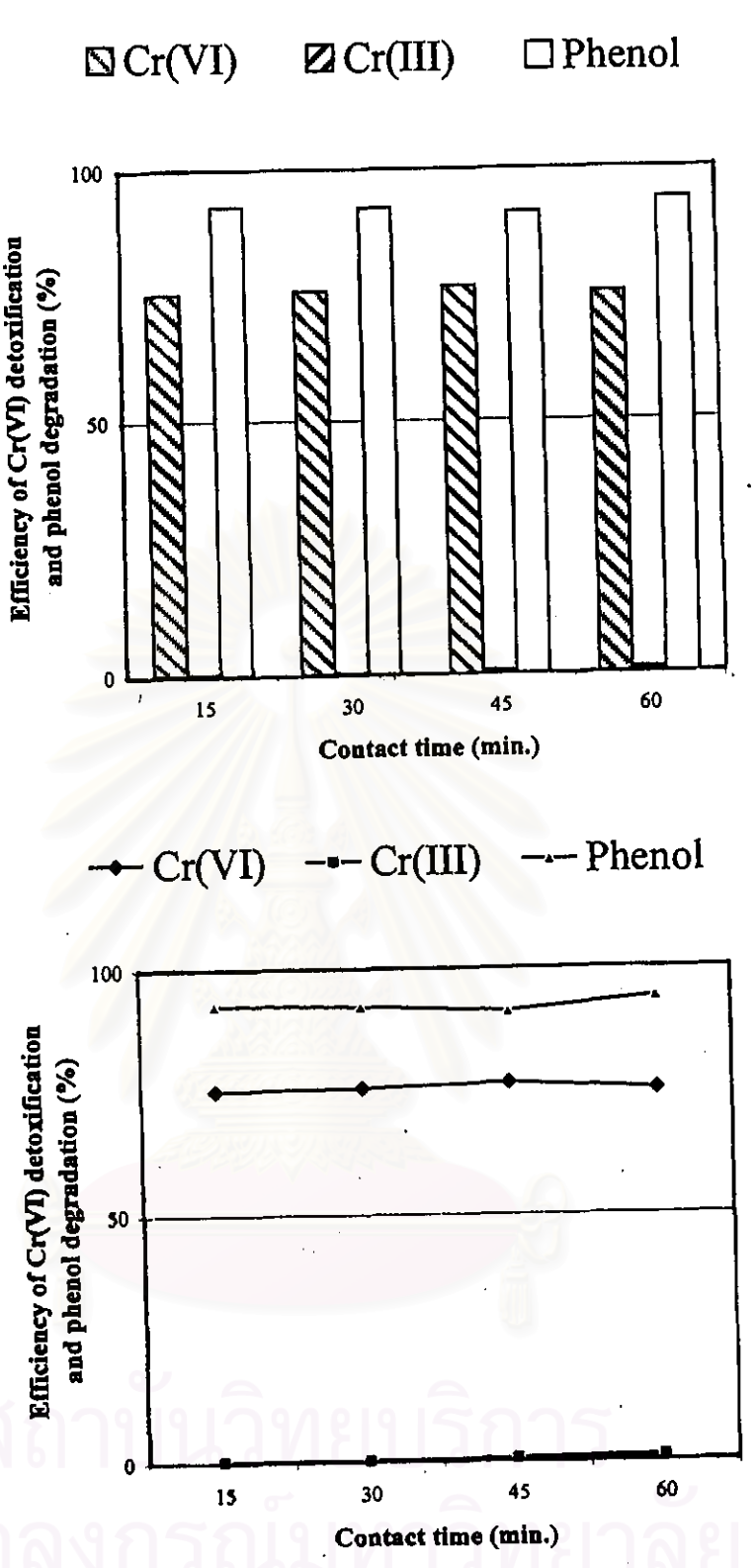


Figure 4.14 Efficiency of Cr(VI) detoxification and phenol degradation at incubation period 6 hr. varied from contact time; 15, 30, 45 and 60 min. by CPR-16

4.3.2 EFFECT OF LOW CONCENTRATIONS OF CHROMIUM DETOXIFICATION AND PHENOL DEGRADATION ON NINE COCULTURES AND THREE SINGLE CULTURES

Efficiency of bacterial isolates, nine cocultures; CrR-2+PhR-26, CrR-2+PhR-33, CrR-2+PhR-64, CrR-14+PhR-26, CrR-14+PhR-33, CrR-14+PhR-64, CrR-15+PhR-26, CrR-15+PhR-33 CrR-15+PhR-64 and three single cultures; CPR-4, CPR-16, CPR-17 at various concentrations; 100, 200, 300 and 400 μg/ml, summarized in **Table 4.8**, on page 90 were shown in **Figure 4.15**, on page 91.

4.3.3 EFFECT OF HIGH CONCENTRATIONS OF CHROMIUM DETOXIFICATION AND PHENOL DEGRADATION ON THREE COCULTURES AND THREE SINGLE CULTURES

The results of Cr(VI) detoxification, Cr(III) production and phenol degradation by bacterial isolate are briefly summarized in **Table 4.9**, on page 92. The Efficiency of bacterial isolates, three cocultures; CrR-2+PhR-26, CrR-14+PhR-33, CrR-15+PhR-64 and three single cultures; CPR-4, CPR-16, CPR-17 at various concentrations; 500, 1000, 1500 and 2000 μg/ml were shown in **Figure 4.16**, on page 93.

Table 4.8 Effect of low concentrations; 100, 200, 300 and 400 µg/ml at contact time 15 min., incubation period 6 hr. by nine cocultures and three single cultures

Strains	Cr(VI) and phenol concentration											
	100 µg/ml (%)			200 µg/ml (%)			300 µg/ml (%)			400 µg/ml (%)		
	Cr(VI)[1]	Cr(III)[2]	Ph[3]	Cr(VI)[1]	Cr(III)[2]	Ph[3]	Cr(VI)[1]	Cr(III)[2]	Ph[3]	Cr(VI)[1]	Cr(III)[2]	Ph[3]
CrR-2+ PhR-26	86±0.3 (86.0)	3±0.1 (3.0)	92±0.3 (92.0)	166±0.5 (83.0)	1±0.0 (0.5)	188±0.6 (94.0)	241±0.7 (80.3)	17±0.5 (5.7)	265±0.8 (88.3)	342±1.0 (85.5)	9±0.3 (2.3)	378±1.1 (94.5)
CrR-2+ PhR-33	85±0.3 (85.0)	2±0.1 (2.0)	92±0.3 (92.0)	170±0.5 (85.0)	2±0.1 (1.0)	188±0.6 (94.0)	225±0.7 (75.0)	1±0.0 (0.3)	267±0.8 (89.0)	345±1.0 (86.3)	7±0.2 (1.8)	379±1.1 (94.8)
CrR-2+ PhR-64	85±0.3 (85.0)	1±0.0 (1.0)	93±0.3 (93.0)	169±0.5 (84.5)	2±0.1 (1.0)	189±0.6 (94.5)	238±0.7 (79.3)	7±0.2 (2.3)	274±0.8 (91.3)	344±1.0 (86.0)	5±0.1 (1.3)	376±1.1 (94.0)
CrR-14+ PhR-26	80±0.2 (80.0)	2±0.1 (2.0)	99±0.3 (99.0)	155±0.5 (77.5)	3±0.1 (1.5)	191±0.6 (95.5)	239±0.7 (79.7)	8±0.2 (2.7)	284±0.8 (94.7)	319±1.0 (79.8)	6±0.2 (1.5)	399±1.2 (99.8)
CrR-14+ PhR-33	75±0.2 (75.0)	2±0.1 (2.0)	99±0.3 (99.0)	153±0.5 (76.5)	5±0.1 (2.5)	196±0.6 (98.0)	274±0.8 (91.3)	12±0.4 (4.0)	290±0.9 (96.7)	316±0.9 (79.0)	4±0.1 (1.0)	385±1.2 (96.3)
CrR-14+ PhR-64	78±0.2 (78.0)	1±0.0 (1.0)	99±0.3 (99.0)	154±0.5 (77.0)	2±0.1 (1.0)	195±0.6 (97.5)	239±0.7 (79.7)	13±0.4 (4.3)	263±0.8 (87.7)	319±1.0 (79.8)	6±0.2 (1.5)	385±1.2 (96.3)
CrR-15+ PhR-26	77±0.2 (77.0)	1±0.0 (1.0)	98±0.3 (98.0)	154±0.5 (77.0)	5±0.1 (2.5)	188±0.6 (94.0)	261±0.8 (87.0)	13±0.4 (4.3)	291±0.9 (97.0)	307±0.9 (76.8)	3±0.1 (0.8)	388±1.2 (97.0)
CrR-15+ PhR-33	76±0.2 (76.0)	1±0.0 (1.0)	99±0.3 (99.0)	157±0.5 (78.5)	5±0.1 (2.5)	192±0.6 (96.0)	229±0.7 (76.3)	3±0.1 (1.0)	256±0.8 (85.3)	311±0.9 (77.8)	2±0.1 (0.5)	376±1.1 (94.0)
CrR-15+ PhR-64	76±0.2 (76.0)	1±0.0 (1.0)	98±0.3 (98.0)	154±0.5 (77.0)	1±0.0 (0.5)	189±0.6 (94.5)	234±0.7 (78.0)	6±0.2 (2.0)	292±0.9 (97.3)	310±0.9 (77.5)	9±0.3 (2.3)	387±1.2 (96.8)
CPR-4	85±0.3 (85.0)	3±0.1 (3.0)	92±0.3 (92.0)	165±0.5 (82.5)	4±0.1 (2.0)	196±0.6 (98.0)	241±0.7 (80.3)	13±0.4 (4.3)	271±0.8 (90.3)	333±1.0 (83.3)	4±0.1 (1.0)	390±1.2 (97.5)
CPR-16	49±0.1 (49.0)	3±0.1 (3.0)	53±0.2 (53.0)	159±0.5 (79.5)	4±0.1 (2.0)	174±0.5 (87.0)	241±0.7 (80.3)	18±0.5 (6.0)	274±0.8 (91.3)	352±1.1 (88.0)	10±0.3 (2.5)	341±1.0 (85.3)
CPR-17	84±0.2 (84.0)	2±0.1 (2.0)	95±0.3 (95.0)	166±0.5 (83.0)	2±0.1 (1.0)	197±0.6 (98.5)	246±0.8 (82.0)	11±0.3 (3.7)	262±0.8 (87.3)	336±1.0 (84.0)	9±0.3 (2.3)	393±1.2 (98.3)

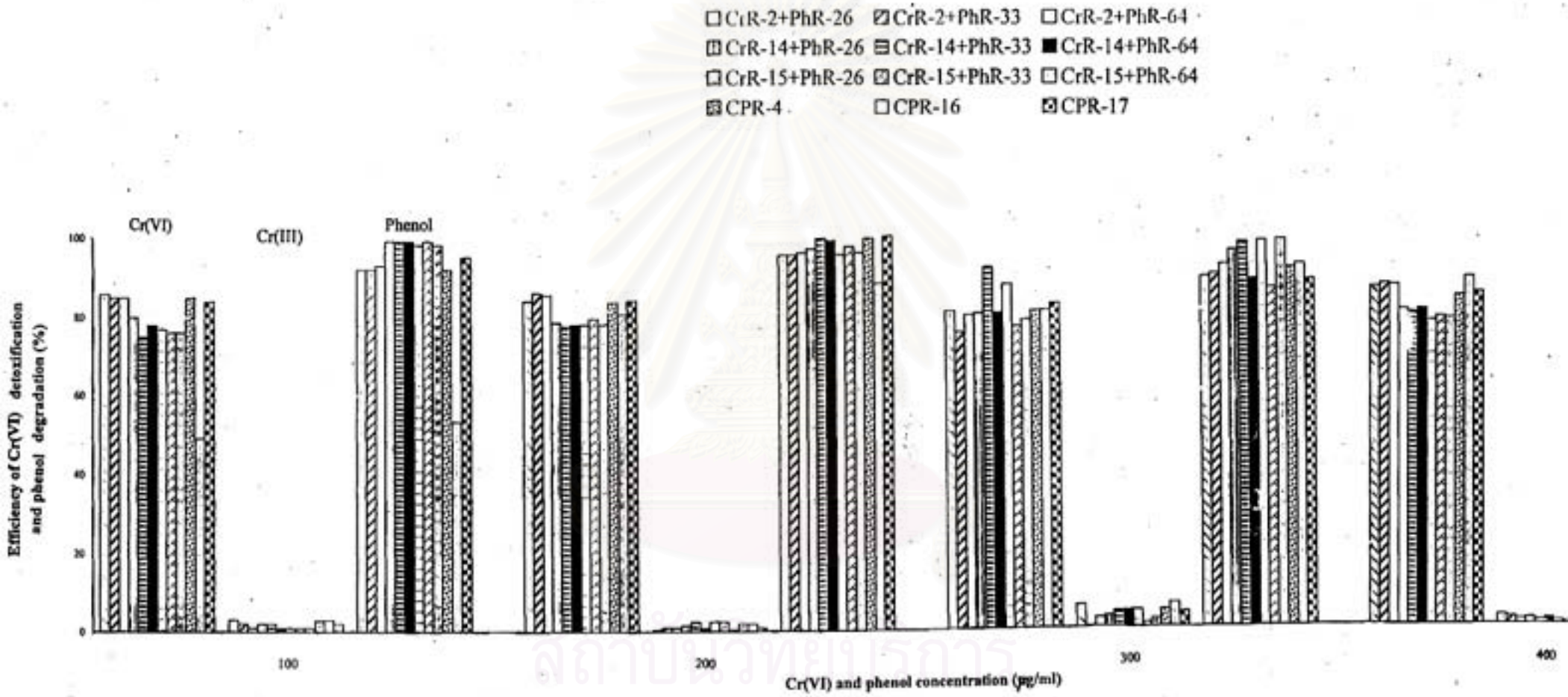


Figure 4.15 Efficiency of Cr(VI) detoxification and phenol degradation at incubation period 6 hr., contact time 15 min. varied from concentration; 100, 200, 300 and 400 µg/ml

Table 4.9 Effect of high concentrations; 500, 1000, 1500 and 2000 μg/ml at contact time 15 min., incubation period 6 hr. by three cocultures and three single cultures

Strains	Cr(VI and phenol concentration											
	500 μg/ml (%)			1000 μg/ml (%)			1500 μg/ml (%)			2000 μg/ml (%)		
	Cr(VI)[1]	Cr(III)[2]	Ph[3]	Cr(VI)[1]	Cr(III)[2]	Ph[3]	Cr(VI)[1]	Cr(III)[2]	Ph[3]	Cr(VI)[1]	Cr(III)[2]	Ph[3]
CrR-2+ PhR-26	390±1.2 (78.0)	100±0.3 (20.0)	461±1.2 (92.2)	920±1.3 (92.0)	80±0.2 (8.0)	783±1.3 (78.3)	1430±1.4 (95.3)	80±0.2 (5.3)	883±1.4 (58.9)	1940±1.2 (97.0)	70±0.2 (3.5)	778±1.3 (38.9)
CrR-14+ PhR-33	390±1.2 (78.0)	100±0.3 (20.0)	494±1.2 (98.8)	900±1.3 (90.0)	100±0.3 (10.0)	767±1.3 (76.7)	1410±1.4 (94.0)	90±0.3 (6.0)	867±1.4 (57.8)	1920±1.2 (96.0)	90±0.3 (4.5)	839±1.4 (41.9)
CrR-15+ PhR-64	400±1.2 (80.0)	90±0.3 (18.0)	456±1.2 (91.2)	910±1.3 (91.0)	90±0.3 (9.0)	911±1.4 (91.1)	1420±1.4 (94.7)	80±0.2 (5.3)	800±1.4 (53.3)	1950±1.2 (97.5)	100±0.3 (5.0)	956±1.4 (47.8)
CPR-4	220±0.7 (44.0)	120±0.4 (24.0)	338±1.0 (67.6)	860±1.2 (86.0)	150±0.4 (15.0)	493±1.2 (49.3)	1380±1.3 (92.0)	70±0.2 (4.7)	662±1.3 (44.1)	1890±1.1 (94.5)	60±0.2 (3.0)	707±1.3 (35.5)
CPR-16	240±0.7 (48.0)	20±0.1 (4.0)	400±1.2 (80.0)	820±1.2 (82.0)	40±0.2 (4.0)	618±1.3 (61.8)	1350±1.3 (90.0)	50±0.2 (3.3)	707±1.3 (47.1)	1890±1.1 (94.5)	40±0.2 (2.0)	707±1.3 (35.3)
CPR-17	190±0.6 (38.0)	40±0.2 (8.0)	480±1.2 (96.0)	810±1.2 (81.0)	50±0.2 (5.0)	889±1.4 (88.9)	1360±1.3 (90.7)	20±0.1 (1.3)	667±1.3 (44.5)	1900±1.1 (95.0)	30±0.1 (1.5)	756±1.3 (37.8)

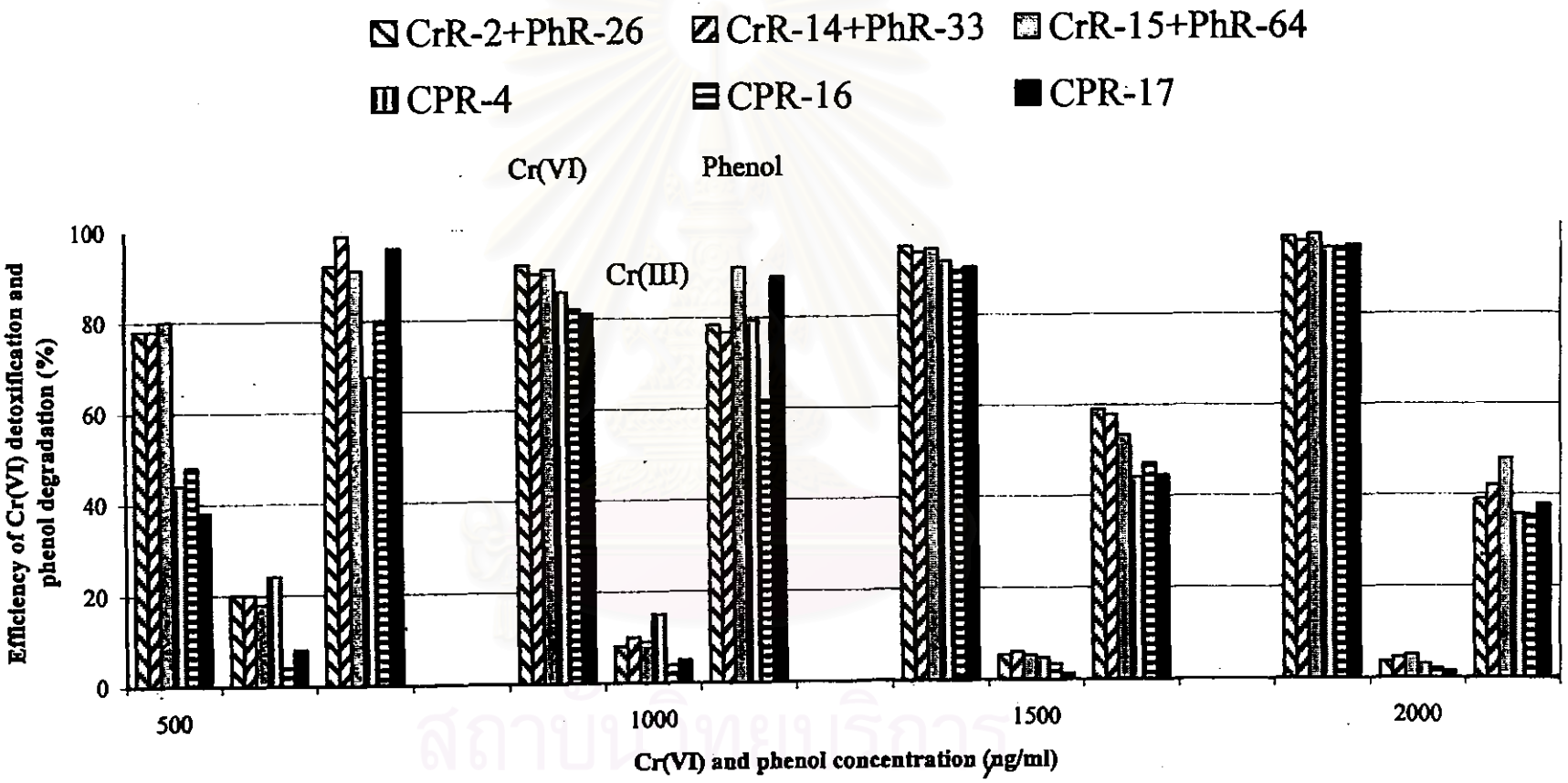


Figure 4.16 Efficiency of Cr((VI) detoxification and phenol degradation at incubation period 6 hr., contact time 15 min. varied from concentration; 500, 1000, 1500 and 2000 µg/ml

4.4 EFFECTS OF SOME ENVIRONMENTAL FACTORS ON CHROMIUM DETOXIFICATION AND PHENOL DEGRADATION

Optimum pH and optimum temperature for growth of three cocultures and three single cultures were neutral or at 7 and 37°C, summarized in **Table 4.10**, on page 95. Comparing between bacterial cells of cocultures with single cultures were shown in **Figure 4-17 and Figure 4-18**, on page 96 and 97.

4.5 EFFICIENCY OF PHENOL DERIVATIVE DEGRADATION BY THE SELECTED BACTERIAL STRAINS

The bacterial strains; PhR-26, PhR-33, PhR-64, CPR-4, CPR-16, CPR-17 were studied the phenol derivative degradation, i. e., p-cresol, p-chlorophenol and p-nitrophenol, the initial concentration 50 μg/ml,, weekly measured samples for three weeks, summarized in **Table 4.11**, on page 98. It found that bacterial isolates can degraded p-cresol better than p-chlorophenol and p-nitrophenol, were shown in **Figure 4.19**, on page 99.

Table 4.10 Effect of pH and temperature on growth of the three cocultures and three single cultures

Strains	Initial no. of Organism ($x10^8$ cells/ml)	Viable Count ($x10^8$ cells/ml)									
		pH							Temp (°C)		
		4	5	6	7	8	9	10	30	37	40
CrR-2 +PhR-26	1.15+1.13	1.32	1.80	1.86	2.45	2.31	1.80	1.48	1.43	1.72	1.68
CrR-14 +PhR-33	1.14+1.15	1.56	1.82	1.96	3.44	2.30	1.37	1.15	1.52	1.64	1.54
CrR-15 +PhR-64	1.15+1.14	1.19	1.32	1.72	2.61	1.91	1.25	1.17	1.33	1.65	1.38
CPR-4	1.14	1.18	1.20	1.31	1.59	1.50	1.17	1.13	1.04	1.40	1.29
CPR-16	1.15	1.27	1.72	1.99	2.04	1.89	1.16	1.09	1.23	1.53	1.47
CPR-17	1.14	1.34	1.45	1.53	1.71	1.48	1.35	1.26	1.06	1.30	1.10

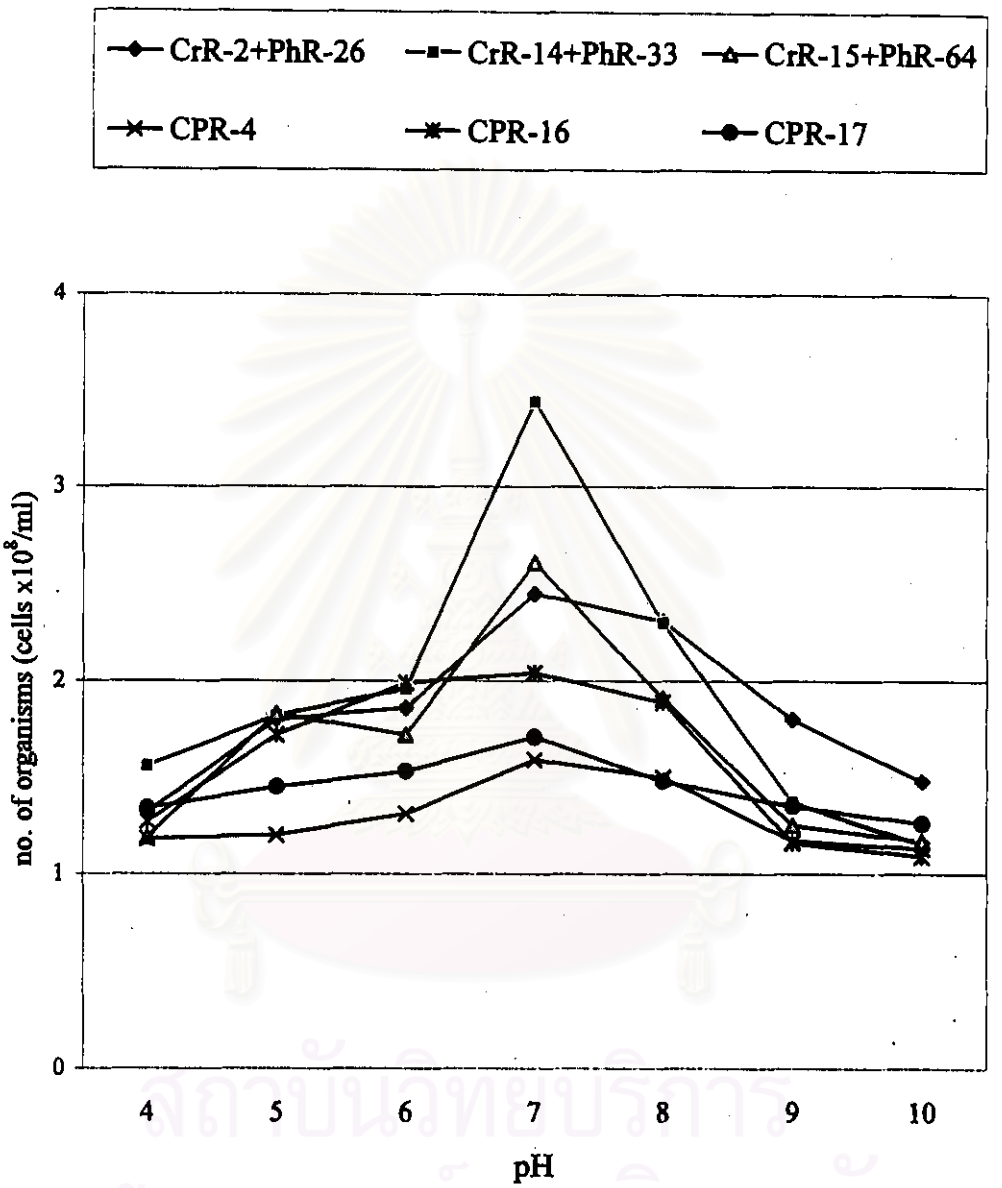


Figure 4.17 Effect of pH on growth of the three cocultures and three single cultures

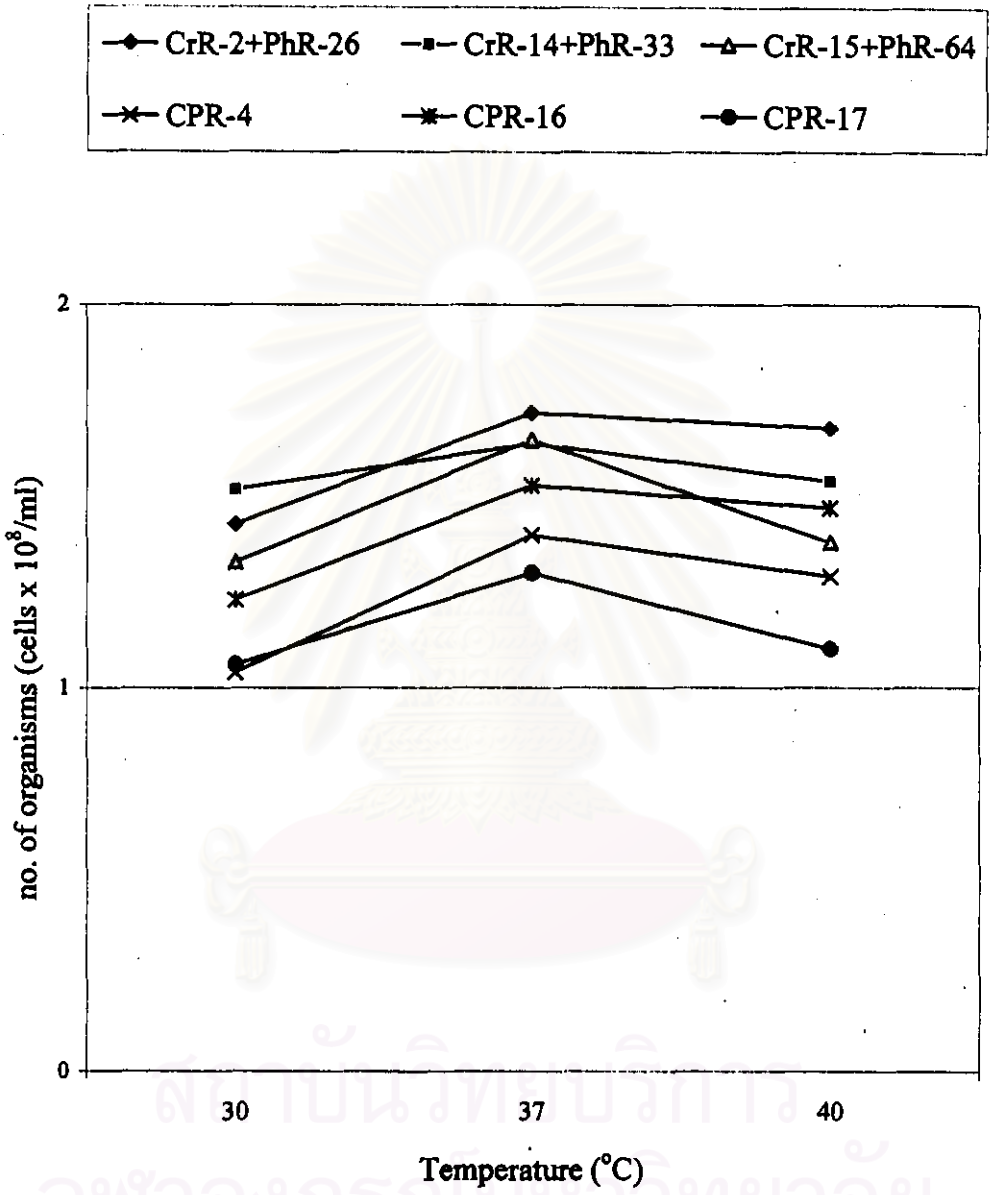


Figure 4.18 Effect of temperature on growth of the three cocultures and three single cultures

Table 4.11 Efficiency of phenol derivative degradation on various weeks; 1, 2 and 3 week.

Strains	Week	Loss of Concentration 50 μg/ml (%*)		
		p-Cresol	p-Chlorophenol	p-Nitrophenol
PhR-26	1	49.0±0.1 (98.0)	1±0.0 (2.0)	8±0.2 (16.0)
	2	49.9±0.1 (99.8)	7±0.2 (14.0)	4±0.1 (8.0)
	3	50.0±0.1 (100.0)	8±0.2 (16.0)	15±0.5 (30.0)
PhR-33	1	49.1±0.1 (98.2)	4±0.1 (8.0)	10±0.3 (20.0)
	2	49.6±0.1 (99.2)	3±0.1 (6.0)	11±0.3 (22.0)
	3	50.0±0.1 (100.0)	11±0.3 (22.0)	12±0.4 (24.0)
PhR-64	1	48.3±0.1 (96.6)	2±0.1 (4.0)	8±0.2 (16.0)
	2	49.7±0.1 (99.4)	9±0.3 (18.0)	8±0.2 (16.0)
	3	50.0±0.1 (100.0)	13±0.4 (26.0)	13±0.4 (26.0)
CPR-4	1	49.4±0.1 (98.8)	3±0.1 (6.0)	13±0.4 (26.0)
	2	50.0±0.1 (100.0)	4±0.1 (8.0)	10±0.3 (20.0)
	3	50.0±0.1 (100.0)	11±0.3 (22.0)	13±0.4 (26.0)
CPR-16	1	49.5±0.1 (99.0)	8±0.2 (16.0)	3±0.1 (6.0)
	2	50.0±0.1 (100.0)	8±0.2 (16.0)	3±0.1 (6.0)
	3	50.0±0.1 (100.0)	14±0.4 (28.0)	18±0.5 (36.0)
CPR-17	1	48.6±0.1 (97.2)	8±0.2 (16.0)	11±0.3 (22.0)
	2	50.0±0.1 (100.0)	5±0.2 (10.0)	6±0.1 (12.0)
	3	50.0±0.1 (100.0)	17±0.5 (34.0)	18±0.5 (36.0)

* Efficiency of Degradation (%)

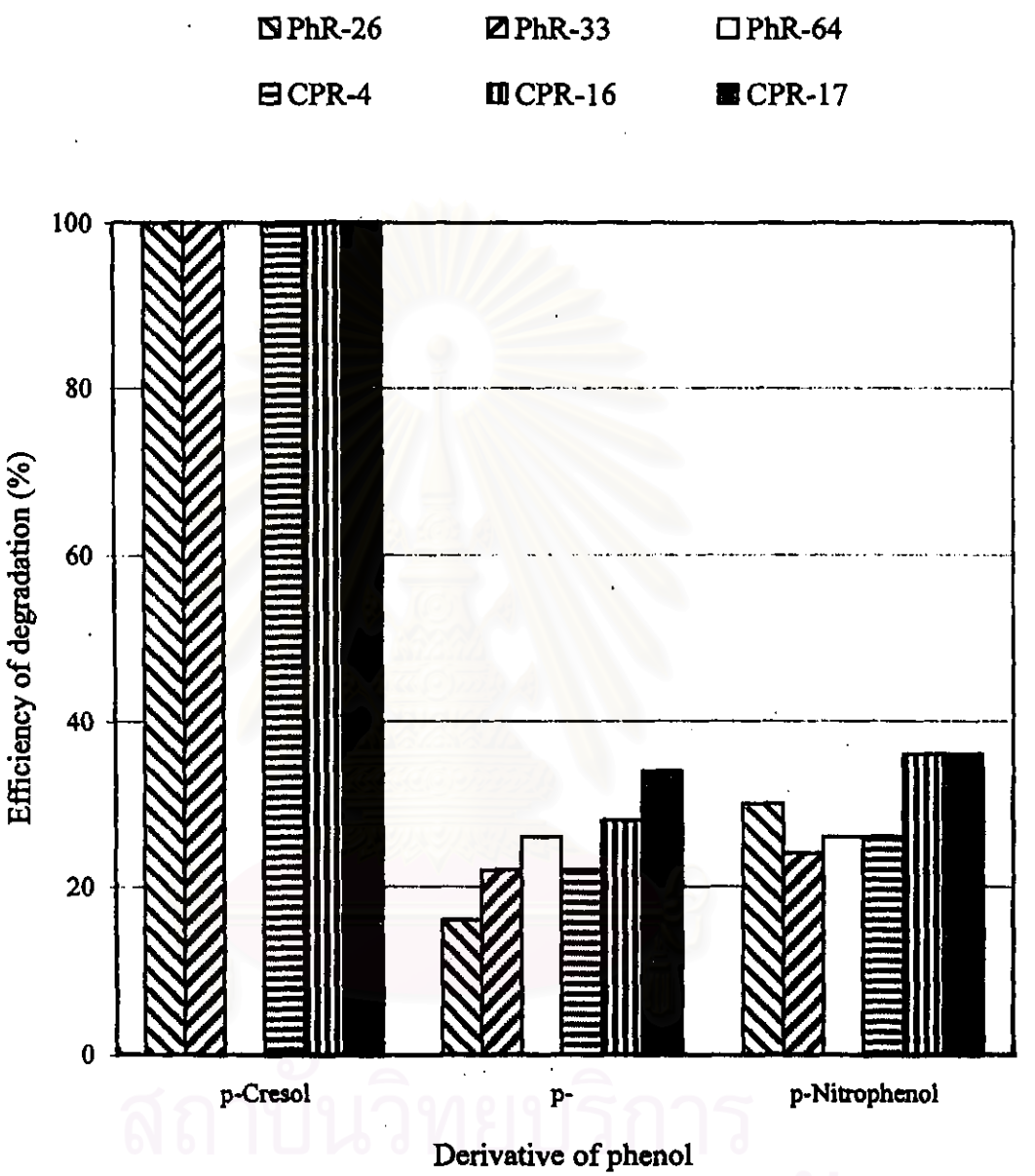


Figure 4.19 Efficiency of degradation of 50 µg/ml p-Cresol, p-Chlorophenol and p-Nitrophenol on the third week