CHAPTER 5

DISCUSSION AND CONCLUSION

The present studies document the removal of soluble arsenic by arsenic-resistance bacterial strains isolated in Thai environment. Arsenic precipitation by those selected bacterial isolates was demonstrated and originated in Thailand.

Three of 219 strains of As-resistance bacterial isolates were selected and named: AsR-17, AsR-19 and AsR-20, and the last two strains were natural consortium. All of them were resistant to arsenic higher than 2,400 μg/ml and able to precipitate arsenic. They were characterized and chosen as the test organisms. Both were gram-negative, rod shape and selected from soil and sediment collected from arsenic contaminated area and industrial sites. For AsR-19 and AsR-20 strains, facultative anaerobic bacteria which must grow and work together, were able to precipitate arsenic as consortium. AsR-17 was obligate anaerobic bacteria, capable of precipiting arsenic by itself.

The study indicated that certain contaminated site might be a favorable habitat of arsenic resistance bacteria according to their growing in highly concentration of arsenic.

Additionally, both of the bacterial strains to resistance to other metal ions showed that were sensitive to all of metal ion especially with Cd, Cu, Cr, Ni and Ag but not strongly resist to Mn and Zn. It is possibly said that sometimes, mechanism of arsenic resistance in those

As-resistance bacterial isolates may be different in resistance to other metals. AsR-17 was able to resist to arsenite, As(III) higher than 500 μg/ml but AsR-19/AsR-20 coculture was unable to resist to this arsenate concentration.

The effect of pH and temperature on growth rate of the selected bacterial isolates can be summarized as follows. They could proliferate at neutral pH (7), and more preferable in the basic than acid condition. It may be depend on pKa of arsenic in basic condition. (**Appendix D**, Lemmo et. al., 1983). The result from this study showed that temperature at 35°C was suitable for growth of AsR-17 and AsR-20 but not for AsR-19. Optimum temperature of AsR-19 was 40 °C but from in 30 °C or 35 °C not different. The number of cell of both AsR-17 and AsR-20 grew in 30 or 40 °C seem to be similar.

The selected bacterial strains were examined to arsenic precipitation by analyze disappearing of total arsenic concentration and confirmed characteristic of arsenic precipitation by X-ray diffraction (**Appendix G**). The product of precipitation was presented as arsenous sulfide (AsS) and dimer of arsenous sulfide (As_2S_2). The efficiency of arsenic precipitation depends on arsenic concentration, pH and temperature and summarized as follows. The suitable arsenic concentration for arsenic precipitation in selected bacterial strains was different. The highest percentage of arsenic precipitation by AsR-17 at 100 μg/ml were 26.64 at 4 days and at 8 days as 49.21 while at the level 200 μg/ml as 34.74 and was unable to precipitate arsenic at 300 μg/ml, after 8-day incubation. The concentration of arsenic on AsR-17 was optimized to precipitate arsenic as 100 μg/ml. For AsR-19/AsR-20, coculture, the percentages of capability arsenic precipitation at 100, 200 and 300 μg/ml of arsenic were 21.67, 24.17 and 35.76 at 4 days and 41.60, 45.24 and 29.98 at 8 days, respectively. The result showed that concentration of arsenic on AsR-19 and AsR-20 was optimized to precipitate arsenic as 200 μg/ml. Possibly, concentration of arsenic at the level 300 μg/ml was not suitable to combine with sulfide by AsR-17 as same as the result of AsR-19/AsR-20 coculture, concentration of arsenic at 100 and 300 μg/ml might not be equivalent sulfide concentration for providing optimum reaction.

The optimized pH for arsenic precipitation in the selected bacterial strains, AsR-17 capable precipitate arsenic for specific pH (7). The percentages of arsenic precipitation were 2.88 and 35.02 after 4 and 8-day incubation, respectively. This implies that arsenic precipitation was sensitive to pH of the culture and the optimum pH was 7. Arsenic precipitation by AsR-19/AsR-20 at pH 6, 7 and 8 were 26.93, 42.21 and 40.75, respectively after 8-day incubation. Like wise, optimum pH on growth might give positive result.

Optimum temperature of arsenic precipitation by AsR-17 and AsR-19/AsR-20 shown that optimum temperature of AsR-17 was 35°C, after 8-day incubation the percentages of removal arsenic was 45.08 and at 30 and 40 °C was 13.12 and 28.45, respectively. It was indicated that at 35 °C provide optimum on growth and capability of arsenic precipitation. For AsR-19/AsR-20, the highest percentage of removal arsenic by coculture was about 40 and was not different at other tested temperature 35 or 40 °C. The optimum temperature for growth of AsR-19 and AsR20 were shown at 40 and 35 °C, respectively. It could be conclude that effect of pH and temperature on capability of arsenic precipitation in each bacterial strain depends on pH and temperature. In addition, concentration of transformation arsenic and soluble sulfide were strongly effect on capability of arsenic precipitation by those selected strains, was similar to the proposed idea of Newman, Beveridge and Morel, 1997, i. e., that is the stability of arsenic precipitation is highly sensitive to small change in pH and sulfide concentration. Some characteristic of the selected bacterial isolates; AsR-17, AsR-19 and AsR-20 was presented in **Appendix F** and the percentage of arsenic removal by bacteria compared to the former investigations was shown in **Table 5.1.**

However, other factors on growth and arsenic precipitation should be to further study, for example, to examine capability in large scale, and chemicals for inhibition and stimulation of arsenic precipitation.

The conclusion, research on removal of soluble arsenic by arsenic-resistant bacterial isolates has made a significant contribution to reduce and protect environmental problem, which extremely and difficult to solve in the future, especially arsenic contaminated area in Thailand.

Table 5.1 The percentage of arsenic removal by bacteria compared to the former investigations.

Organisms	**Conc.[1] (μg/ml)**		**% removal**	**Product**	**Reference**
	Max.[2] Resist.	**Initial**	**Arsenic /days**		
SRB	-	70	~97/19	As_2S_5	Belin,Dinsdale and Altringer, 1993
SRB	-	10	~96/6	AsS, As_2S_3	Uhrie et. al., 1996
Desulfotomaculum auripigmentum	>700	75	~40-50/12	As_2S_3	Newanan et. al., 1997
SRB	-	14	~96.43/-	Arsenic sulfide forms	Adam, Pickett and Nilsen, 1999
AsR-17	**>2400**	**100**	**~36.15/8**	**AsS, As_2S_2**	**This study**
AsR-19/AsR-20	**>2400**	**200**	**~41.73/8**	**AsS, As_2S_2**	**This study**

[1]Conc. = Concentration

[2]Max. Resist. = Maximum Resistance