CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusion

The main conclusions from this work can be summarized as follow:

▪ **The comparison of microorganisms from five sources:** septic tank, the coastal area, a brewery wastewater treatment plant, acidic sulfate soil, and leachate from a landfill site; showed that a mixed-bacterial culture from the brewery wastewater treatment plant has the highest potency in facilitating recovery of system in terms of COD removal by metal sulfide precipitation by avoiding system inhibition. This mixed-culture was found to be the most appropriate bacterial assemblages due to its ability to show mutualistic interaction between sulfate reducing bacteria and methane producing bacteria and useful for application in anaerobic process of waste containing both high sulfate and heavy metals. This mixed-culture was able to degrade volatile fatty acids effectively, metal-resistant, able to degrade sulfate in acidogenic phase for preventing methane producing bacteria by heavy metal sulfide precipitation and produced the high amount of methane. The anaerobic system with this mixed-bacterial culture was found to be easy to operate and provided a short lag time to start up. The major types of this mixed-bacterial culture was *Sporosarcina* sp. PIC-C28, Alicycliphilus sp. R-24604, and *Micrococcus luteus*. The sulfate reducing bacterial type found was *Clostridium ganghwense* strain HY-42-06. The overall process performance data obtained from this culture were:

•The methane production rate and the amount of methane production obtained were 0.0072 ml/(mgMLVSS/l)-hour and 324 ml/gram of COD removed, respectively (expressed at STP).

•The endogenous decay coefficient was not found during the experimental period.

•The glucose synthetic waste reduction rate was $3x10^{-7}$ liter/mgMLVSS-hour.

•The highest performance in system recovery from metal sulfide precipitation was shown. The sulfate reduction rate in the reactor which contained copper occurred at $9x10^{-7}$ liter/ mgMLVSS-hour.

▪ **The optimum reactor conditions for simultaneous control of sulfate and heavy metals in complex wastewater streams** depend on the COD:S ratio. With the high performance bacterial assemblage from a brewery wastewater treatment plant, the most effective condition was found at COD:S of 9. Under this optimum condition, the outcome of the mutualism between sulfate reducing bacteria and methane producing bacteria could be achieved. This COD:S ratio of 9 suppressed the competition between sulfate reducing bacteria and methane producing bacteria, and showed mutualistic interaction between these two types of bacteria for glucose synthetic waste. At COD:S of 9, the following conclusions can be drawn.

• COD reduction reaction and methanogenesis were promoted.

• The maximum specific methanogenic actvity could be achieved at 0.5815 (gCH_4gasCOD)/(gCOD removal-gMLVSS).

• The CH_4 produced was 196 ml (at STP) per gram of COD removed.

• The sulfate reduced was 0.356 gram per gram of COD removed.

• The COD reduction by SRB was 20% and by MPB was 48% (the less was in the effluent COD and COD which was assumed to be biomass).

• The highest system performance for COD removal could be achieved (86%) while sulfate reduction still occurred (89%).

• The final pH of the effluent was elevated but did not exceed a pH of 8, which was appropriate for metal sulfide precipitation.

This result illustrated the system performance for a reactor involved in simultaneous methane production, sulfide detoxification and a heavy metal remediation process.

▪ **Heavy metals could negatively impact the activities of methane producing bacteria and sulfate reducing bacteria in anaerobic bioreactors.** With high performance bacteria from the brewery wastewater treatment plant at the COD:S of 9, the following conclusions could be drawn.

• The relative toxicity of heavy metals in the organic degradation was Cu > Cd >Zn.

• Combined heavy metals caused either synergism or antagonism, or both on the bacterial activities depending on the type and concentration of heavy metal. In most cases, combined heavy metals caused synergistic inhibition on the bacterial activity factor. For the system that contained either Cd or Zn less than 5 mg/l, the inhibition could be antagonized by Cu which the concentration was not more than 1 mg/l. The antagonism was not observed when Cd combined with Zn. This deduced that Cd and Zn should be separately discarded.

• A tool for the design and operation of anaerobic system and for treating heavy metals commingling with high sulfate wastewaters was proposed as a model to prevent synergistic inhibition by the mechanism of metal sulfide precipitation before or during biological uptake. The metal loading, K, was (Zn/32.7+Cd/56.2+Cu/31.8)/W. The value of K in wastewater has to be less than 15 meq/kg MLVSS. Where K is the ratio of the sum of Zn, Cd, and Cu(in meq/l) divided by the MLVSS, W(in kg/l). Zn, Cd, and Cu are the concentration of the metal in solution in mg/l. At this metal loading, the relative methane production activity was not lower than 80% and the synergistic inhibition was not observed. The residual heavy metal concentration in the effluent was not detectable. The final pH was elevated but did not exceed 8. These experimental results elucidated the performance of the reactor involved in sulfide detoxification and heavy metal remediation processes.

7.2 Contributions of this work

This work attempted to propose the criterion model to prevent anaerobic treatment process from heavy metal inhibition and synergism. The equation proposed is a tool to the designer and operator to control the anaerobic treatment process and also gives the possibility to formulate consent conditions for a suitable industrial effluent control. The information obtained from this work can be further employed in the design of the control technique and management strategies for the wastewater treatment plant. This finding should be beneficial for most existing anaerobic wastewater treatment plants as it indicates the metal loading that can maintain the treatment process by preventing the system before synergism occurred. The system performance could be well enhanced just by adjusting operational parameter of COD:S, heavy metal and MLVSS without the need to invest in new construction or equipment. It should be mentioned, however, that the conclusions obtained from this work were based on the mixed-bacteria from the brewery wastewater treatment plant which consist of the high potential methane producing and sulfate reducing bacteria. The organic waste used was the readily glucose synthetic waste and the system was operated under the batch experiment.

In addition, it was proven that the COD:S, heavy metals, and the biomass is a proper condition to show mutualistic interaction between sulfate reducing and methane producing bacteria.

Another point of importance, the kinetic coefficients of five mixed-bacterial types from natural sources which obtained from this study could be very well employed to the scientists, environmental engineers and the end users. They can select an appropriate bacterial type for more specific purpose to apply to their work efficiently, feasibly and economically. Bacteria from the coastal area is the example that show high performance in substrate degradation. Bacteria from the leachate is the example that show high performance in methane production but need more time to start up.

Saving cost in industry without generating toxic waste to environment simultaneous providing the fuel gas, this research dissertation could lead to the sustainable development.

7.3 Application for Industry

Today indiscriminate and uncontrolled discharge of metal contaminated industrial effluents into the environment has become an issue of major concern. Heavy metals, being non-biodegradable and persistent, beyond a permissible concentration from unspecific compounds inside the cells thereby causing cellular toxicity. Also, under anaerobic conditions, reduction of sulfate will produce hydrogen sulfide (H_2S) that also inhibits biological activity. An alternative to remove heavy metals, sulfate, sulfide and organic waste from wastewater is by allowing the two waste streams to react together. The overall toxicity can be reduced by precipitation of heavy metal sulfides. The potential biotechnological application is the use of sulfate reducing bacteria (SRB) to utilize the organic waste in anaerobic bioreactors of organic substrates for cell growth, reducing sulfate (SO_4^{2-}) to sulfide (S^{2-}) and organic substrate to methane (CH_4).

Here microbial biomass offers an economical option for reduction of SO_4^{2-} to S^{2-}, and the heavy metal sulfide will be precipitated. The biomass removes heavy metals by the phenomenon of precipitation and biosorption. The process can be made economical by procuring the mixed-culture of biomass from industry or naturally available SRB. A batch or a continuous process of removal of heavy metals and sulfate directly from wastewater can be developed using the appropriate biomass (Biomass from a brewery wastewater treatment plant is recommended). Further potential of the system can be improved by the optimization of the ratio of COD:S and heavy metal in the influent wastewater before treatment. The findings of the research makes it an economical and sustainable option for developing wastewater treatment process for removal of heavy metals, sulfate, sulfide and organic substances.

7.4 Recommendations for further work

This work was successful in preventing the problematic heavy metals for three out of the five most consistently metals found in Thailand and in the world. However, it will be completed if all types of the most consistently found heavy metals are studied. Further work should be conducted to

- Investigate the combined effect of five combined metals of Cd, Cu , Zn, Pb and Ni and then find the criterion model to prevent the synergistic inhibition and maintain the anaerobic wastewater treatment system.
- Examine the metal sulfide detoxification process by increasing higher amount of sulfate concentrations than the optimum amount with the expectation to obtain the higher amount of methane.
- Study the prevention of system inhibition by the mixed-bacterial culture from other potential bacterial sources such as bacteria from the Para Industry.
- Study the prevention of system inhibition by pure culture.