CHAPTER I



INTRODUCTION

1.1 Motivations

Many industrial discharges of heavy metals are ultimately dispersed to the environment via wastewater treatment plants (Christensen et al., 1996; Chang et al., 2000). The dissemination of heavy metals into the environment needs to be prevented. The ability of treatment plants to remove metals is an important consideration for both environmental protection and wastewater management. The presence of significant quantities of heavy metals in sewage is due to industrial, commercial and domestic activities. Some water contains some levels of heavy metals, such as cadmium (Cd), copper (Cu), and zinc (Zn), inorganic pollutants, such as sulfate (SO_4^{2-}) , and large amounts of organic substances as well as mixed microbial assemblages. The accumulation of sulfate-rich wastewater (Rinzema 1988; Rinzema and Lettinga 1988; Yoda et al., 1987) may cause the release of toxic sulfides that can provoke damage to the anaerobic system. Various processes for their treatment have been developed such as reverse osmosis operations and chemical precipitation (Edwards et al., 1992; Gorontzy et al., 1993). These systems are generally expensive, and moreover, produce chemical wastes that need to be disposed. In order to decrease the environmental impact and resource use, biological waste treatment for industrial treatment is often advocated. Anaerobic systems with simultaneous production of energy have to potential to provide improvements for both the environment and its sustainability, because they can result in low environmental impact, reduced energy demand and smaller quantities of surplus sludges.

There is increasing interest in the potential biotechnological applications of bacterial sulfate reduction as an alternative method for sulfate (SO_4^{2-}) and heavy metal removal from environmental contamination (Chang et al., 2000; Elliott et al., 1998; Kim et al., 1996; Machemer et al., 1992; Dvorak et al., 1992). The existence of heavy metals and/or sulfate is unfavorable to the growth of microorganisms. The various types of biomass used in wastewater treatment have a certain capacity for the

removal of metals (Leighton and Forster, 1997). Knowledge of the effect of heavy metals on the anaerobic treatment processes is required.

Anaerobic digestion has been considered as a highly integrated process because of the close collaboration existing between all the members of bacterial population. Anaerobic process and the resulting formation of methane arise from the co-operative action of many micro-organisms. A mixed culture is much more efficient in degrading substrate than each strain alone. The performance of the mixed bacterial populations was investigated in this study.

The process of interest is the use of sulfate reducing bacteria (SRB) to utilize the substrate in anaerobic bioreactors of organic substrates for cell growth, reducing sulfate $(SO_4^{2^-})$ to sulfide (S^{2^-}) , and organic substances to methane (CH_4) . Concurrent with the sulfate reduction, alkalinity would be generated, resulting in an increase in the pH and the precipitation of many of the heavy metals as sulfides. Thus, not only will heavy metals precipitate but also the $SO_4^{2^-}$, S^{2^-} , and COD will be reduced. However, CH_4 will also be produced simultaneously. In addition, the influence of heavy metals is investigated by performing microbial toxicity test using an appropriate selected bacterial mixed-culture. The results which referred to inhibition by individual and combined metals were complied and the criterion model to prevent the system suffering from $SO_4^{2^-}$ and heavy metals was proposed.

The goal of this project is to facilitate the use of SRB to utilize sulfate as an electron acceptor for cell growth, resulting in the reduction of sulfate to sulfide and the transformation of organic substances to methane, with concurrent lowering of the sulfide and heavy metal toxicity effect through metal precipitation as metal sulfides. This application can lead to use of one kind of toxic waste to aid in the treatment of another kind of toxic waste. To accelerate the start-up process, increase gas production, increase the percentage of COD removal, and retard volatile fatty acid (VFA) production, microbial selection was conducted at the beginning of the study. Five sources of microorganisms, i.e., sludge from septic tank, sediment from the coastal area at Pattaya (Chonburi), sludge from a brewery wastewater treatment plant, acidic sulfate soil from Ongkarak (Nakornayok), and leachate from a landfill site at Nongkham (Bangkok) were examined. The highest performance microorganism was selected and used as seed in the second phase of the toxicity batch study.

From previous work, it has been customary to study the inhibitory effect of one metal at a time on anaerobic digestion. Information about the concentrations of these metals at which these effects become apparent is still contradictory. Moreover, municipal or industrial digesters present a more complex situation and in this case it is more useful to find the impact of different metals in combination, which has not yet been examined vigorously. Cd, Cu and Zn were the most consistently heavy metals found in wastewaters (USEPA, 1981) and they are the three metals that were used in this study.

1.2 Objectives

One of the main objectives of this thesis is to determine the optimum ratios of sulfate, COD, and heavy metals that facilitate, in anaerobic bioreactors, simultaneous lowering of COD and sulfate with precipitation of metal sulfides, with CH_4 production using the high performance mixed microbial assemblages. Another main objective is to propose a tool to the designer and operator to control the anaerobic process and also give the possibility to formulate consent conditions for a suitable industrial effluent control. The sub-objectives are:

- 1) Comparison of the biological treatment potential of microbial assemblages selected from various areas with presently occurring levels of both sulfate and heavy metals and selection of the microbial assemblage that shows the best performance in terms of COD removal, sulfate reduction, tolerance of heavy metals, with simultaneous methane production.
- 2) Use of the selected assemblage for seeding batch reactor processing to determine the levels where toxicity becomes a problem and the conditions under which heavy metals can be removed to acceptable levels.
 - 3) Determination of key process variables and limits that lead to development of a criterion model to facilitate the design and operation of an anaerobic reactor capable of treatment of mixed wastes containing problematic levels of both sulfate and of heavy metals.

1.3 Scope of the Study

- 1) Glucose was used as the carbon source.
- 2) Bacterial assemblages from 5 different locations, already acclimated through natural selection to significant levels of both heavy metals and sulfate were tested by conventional anaerobic reactors with a semi-continuous operation. The most appropriate bacterial assemblages was selected to use in the toxicity test.
- 3) Toxicity study was tested in batches.
- 4) Optimum ratio of COD and sulfate was determined to facilitate the bioreactors.
- Cadmium, copper and zinc were studied in the form of single and combined metal(s). These metals were in the solution-phase.
- 6) The toxic effect of the soluble metals after mitagation with metal sulfide precipitation was investigated. The data interpretation would come up with the development of the model to predict the system inhibition.

1.4 References

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