CHAPTER 4: DISCUSSION

Microbial activity in activated sludge is sensitive towards temperature and pH. The observed temperature in our samplings were in the range from 20°C to 30°C throughout the year, and is typical of tropical regions. This temperature range is sufficient for aerobic processes (Sperling, 2007). The pH in influent and aeration tank of all the STPs were in the acceptable range (7.0 to 8.5) (Gerardi, 2002) except for the aeration tank in STP 3 (5.0 ± 0.7).

4.1 Influent characteristics

Removal of carbonaceous compounds and ammonia start when the sewage flows into wastewater treatment plant. A minimum DO concentration of 1.5 to 2.0 mg l^{-1} is required to ensure maximum BOD removal in influent (Vesilind, 2003). DO concentration in the influent at STP 1 and STP 2 were generally above 2 mg l^{-1}. On the other hand, the influent at STP 3 experienced low DO of less than 1 mg l^{-1}, suggesting an oxygen limiting condition (Woodside and Kocurek, 1997).

In domestic wastewater, ammonia is one of the major compositions and concentration normally range from 10 to 50 mg l^{-1}, but can be as high as 200 mg l^{-1} in industrial wastewater. Ammonia concentration in the influent of all the STPs was below 50 mg l^{-1} which was typical of domestic wastewater (Korner *et al.,* 2001). Nitrite and nitrate are also normally present in raw sewage in low concentrations. In our study, the average nitrite and nitrate concentrations in the influent were $< 0.35 \pm 0.06$ mg l^{-1} and $< 2.52 \pm 3.79$ mg l^{-1}, respectively. Another ubiquitous constituent of wastewater is phosphorus which is a macronutrient in all living cells and is primarily present in the form of phosphate (Srinivasan, 2009). Phosphate in raw sewage is approximately 30 mg l^{-1} (Bridges, 1922), and our results were higher i.e. from 50.30 ± 11.80 mg l^{-1} to 62.80 ± 12.40 mg l^{-1}.

Influent BOD and COD were not different among the three STPs and were typical of domestic and industrial levels. Typical domestic wastewater BOD can range from 100 to 300 mg l^{-1} whereas COD of industrial wastewater range from 420 up to 5600 mg l^{-1} (Lindeburg, 2009).

There are heavy metals that may be present in wastewater such as Cu, Pb, Hg, As, Cr and Cd (Selivanovskaya *et al.,* 2010). All these compounds have high atomic masses and are toxic to microorganisms. In this study, our results suggested that there were Cu and Pb pollution in the influent at STP 3 which were above Standard A criteria in Environmental Quality Act 1974. This Cu and Pb pollution was most likely from light industries in the area e.g. auto-service. Studies have shown that the detected heavy metal pollution in wastewater treatment plants are probably caused by nearby industries (Binkley and Simpson 2003; Chipasa 2003; You *et al*., 2009). The Cu and Pb pollution could explain the lower pH in the influent of STP 3 as Sukreeyapongse (2002) has shown that Cu and Pb are negatively correlated with pH.

4.2 Characteristics of aeration tank

Cu and Pb concentration were higher in aeration tank especially at STP 3. The increased heavy metal concentration in the aeration tanks was probably due to the absorption of heavy metals by the extracellular polymers in activated sludge (Loaec *et al*., 1997; Alkan *et al*., 2002). The high concentration of Cu and Pb in aeration tank at STP 3 further confirmed our observation of Cu and Pb pollution at STP 3. As the other heavy metals were generally similar among the STPs, the obvious Cu and Pb pollution in STP 3 allowed comparative studies to suggest the effects of Cu and Pb pollution in activated sludge system.

Ammonia undergoes nitrification, denitrification and release nitrogen gas in aeration tank Nitrification occurs when ammonia is oxidized by autotrophic nitrifying bacteria to nitrate via nitrite (Lin *et al.,* 2009) whereas denitrification is a biological reduction of nitrate to nitrogen gas by facultative heterotrophic bacteria. Denitrification occurs when oxygen levels are depleted (< 0.1 mg l^{-1}) (Focht and Chang, 1975; Krul, 1976). In this study, ammonia concentration in the aeration tanks was lower than the influent whereas nitrate concentration increased. From our results, we speculated that ammonia was oxidized to nitrate.

Settling property of a biological suspension, as measured by SVI, is one useful parameter to evaluate sludge health. SVI in both STP 1 and STP 2 were in acceptable range of sludge settleability (< 150 mg l^{-1}) (Gray, 2004), whereas STP 3 had poor settling sludge. This resulted from pin point flocs that do not settle well (Gerardi, 2002). Heavy metal pollution at STP 3 could have affected the floc development. (Principi *et al.,* 2009) have shown how Cu pollution resulted in biomass deflocculating phenomena or pin point flocs. SSV in STP 1 was the highest followed by STP 2 and then STP 3. Meanwhile, MLSS and MLVSS represent concentration of activated sludge or microorganisms present in the aeration tank (Spellman, 2011). Healthy sludge has

MLSS range of 3000 to 5000 mg l^{-1} (Eckenfelder and Grau, 1998) and STP 1 showed the healthiest sludge range in this study. STP 3 showed consistently low SSV, MLSS and MLVSS, and might indicate low biomass in the plant. Studies have shown how Cu and Pb have a cumulative negative effect on the biomass and sludge production (Li and Fang, 2007; Lin and Shei, 2008), and the Cu and Pb pollution could be the reason for the poor sludge, MLSS and MLVSS levels at STP 3.

Similar with SSV, MLSS and MLVSS, DOUR was the highest in STP 1 compared with STP 2 and STP 3. DOUR is known to increase with sludge concentration (Mineta *et al.*, 2011), and in this study, we found that DOUR increased significantly with MLVSS ($R^2 = 0.526$, $df = 12$, $p < 0.01$). Therefore, the low DOUR at STP 3 was most likely cause by lower microbial biomass as indicated by MLVSS than heavy metal toxicity.

4.3 Effluent characteristics

Our results suggested that STP 1 was the most efficient plant among the STPs with the highest BOD, COD and TSS removal. According to Environmental Quality (sewage and industrial effluents) Regulations, 1979, TSS in the effluent in STP 1 was within Standard A (< 50 mg l^{-1}) whereas STP 2 and STP 3 were within Standard B (< 100 mg l^{-1}). The average BOD removal efficiency for an extended aeration system is 75 to 90% and STP 1 removal efficiency was within acceptable range (Wang *et al.*, 2008). By contrast, effluent in both STP 2 and STP 3 exceeded Standard B limit for BOD. Generally, STP 2 and STP 3 suffered from low BOD reduction rate (< 60%). Similarly, COD removal efficiency was highest in STP 1 but not in STP 2 and STP 3. An acceptable range for COD removal efficiency for activated sludge is 73 to 80% (Wang *et al.,* 2008).

In our study, STP 1 was the most efficient plant with the healthiest activated sludge. Apart from the DO disruption at STP 2 that might cause poor efficiency, it also had a malfunctioning scraper in the clarifier tank. Without the scraper, the sludge was not removed properly and often caused the sludge blanket to spill into the effluent of STP 2 (Jenkins *et al.*, 2004). STP 3 suffered from Cu and Pb pollution that subsequently caused low pH condition and pin point floc problem in the aeration tank that caused high TSS in effluent (Richard, 2003).

4.4 Bacterial community composition in activated sludge

DGGE have been frequently used to examine the microbial diversity in activated sludge and monitor change in microbial communities. From a DGGE gel, the number, precise position and intensity of the bands give an estimation of the number and relative abundance of numerically dominant ribotypes in the sample (Muyzer and Smalla, 1998). In this study, we observed that the bacterial communities composed of eight groups of bacteria i.e. *α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, Bacteroidetes, Acidobacteria, Nitrospirae* and *Chloroflexi.* The most dominant group of bacteria in all the STPs was *β-Proteobacteria,* and this group of bacteria is commonly found in activated sludge community and is responsible for ammonia oxidization of the sewage (You *et al.,* 2009).

Among the 8 groups of bacteria detected, *Chloroflexi* was only found in STP 1. *Chloroflexi* was common in activated sludge (Björnsson *et al.,* 2002; Beer *et al.,* 2002; Kragelund *et al.,* 2006), and is related to floc forming and macromolecules degradation (Reichenbach, 1992; Bossier and Verstraete, 1996; Björnsson *et al.,* 2002). The presence of this floc forming group of bacteria might have contributed to the healthy SSV, MLSS and MLVSS levels at STP 1.

The presence of *Flavobacterium* sp. and *Acidobacteria* in STP 3 have been reported in heavy metal contaminated environments such as wastewater stream (Yoon *et al.,* 2009), oxidation ponds and sewage sludge (Anyanwu and Ugwu, 2010), and contaminated river water (Branco *et al,* 2006). On the other hand, *Thiobacillus* sp. functions as chemosynthetic sulfur oxidizer (Wetzel, 2001) which could help in activated sludge process at STP 1. *Sterolibacterium* sp. and *Saprospiraceae* sp. which were more prevalent in STP 1 and STP 2, respectively, have been reported from activated sludge systems where they play a role in phosphorus and nitrate removal (Tarlera and Denner, 2003; Xia *et al*., 2008).

SIMPER test showed the predominance of heavy metal resistant bacteria at STP 3. However, SIMPER test does not take into account the physico-chemical variables at each STP that affects the bacterial distribution and hence we analyzed the ecological distance of the bacterial community structure by the ordination technique of CCA. The two distinct groups of bacteria that were placed away from the rest mirrored the effects of Cu and Pb pollution and the variable SSV. Both uncultured *Sphingobacteriales* and uncultured *Thermomonas* sp. which mirrored the effects of Cu and Pb, have been reported to have resistance to heavy metals e.g. *Sphingobacteriales* was found in acid mine drainage waters associated with zink mine tailings (Almeida *et al.,* 2009) whereas *Thermomonas* related species was found in copper resistant plant species in copper mine wasteland (Sun *et al*., 2009). The group related with SSV comprised uncultured *Chloroflexi*, uncultured *Sterolibacterium* sp. and uncultured *Bradyrhizobium* sp. Most of the bacteria in this group help in the wastewater treatment process and exhibit filamentous morphotypes that can form the backbone of microbial flocs (Miura *et al*., 2007).

Cluster analysis of the DGGE profiles showed that on most occasions, the different sampling dates of the same STP were grouped together at a similarity > 0.5. As sampling was carried out over a period of more than one year, the results from the cluster analysis showed relative stability of the bacterial community structure at each STP. Therefore, our results suggested that the bacterial community in STP 3 was acclimatized to the Cu and Pb pollution but were of the type that could not form good flocs.

In this study, we found that bacteria could tolerate heavy metal pollution, but plant efficiency was still affected when important floc forming bacteria were not present in the bacterial community. We found *Chloroflexi* in a healthy plant (i.e. STP 1) but not in unhealthy and heavy metal polluted plants. It will be interesting to investigate the effectiveness of *Chloroflexi* to improve the activated sludge process in plants impacted by heavy metal pollution in the future.

CHAPTER 5: CONCLUSION

In this study, our results showed that Cu and Pb pollution at STP 3 had a negative effect on the sludge production and microbial biomass. A predominance of heavy metal resistant bacteria was found in STP 3.

By contrast, STP 1 had the healthiest sludge with high waste plant removal efficiency. Moreover *Chloroflexi* was found only in STP 1 which is known to contribute to floc formation.

Canonical Correspondence Analysis with the variables SSV, DOUR, Cu and Pb showed two distinct groups of bacteria that were placed away from the center. One group reflected the effects of Cu and Pb pollution whereas the other group mirrored the variable SSV.