UNIVERSITI TEKNOLOGI MARA

POLYCYCLIC AROMATIC HYDROCARBONS
BIODEGRADATION USING ISOLATED STRAINS UNDER INDIGENOUS CONDITION

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Doctor of Philosophy

Faculty of Civil Engineering

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The treatment and disposal of domestic sludge is an expensive and environmentally sensitive problem. It is also a growing problem since sludge production will continue to increase as new wastewater treatment plants are built due to population increase. The large volume of domestic sludge produced has made it difficult for many countries including Malaysia to assure complete treatment of the sludge before discharging to the receiving environment. Domestic sludge contains diverse range of pollutants such as pathogen, inorganic and organic compounds. These pollutants are toxic, mutagenic or carcinogenic and may threaten human health. Improper disposal and handling of sludge may pose serious impact to the environment especially on soil and water cycles. Previous studies on Malaysian domestic sludge only reported on bulk parameters and heavy metals. Thus, no study reported on organic micro pollutants, namely, polycyclic aromatic hydrocarbons (PAHs). Their recalcitrance and persistence make them problematic environmental contaminants. Microbial degradation is considered to be the primary mechanism of PAHs removal from the environment. Much has been reported on biodegradation of PAHs in several countries but there is a lack of information quantitative on this subject in Malaysia. This study is carried out to understand the nature of domestic sludge and to provide a better understanding on the biodegradation processes of PAHs. The methodology of this study comprised field activities, laboratory work and mathematical modelling. Field activities involved sampling of domestic sludge from Kolej Mawar, Universiti Teknologi MARA, Shah Alam, Selangor. Laboratory activities include seven phases of experimental works. First phase is characterization study of domestic sludge based on bulk parameters, heavy metals and PAHs. Second phase is enrichment and purification of bacteria isolated from domestic sludge using single PAHs and mixed PAHs as growth substrate. This was followed by identification of bacteria using BIOLOG system. The fourth phase focussed on turbidity test to monitor growth rate of the isolated bacteria. Preliminary degradation study involves optimization of the process at different substrate concentration, bacteria concentration, pH and temperature. The optimum conditions established from optimization study were used in degradation study. In biodegradation study, two experimental conditions were performed. These conditions include using bacteria isolated from single PAHs as substrate and bacteria isolated from mixed PAHs. Protein and pH tests were done during degradation study. Final activity is mathematical modelling of the biodegradation process. In general results on bulk parameters are comparable to previous studies. Zinc was the main compound with a mean concentration of 1196.4 mg/kg. PAHs were also detected in all of the samples, with total concentration between 0.72 to 5.36 mg/kg dry weight for six PAHs. In the examined samples, phenanthrene was the main compound with a mean concentration of 1.0567 mg/kg. The results from purification studies of bacteria strains successfully isolated 13 bacteria strains from single PAH substrate while three bacteria were isolated from the mixed PAHs substrate. Based on bacteria growth rates, only six strains grown on single PAHs and three strains grown on mixed PAHs were used for further studies. Results from the optimization study of biodegradation indicated that maximum rate of PAHs removal occurred at 100 mg L\(^{-1}\) of PAHs, 10% bacteria concentration, pH 7.0 and 30°C. The results showed that bacteria grown on lower ring of PAHs are not able to grow on higher ring of PAHs. As for example Micrococcus diversus grown on napthalene as sole carbon source was unable to degrade other PAHs like acenaphthylene, acenapthene, fluorene, phenanthrene and anthracene. In the case of bacteria isolated from mixed PAHs, the results showed that most of the napthalene was degraded by isolated strains with the highest average degradation rate followed by acenaphthylene, acenapthene, fluorene, phenanthrene and anthracene.
Different degradation trends were observed in the study could be attributed to the different substrates provided during isolation process. Interaction through cometabolism and synergistic occurred for bacteria strains isolated from single substrate. Thus, only synergistic interaction was observed for bacteria isolated from mixed substrate. *Corynebacterium urolyticum* revealed to be the best strain in degrading PAHs. The experimental results have led to a model concept describing PAHs degradation.
# TABLE OF CONTENT

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
</tr>
<tr>
<td>CANDIDATE DECLARATION</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
</tr>
<tr>
<td>TABLE OF CONTENT</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
</tr>
<tr>
<td>LIST OF SYMBOLS</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
</tr>
</tbody>
</table>

## CHAPTER 1: INTRODUCTION

1.1 Background of Study

1.2 Problem Statement

1.3 Objectives of Study

1.4 Scope of Work

1.5 Significance of the Study in Malaysia

1.6 Limitation of Study

1.7 Concluding Remarks

## CHAPTER 2: LITERATURE REVIEW

2.1 Introduction to Sludge Management in Malaysia

2.1.1 Advantages and disadvantages of sludge for agriculture and health

2.1.1.1 Advantages of sludge for agriculture

2.1.1.2 Disadvantages of sludge for agriculture

2.1.1.3 Impact of heavy metal in sludge

2.1.1.4 Effect of heavy metals on biological systems

2.2 Problem Associated to Sludge Management in Malaysia

2.2.1 Sludge quantity and associated problems
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3</td>
<td>Sludge quality and problems</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Sludge Characteristics</td>
<td>26</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Studies on sludge characteristics in Malaysia</td>
<td>26</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Chemical characteristics of municipal sludge</td>
<td>28</td>
</tr>
<tr>
<td>2.4</td>
<td>Characterization and Treatment Types in Malaysia</td>
<td>29</td>
</tr>
<tr>
<td>2.5</td>
<td>Bioremediation</td>
<td>31</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Principles of bioremediation</td>
<td>33</td>
</tr>
<tr>
<td>2.5.2</td>
<td>Methods of bioremediation</td>
<td>34</td>
</tr>
<tr>
<td>2.5.2.1</td>
<td>In-situ bioremediation</td>
<td>35</td>
</tr>
<tr>
<td>2.5.2.2</td>
<td>Ex-situ bioremediation</td>
<td>35</td>
</tr>
<tr>
<td>2.5.3</td>
<td>Microbes as degradation agent</td>
<td>37</td>
</tr>
<tr>
<td>2.5.4</td>
<td>Factors affecting the bioremediation</td>
<td>39</td>
</tr>
<tr>
<td>2.5.4.1</td>
<td>Abiotic factors</td>
<td>40</td>
</tr>
<tr>
<td>2.5.4.2</td>
<td>Physiological factors</td>
<td>42</td>
</tr>
<tr>
<td>2.5.5</td>
<td>Advantages and limitations of bioremediation</td>
<td>45</td>
</tr>
<tr>
<td>2.6</td>
<td>Biodegradability of Organic Pollutants</td>
<td>48</td>
</tr>
<tr>
<td>2.6.1</td>
<td>Polycyclic aromatics hydrocarbons</td>
<td>49</td>
</tr>
<tr>
<td>2.6.2</td>
<td>Degradation of PAHs</td>
<td>52</td>
</tr>
<tr>
<td>2.6.2.1</td>
<td>Biological degradation of PAHs</td>
<td>52</td>
</tr>
<tr>
<td>2.6.2.2</td>
<td>Chemical degradation of PAHs</td>
<td>55</td>
</tr>
<tr>
<td>2.6.3</td>
<td>Study on Biodegradation of PAHs</td>
<td>56</td>
</tr>
<tr>
<td>2.7</td>
<td>Isolation and Identification of PAH Degradating Bacteria</td>
<td>57</td>
</tr>
<tr>
<td>2.7.1</td>
<td>Enrichment for isolation of PAHs degrading bacteria</td>
<td>57</td>
</tr>
<tr>
<td>2.7.2</td>
<td>Culturing bacteria</td>
<td>58</td>
</tr>
<tr>
<td>2.7.3</td>
<td>Strain identification</td>
<td>59</td>
</tr>
<tr>
<td>2.8</td>
<td>Extraction Method for PAHs</td>
<td>60</td>
</tr>
<tr>
<td>2.8.1</td>
<td>SPME device</td>
<td>61</td>
</tr>
<tr>
<td>2.8.2</td>
<td>Mode of SPME operation</td>
<td>61</td>
</tr>
<tr>
<td>2.8.3</td>
<td>Instrumental tools in analyzing PAHs</td>
<td>62</td>
</tr>
<tr>
<td>2.8.3.1</td>
<td>SPME-GC</td>
<td>62</td>
</tr>
<tr>
<td>2.8.3.2</td>
<td>SPME-HPLC</td>
<td>62</td>
</tr>
<tr>
<td>2.9</td>
<td>Biodegradation Kinetic</td>
<td>63</td>
</tr>
</tbody>
</table>
CHAPTER 3: METHODOLOGY

3.1 Sampling Location

3.2 Sampling Method

3.3 Overview of Experiments

3.4 Materials and Apparatus

3.4.1 Bacteria strains

3.4.2 Apparatus used in this study

3.4.3 Chemicals

3.4.3.1 Apparatus cleaning method for heavy metals analysis

3.4.3.2 Apparatus cleaning method for PAHs

3.4.3.3 Apparatus cleaning method for microbiology Tests

3.4.4 Equipments used in this study

3.5 Characterisation of Municipal Sludge

3.5.1 Analysis on bulk parameters

3.5.2 Analysis on heavy metals

3.5.2.1 Sample pretreatment

3.5.2.2 Digestion of samples for metal analysis

3.5.2.3 Preparation of heavy metals standard aqueous solution

3.5.2.4 Analytical Instrument for heavy metals

3.5.2.5 Quality control

3.5.3 Analysis of PAHs

3.5.3.1 Sample pretreatment for PAHs analysis

3.5.3.2 Preparation of PAH standard solution

3.5.3.3 Sample extraction for PAHs

3.5.3.4 Analytical Instruments for PAHs

3.6 Isolation and Identification of PAHs Degrading Bacteria

3.6.1 Enrichment processes

3.6.2 Isolation of bacteria
3.6.3 Identification of strains
  3.6.3.1 Gram staining
  3.6.3.2 Preparing inoculum
  3.6.3.3 Inoculating and incubating on micro plate
  3.6.3.4 Reading micro plate & determine ID
3.7 Turbidity Test
3.8 Preparation of Bacterial Strain and PAH Degradation
3.9 Effect of Abiotic Factors on PAHs Degradation
  3.9.1 Effects of initial bacterial strain concentration on PAHs degradation
  3.9.2 Effect of substrate concentration on PAHs degradation
  3.9.3 Effects of pH on PAHs degradation
  3.9.4 Effects of temperature on PAHs degradation
3.10 Sample Extraction
3.11 Sample Analysis
3.12 Protein Test
3.13 pH Measurement
3.14 Concluding Remarks

CHAPTER 4: RESULTS AND DISCUSSION
4.1 Introduction
4.2 Municipal Sludge Characterization
  4.2.1 Bulk Parameters
    4.2.1.1 Analysis on pH
    4.2.1.2 Analysis on total solid and volatile solid
    4.2.1.3 Analysis on COD
    4.2.1.4 Analysis on nutrients
    4.2.1.5 Comparison of bulk parameters analysis with reported studies on Malaysian sludge
  4.2.2 Heavy metals
    4.2.2.1 Calibration curves
    4.2.2.2 Concentration of heavy metals in municipal sludge
4.2.2.3 Comparison of Heavy Metals with reported studies in Malaysian sludge

4.2.3 Polycyclic Aromatic Hydrocarbons (PAHs)

4.2.3.1 Calibration curves

4.2.3.2 PAHs concentration in municipal sludge

4.3 Enrichment Process for PAH Degrading Bacteria

4.3.1 Isolation of bacteria

4.4 Turbidity Test

4.5 Identification of Bacteria

4.6 Optimization of Degradation Conditions

4.6.1 Effect of Initial Bacteria Concentration on PAHs degradation

4.6.2 Effect of pH on PAHs degradation

4.6.3 Effect of temperature on PAHs degradation

4.6.4 Effects of Initial substrate concentration on PAHs degradation

4.7 Degradation Study

4.7.1 Degradation study by bacteria isolated from single substrate

4.7.1.1 PAHs degradation by Micrococcus diversus (isolation substrate-naphthalene)

4.7.1.2 PAHs degradation by Rhodococcus rhodochrous (isolation substrate-acenaphthene)

4.7.1.3 PAHs degradation by Corynebacterium agropyri (isolation substrate-acenaphthene)

4.7.1.4 PAHs degradation by Micrococcus diversus (isolation substrate-fluorene)

4.7.1.5 PAHs degradation by Corynebacterium urolyticum (isolation substrate-phenanthrene)

4.7.2 Degradation study by bacteria isolated from mix substrate

4.7.2.1 Identification of most preferred PAHs degrading bacteria

4.7.2.2 Identification of highly performance
CHAPTER 5: MODEL DEVELOPMENT
5.1 Introduction 171
5.2 Development of the Model Concept 171
5.3 Parameter Estimation 172
5.4 Model calibration for PAHs Degradation 175
5.5 Application of PAHs degradation Model to Engineering Problems 186
5.6 Concluding Remark 188

CHAPTER 6: CONCLUSION AND RECOMMENDATION
6.1 Summary of Finding 189
6.2 Conclusion and Contribution from This Study 190
6.3 Recommendation for Further Works 192

REFERENCES
Appendix A
Appendix B
Appendix C
Appendix D
Appendix E
Appendix F
### LIST OF TABLE

<table>
<thead>
<tr>
<th>TABLE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Effects of pollutants in sludge on human health</td>
<td>19</td>
</tr>
<tr>
<td>2.2</td>
<td>Chemical characteristic of municipal sludge from different countries</td>
<td>29</td>
</tr>
<tr>
<td>2.3</td>
<td>Advantages of bioremediation technology</td>
<td>46</td>
</tr>
</tbody>
</table>

### CHAPTER 2

| 3.1   | Major phases of experimental works | 72   |
| 3.2   | Apparatus used in sample preparation and running of experiments | 73   |
| 3.3   | Chemicals used in this study | 74   |
| 3.4   | Equipments used for sample preparation | 76   |
| 3.5   | Equipments used in sample analysis | 76   |
| 3.6   | Determination of Bulk parameters | 77   |
| 3.7   | Specific wavelength for heavy metals | 79   |
| 3.8   | Summary of abiotic factors | 90   |

### CHAPTER 3

| 4.1   | pH value of sample | 97   |
| 4.2   | Total solid and volatile solid in samples | 99   |
| 4.3   | COD value of sample | 101  |
| 4.4   | Nutrients content in sample | 102  |
| 4.5   | Bulk parameters of municipal sludge in Malaysia | 103  |
| 4.6   | Concentration of heavy metals in municipal sludge (mg/kg) | 105  |
| 4.7   | Concentrations of heavy metals reported in the literature | 107  |
| 4.8   | Limit for land application of heavy metals | 108  |
| 4.9   | Concentration of PAHs in municipal sludge | 109  |
| 4.10  | Concentrations of PAHs reported in the literature | 110  |
4.11 Total concentration of six PAHs with different extraction method
4.12 Medium for enrichment process of PAHs degrading bacteria
4.13 Results for gram staining (single source of PAHs)
4.14 Results for gram staining (mix sources of PAHs)
4.15 Average growth rate for isolated strains
4.16 Identification of bacteria using Biolog
4.17 PAHs degradative bacteria from literature
4.18 Previously reported optimum amount of bacteria concentration for various bacteria
4.19 Phenanthrene degradation by Corynebacterium uroalyticum
4.20 Previously reported optimum temperature for various PAHs degradation
4.21 Previously reported optimum concentration on PAHs for various biodegrader
4.22 Optimization results for all isolated bacteria
4.23 Retention time of PAHs
4.24 Type of interaction between substrate and bacteria
4.25 Interaction between Micrococcus diversus and PAHs
4.26 Interaction between Rhodococcus rhodochrous and PAHs
4.27 Interaction between Corynebacterium agropyri and PAHs
4.28 Interaction between Micrococcus diversus and PAHs
4.29 Interaction between Corynebacterium uroalyticum and PAH
4.30 Interaction between Pediococcus pentosaceus and PAH
4.31 Interactions of isolated strains with PAHs
4.32 Interaction between Corynebacterium uroalyticum and PAHs
4.33 Interaction between Sphingobacterium spiritovorum and PAHs
4.34 Interaction between Tsukamurella inchenensis and PAHs
4.35 Interaction between isolated strains and PAHs
4.36 PAHs degradation rate by isolated strains (isolation substrate-mix PAHs)
4.37 Degradation rate by various degrading bacteria
<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Summary of Experiments Conducted during laboratory Activities</td>
<td>9</td>
</tr>
<tr>
<td>2.1</td>
<td>The Three-stage Strategy for Sludge Management formulated by IWK</td>
<td>13</td>
</tr>
<tr>
<td>2.2</td>
<td>Acts, Standard and Guidelines for Sludge Management in Malaysia</td>
<td>16</td>
</tr>
<tr>
<td>2.3</td>
<td>The Volume of Sludge and urban population at different years</td>
<td>24</td>
</tr>
<tr>
<td>2.4</td>
<td>Sludge quality parameters as reported by various researchers on Malaysian sludge</td>
<td>27</td>
</tr>
<tr>
<td>2.5</td>
<td>Biochemical Pathway for Oxidation of PAHs</td>
<td>54</td>
</tr>
<tr>
<td>3.1</td>
<td>Map of sampling location</td>
<td>69</td>
</tr>
<tr>
<td>3.2</td>
<td>Schematic diagram of Mawar wastewater treatment plant and the location of sampling</td>
<td>70</td>
</tr>
<tr>
<td>3.3</td>
<td>Schematic diagram of digestion apparatus and digestion procedure</td>
<td>78</td>
</tr>
<tr>
<td>3.4</td>
<td>SPME procedure for PAHs extraction introduced and optimized by Othman et al (2008)</td>
<td>81</td>
</tr>
<tr>
<td>3.5</td>
<td>SPME device with the specific fibre used in this study</td>
<td>82</td>
</tr>
<tr>
<td>3.6</td>
<td>Dilution process</td>
<td>85</td>
</tr>
<tr>
<td>3.7</td>
<td>Colonies of microbes on minimal media agar</td>
<td>86</td>
</tr>
<tr>
<td>3.8</td>
<td>The Biolog identification process</td>
<td>87</td>
</tr>
<tr>
<td>3.9</td>
<td>Changing of color in micro plates after incubation</td>
<td>88</td>
</tr>
</tbody>
</table>
CHAPTER 4

4.1 Cumulative pH value of samples
4.2 Relationship between volatile and total solid
4.3 Solid content and COD in sample
4.4 Relationship between volatile solid and COD
4.5 Concentration of nutrients in sample
4.6 Concentration of heavy metals in municipal sludge
4.7 Agar plates with pure culture
4.8 Agar plate with streaking
4.9 Clear zone on minimal salt agar
4.10 Four distinct colour of strains (a:pink ; b:lightly pink ; c:cream; d:yellow)
4.11 Absorbance value for single source of PAH
4.12 Absorbance value for mix PAHs
4.13 Phenanthrene degradation by different concentration of Corynebacterium ureolyticum after 14 days of incubation
4.14 Normalise PAHs degradation for inoculum concentration (single PAHs substrate)
4.15 Normalise PAHs degradation for inoculum concentration (mix PAHs substrate)
4.16 Effects of pH on phenanthrene degradation by Corynebacterium ureolyticum
4.17 Phenanthrene degradation by Corynebacterium ureolyticum
4.18 Normalise PAHs degradation for pH value (single PAHs substrate)
4.19 Normalise PAHs degradation for pH value (mix PAHs substrate)
4.20 Effects of temperature on phenanthrene degradation by Corynebacterium ureolyticum
4.21 Normalise PAHs degradation for temperature (single PAHs substrate)
<table>
<thead>
<tr>
<th>Page</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.22</td>
<td>Normalise PAHs degradation for temperature (mix PAHs substrate)</td>
</tr>
<tr>
<td>4.23</td>
<td>Effects of substrate concentration on phenanthrene degradation</td>
</tr>
<tr>
<td>4.24</td>
<td>Average degradation rate for different concentration</td>
</tr>
<tr>
<td>4.25</td>
<td>Normalise PAHs degradation for substrate concentration (single PAHs substrate)</td>
</tr>
<tr>
<td>4.26</td>
<td>Normalise PAHs degradation for substrate concentration (mix PAHs substrate)</td>
</tr>
<tr>
<td>4.27</td>
<td>PAHs degradation using <em>Micrococcus diversus</em> (isolation substrate-naphthalene)</td>
</tr>
<tr>
<td>4.28</td>
<td>PAHs degradation using <em>Rhodococcus rhodochrous</em> (isolation substrate-acenaphthylene)</td>
</tr>
<tr>
<td>4.29</td>
<td>PAHs degradation using <em>Corynebacterium agropyri</em> (isolation substrate-acenaphthene)</td>
</tr>
<tr>
<td>4.30</td>
<td>PAHs degradation using <em>Micrococcus diversus</em> (isolation substrate-fluorene)</td>
</tr>
<tr>
<td>4.31</td>
<td>PAHs degradation using <em>Corynebacterium urolyticum</em> (isolation substrate-phenanthrene)</td>
</tr>
<tr>
<td>4.32</td>
<td>PAHs degradation using <em>Pediococcus pentosaceus</em> (isolation substrate-anthracene)</td>
</tr>
<tr>
<td>4.33</td>
<td>PAHs degradation by <em>Corynebacterium urolyticum</em> (isolation substrate-mix PAHs)</td>
</tr>
<tr>
<td>4.34</td>
<td>PAHs degradation by <em>Sphingobacterium spiritovorum</em> (isolation substrate-mix PAHs)</td>
</tr>
<tr>
<td>4.35</td>
<td>PAHs degradation by <em>Tsukamurella inchoensis</em></td>
</tr>
<tr>
<td>4.36</td>
<td>Degradation of phenanthrene by different bacteria</td>
</tr>
<tr>
<td>4.37</td>
<td>Change of protein during degradation (bacteria isolated from single substrate)</td>
</tr>
<tr>
<td>4.38</td>
<td>Change of pH during degradation (bacteria isolated from single substrate)</td>
</tr>
<tr>
<td>4.39</td>
<td>Change of protein during degradation (bacteria isolated from mix substrate)</td>
</tr>
<tr>
<td>4.40</td>
<td>Change of pH during degradation (bacteria isolated from mix substrate)</td>
</tr>
</tbody>
</table>
CHAPTER 5

5.1 Concentration of remaining phenanthrene predicted and observed for data set one
5.2 Concentration of remaining phenanthrene predicted and observed for data set two
5.3 Concentration of remaining phenanthrene predicted and observed for data set three
5.4 Concentration of remaining phenanthrene predicted and observed for data set four
5.5 Concentration of remaining phenanthrene predicted and observed for data set five
5.6 Concentration of remaining phenanthrene predicted and observed (substrate-50mg/l phenanthrene)
### LIST OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2</td>
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<tr>
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<tr>
<td>RO2•</td>
<td>peroxy radicals</td>
</tr>
<tr>
<td>HO•</td>
<td>hydroxyl radicals</td>
</tr>
<tr>
<td>μ</td>
<td>growth rate</td>
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<tr>
<td>q</td>
<td>substrate utilization rate</td>
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<td>μmax</td>
<td>maximum specific growth rate</td>
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</tr>
<tr>
<td>X0</td>
<td>initial biomass concentration</td>
</tr>
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<td>initial substrate concentration</td>
</tr>
<tr>
<td>dS/dt</td>
<td>substrate disappearance rate</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background of Study

Sludge generated during wastewater treatment had been traditionally regarded as a necessary nuisance byproduct. Sludge can be defined as an active biological solid residue resulting from the treatment of wastewater. There are three types of sludge, namely, sewage sludge from septic tank, sludge from municipal treatment works and industrial sludge (Hope, 1986; Schnaak et al., 1997; Nathan and Robert, 2003). All three constitute growing management problems in sewerage industries throughout the world.

Sludge production will continue to increase as new sewerage treatment facilities are built to cater for the increase in population especially in urban areas. The large volume produced had made it impossible for many countries to assure complete treatment of the sludge before discharging to the receiving environment (Abdul-Hamid and Muda, 1999; Mahmoud et al., 2002). Indah Water Konsortium reported that in 2005 the total amount of sewage sludge generated in Malaysia was estimated at 6.5 million cubic meters (IWK, 2005). Recent data in 2007 reported that the volume increased to 7 million cubic meters (IWK, 2008).

Land application is a favorable practice to dispose off sludge in Malaysia and is thought to be very effective and efficient method. Sludge may be applied to agriculture land, forest, disturbed land or dedicated disposal sites (Plaquart et al., 1999; Alvarez et al., 2002; Walker and Kelleg, 2003). In this case, wastewater sludge is viewed as a valuable resource especially as soil conditioner and nutrients for non food crop.

However, large volume of sludge will create problem to sludge management industry especially to ensure safe disposal of sludge because it contains diverse range of pollutants such as pathogen, inorganic and organic compounds. These pollutants are
toxic, mutagenic or carcinogenic and may threaten crop yields, long term soil quality, wild life health or eventually constitute health hazard to human (Chang et al., 1997; Rathbone et al., 1998; Jensen and Jepsen, 2005). Improper disposal and handling of sludge may pose serious impact to the environment especially on soil and water cycles (Lazzari et al., 2000; Dhanagunan and Narendran, 2001; Abdullah and Baki, 2006).

There are several remediation and reclamation techniques that are currently used to clean up land contaminated with hazardous material. The choice of technology depends on various factors such as type and concentration of contaminants, soil properties and geological conditions. However, the choice of treatment normally is decided base on absolute cost and circumstances (Rakesh et al., 2005).

Many remediation methods for heavy metals removal from sludge have been proposed in the last 25 years (Petrasek et al., 1983; Chan et al., 2003). However, the removal of persistent organic pollutant (POP) is much more difficult because of their stable physical and chemical properties. Polycyclic aromatic hydrocarbons (PAHs) constitute an important class of POP in causing environmental problem due to their high potential of toxicity, mutagenicity and carcinogenicity to mammals and aquatic organism (Wilson and Jones, 1993; Mangas et al., 1998; Jardé et al., 2005).

During this decade, bioremediation has gained acceptance as an alternative technology for pollutant removal. It can be defined as the use of microorganism or plants to detoxify any environmental components by degradation of pollutants. Bioremediation techniques have been used at a number of sites contaminated with organic compounds, including the well published clean up of oil spill from Exxon Valdez in Prince William Sound, Alaska in 1989 (Claxton et al., 1991; Young and Cerniglia, 1995; Venkata et al., 2006).

Bioremediation has advantage over thermal and some physical chemical techniques in term of cost. In this case, cost association with excavation and disposal of contaminated material are not incurred. In addition, soil as life support system suitable for plant growth is not destroyed. Furthermore, the contamination is not transferred elsewhere, as it can be degraded on site (Rakesh et al., 2005).
More research needs to be pursued prior to the successful application of on site bioremediation. The presence of microorganism with catabolic potential to degrade the target pollutant is indispensable. Many efforts are made to isolate novel microorganism that can degrade different pollutants (Mishra et al., 2001; Marin et al., 2005; Dzulkifli, 2007). Moreover, it is important to understand the factors limiting or promoting the biodegradation process such as bioavailability, toxicity, temperature and nutrients. Once basic knowledge is established, bioremediation can be applied with better efficiency and effectiveness.

Biodegradation of individual PAHs compounds by pure and mixed microbial communities have been reported by several researchers mostly in developed countries (Cemiglia 1992; Boldrin et al., 1993; Moody et al., 2001). However, very little information is available on PAHs degradation from developing countries like Malaysia. Thus, no evidence has been found indicating that the isolated stains from other countries are effectively used in Malaysia. Furthermore, there is limited report on capability of indigenous microbes in degrading PAHs. Therefore, a study on indigenous microbes isolated from PAHs contaminated sites locally is necessary.
1.2 Problem Statement

In many countries, sludge management represents 50% of the total wastewater treatment cost. Disposal techniques adopted fall into three categories: application on land, disposal in a landfill or thermal technology (Campbell et al., 1996; Bradley and Dhanagun, 2004). The selection of disposal method mainly depends on cost effectiveness factor.

At the moment, most of the municipalities in Malaysia are adopting land application as the main method for final disposal of municipal sludge. Land has long been a repository for sludge, often with few constrains attached to the practice. In some cases, sludge was applied onto land at extremely high loading rates. Currently, no attempt is made to use sludge as edible crop fertilizer on a large scale. Municipal sludge from Pantai wastewater treatment plant is being used as fertilizer for landscaping plants. Whatever disposal choices adopted, sludge removal from treatment facilities need to be well managed and controlled in order to protect human health and the environment.

The problems of sludge handling and disposal are further complicated by the fact that different wastewater treatment facilities, used in Malaysia, will produce sludge of different characteristics (Bradley and Dhanagun, 2004; Abdullah and Baki 2006). An investigation on the characteristic of pollutants should be done in order to gain better understanding on the nature of the sludge. Knowledge on sludge characteristics is necessary to determine and decide the best option before treating and disposing sludge (Hepe, 1986; Mahmoud et al., 2002).

Several studies had reported on characteristics of Malaysian sludge (Dhanagun and Narendra, 2001; Bradley and Dhanagun, 2004; Abdullah and Baki 2006). However, these studies only reported on bulk parameters and heavy metals in sludge. There is no information on organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) reported in these studies. Nowadays, PAHs can be found in many household products such as mothballs, cosmetics and hair dyes. In addition, some food products such as roasted coffee, peanut and barbecued meat also constitute other source
of PAHs in domestic setting. These products at the end of their used would eventually end up in the wastewater treatment plants thus contributing to PAHs presence in sludge.

A variety of methods had been applied to handle and treat sludge in Malaysia, namely, drying beds, trenching, sludge lagoons, mobile mechanized dewatering and centralized treatment facilities (Mohd-Din and Velayutham, 1999; Abdullah and Baki, 2006). The treatment methods are carried out mainly to reduce water content in sludge before disposal. The design of treatment methods basically focus on physical and chemical processes but somehow neglect biological processes. It must be noted that trenching, sludge lagoons and centralized treatment facilities involve biodegradation which relies on microbial communities to break down organic substances in municipal sludge (Faehrul-Razi et al., 2005).

Studies on the microbial processes to degrade organic pollutants in several countries utilizing microbes from different sources have established information on the best microorganism to be used (Churchill et al., 1998; Yuan et al., 2000; Gaskin et al., 2005; Yu et al., 2005). Churchill et al., (1998) studied riverine sediment sample in USA. Meanwhile, Yuan et al., (2000) studied PAH degrading bacteria from petrochemical effluent in Taiwan. Beside that, Gaskin et al., (2005) studied on biodegradation of PAHs using bacteria from soil sample taken from coal gasification site in South Australia. PAHs degradative bacteria isolated from mangrove sediment had been reported by Yu et al., (2005). Very few researches have been reported on studies in tropical countries like Malaysia (Ghazali et al., 2004 and Dzulkifli, 2007). It is noted that bacteria strain isolated from different sources, places and environment will show diverse potential in treating PAHs contaminated sites. Lack of information on microbial consortium to degrade PAHs in Malaysia currently impedes further development on bioremediation efforts to treat PAHs contaminated sites.

Thus, there is a need to study on the nature of local municipal sludge and identify microbial consortium that can effectively degrade PAHs. For bioremediation to be successfully implemented as a remediation technology, it is essential to understand environmental factors that affect biodegradation of PAHs. Information on diversity of PAHs mixtures and biodegradation kinetics of PAHs components will provide better
understanding on PAHs biodegradation. Integrated information on qualitative and quantitative studies will lead to better knowledge on bioremediation.

1.3 Objectives of Study

The overall goal of this research is to study biological treatment of organic pollutants in municipal sludge focus on the biodegradation of PAHs. The research is also aimed at providing a better understanding on the biodegradation processes, rates and mathematical equation for biodegradation rate of PAHs under aerobic condition. The equation established will be useful in determining the fate of PAHs in the environment.

The specific objectives of this study are:

i. To determine concentration of bulk parameters, heavy metals and PAHs in municipal sludge.

ii. To screen, isolate, identify and evaluate growth rate of microbes from municipal sludge that can metabolize PAHs.

iii. To optimize conditions, namely PAHs concentration, inoculum concentration, pH and temperature for biodegradation of PAHs.

iv. To quantitatively evaluate biodegradation rate of selected PAHs compounds

v. To evaluate protein content and change of pH during degradation study

vi. To introduce a mathematical equation for biodegradation of PAHs
1.1 Scope of Work

This study consists of field activities, laboratory work and mathematical modeling. Field activities involved sampling of municipal sludge from Kolej Mawar Universiti Teknologi MARA, Shah Alam Selangor. Laboratory activities include seven phase of experiments as illustrated in Figure 1.1. The seven phases included characterization study, enrichment and purification, identification of bacteria, turbidity test, optimization study, biodegradation study, protein and pH test. Results from laboratory activities will be used in formulating a mathematical model that describes the biodegradation process.

Sludge characteristics of bulk parameters only focus on total solid, volatile suspended solid, pH, COD, total nitrogen and total phosphorus. While for heavy metals, only five elements had been analyzed namely zinc, lead, chromium, cadmium and nickel.

This study also focuses on low chain PAHs namely naphthalene, acenaphthene, acenaphthene, fluorene, phenanthrene and anthracene. Studies were conducted under aerobic condition. Identification of bacteria was done using a standardized identification system (BIOLOG) not a molecular technique. The optimum biodegradation conditions were determined as a function of concentration of PAHs (substrate), concentration of microbes, pH and temperature.
Stage 1
Characteristic study on municipal sludge

Purpose:
To determine concentration of bulk parameters, heavy metals, PAHs in municipal sludge.

1. Analysis of bulk parameters based on Standard Method for Water & Wastewater.
2. Analysis of heavy metals were run using flame-AAS
3. Analysis of PAHs were run using GC-PID
   (Pretreatment processes were done for each specific method before analysis)

Stage 2
Enrichment & Purification

Purpose:
To screen & isolate microbes from municipal sludge that can metabolize PAHs.

(a) Enrichment
   i. Preparation of minimal media (MM)
   ii. Add 10g of municipal sludge
   iii. 0.1% of PAHs inoculated to MM
   iv. Incubate for two months, at 30°C, 150rpm
      (turbid culture were taken for isolation)

(b) Purification & Isolation
   Perform multi steps of purification until pure strains were obtained

Stage 3
Bacteria Identification

Purpose:
To identify bacteria using BIOLOG

i. Gram staining
ii. Preparation of liquid inoculating fluid
iii. Dip a sterile swab into inoculating fluid
iv. Lift cell from pure culture agar using sterile swab
v. Dip the swab into the fluid & adjust inoculum density with specific turbidity range.
vi. Fill all microplate well with cell suspension.
vii. Incubate microplate for 24 hours at 30°C
viii. Read microplate using Micolog Software

Stage 4
Turbidity Test

Purpose:
To evaluate the growth rate of bacteria isolated

i. Used minimal media broth
ii. Determination of turbidity daily for two weeks
iii. Instrument: Spectrophotometer
Stage 5
Optimization study

Purpose:
To optimize conditions, namely PAHs concentration, pH and temperature for biodegradation of PAHs
i. Batch experiments
ii. Concentration of PAHs
iii. pH
iv. Temperature

Stage 6
Biodegradation test

Purpose:
To quantitatively evaluate PAHs degradation rate of selected PAHs compounds
i. Perform PAHs extraction using SPME
ii. Analyze PAHs using GC-FID

Stage 7
Protein & pH test

Purpose:
To evaluate protein content and change of pH during degradation study
i. Perform protein test using Lowry Method
ii. Observe pH change using pH meter

Figure 1.1: Summary of Experiments Conducted during Laboratory Activities
1.5 Significance of the Study in Malaysia

Current practice of sludge disposal in Malaysia is on land. Lack of understanding and mismanagement of land application of sludge has resulted in more contaminated sites. Therefore, an in depth studies especially on sludge quality need to be performed. Study on sludge quality needs to incorporate sludge characteristics on bulk parameters, heavy metals and organic pollutants. Recent studies and published literatures showed that not much information was reported on organic contaminant in Malaysian sludge (Dhanagunan and Narendran, 2001; Bradley and Dhanagunan, 2004; Abdullah and Baki 2006). The finding from this research may eventually lead to the development or improvement of the existing database on sludge characteristics in Malaysia especially in terms of organic contaminants.

As stated earlier, bioremediation is emerging as an alternative technology for cleaning up sites contaminated with organic pollutants. Bioremediation involves microbial transformation processes that occur under aerobic or anaerobic condition. Laboratory scale studies related to bioremediation of PAHs using indigenous microbial strains will provide better knowledge and understanding of bioremediation process. Such knowledge is essential before bioremediation concept in any PAHs contaminated site in Malaysia could be applied. It is also noted that using isolated strains from other countries for bioremediation purpose is not feasible as geographical and environmental factors in Malaysia is much different.

It is not possible to control environmental factors on treatment site. Therefore studies should be directed to explore appropriate treatment method for PAHs such as bioremediation process using local strains. The results will help in minimizing PAHs in municipal sludge so that concentration of these compounds getting into soil during disposal is minimized. Having knowledge on sludge characteristics and microbial processes in biodegradation will enable effective treatment processes to be proposed.
1.5 Limitation of Study

This study was carried out to analyze one type of sludge namely, sludge from a municipal wastewater treatment plant located at Mawar College, UiTM Shah Alam. Thus, sludge from septic tank and industrial wastewater treatment plants are not analyzed. In this case, it is assumed that experimental results are narrower. It is also assumed that the condition of replicates in all sampling bottles are similar.

The biodegradation process was also limited by type of carbon sources. Only one type of carbon source that is PAH is used. Thus, results from the studies are based on laboratory experiments. For modelling purpose, only results from single substrate of biodegradation were used.

In addition, no experiments were conducted to study the pathway of chemical degradation for each pollutant. Results from this study will be compared against studies reported in literature where appropriate.

1.7 Concluding Remarks

The increased of population especially in urban areas generates more municipal sludge. A large quantity of municipal sludge will create problem to the environment if pollutants in the sludge is not well treated before dispose off. A complete sludge characteristic is needed in order to understand the nature of sludge. Thus, suitable treatment methods could be applied to specific pollutants in the sludge. This study is important and timely to provide basic knowledge and understanding on how characteristics of sludge could influence treatment method adopted. With the understanding of characteristics and treatment method of specific pollutants, an environmental friendly treatment system could be applied in treating sludge.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Sludge Management in Malaysia

Before 1993, sewerage services in Malaysia were under the jurisdiction of 144 Local Authorities. It was reported that 80% of the public sewage treatment facilities did not meet the standard set by Department of Environment. Though land application was applied to dispose sludge but some portions of sludge remain untreated. It was reported that about sixty five percent of the five million cubic meters of sludge produced in Malaysia was untreated and discharge into waterways causing serious pollution to rivers in Malaysia (Abdul-Hamid and Muda, 1999).

Realizing that sewerage system in Malaysia were in a deplorable condition, an Act was introduced in 1993 to deal with the management of sewerage services in order to avoid problems of sewage pollution of receiving waters. The Sewerage Service Acts (Sewerage Services Act (Act 518), 1993) empowers the Federal Government to take control of sewerage services in Malaysia. Department of Sewerage Services was formed as the regulator of the sewerage industry thus relieving the Local Authorities of this responsibility (Abdul-Hamid and Muda, 1999).

Furthermore, SSA (1993) allows for privatization of sewerage services, and subsequently Indah Water Konsortium (IWK) was offered a 28 years (later extended to 40 years) concession to operate and maintain the national sewerage system. In 2001, due to financial problems faced by IWK, The Federal Government through The Ministry of Finance acquired the entire private equity of IWK.

Upon being awarded the concession to undertake the management of the sewerage services in Malaysia, IWK outlined a three-stage strategy for sludge management, namely, immediate, short term and long term strategies as illustrated in Figure 2.1. The
Figure 2.1: The Three-stage Strategy for Sludge Management formulated by IWK (Abdullah et al., 2005a; Ahmad-Janí, 2010)

These methods include installation of reception facilities, drying beds, trenching, sludge lagoons, mobile mechanized dewatering and centralized sludge treatment facilities (CSF). Brief descriptions and relative merits of these methods will be discussed.
in the following sub-section (Section 2.4). It is interesting to note that trenching method is applicable under immediate and short term strategy, while sludge lagoons and mobile mechanized dewatering methods are applicable under both short and long term strategy. This indicated flexibility in terms of location and economy. Suitable methods were selected based on ease of operation and maintenance.

In Malaysia, there are various Acts, Standard and Guidelines, that govern sludge management (Othman et al., 2007). Figure 2.2 illustrates regulatory instruments for sludge management. The Environmental Quality Act (EQA, 1974) is one of the major legislations that govern sludge management. The EQA (1974) is a general law that specifies the standard for effluent discharges. This Act also addresses aspect of handling, treatment and disposal of sewage sludge. The EQA (Sewage and Industrial Effluents), Regulations 2009 was introduced to regulate sewage and industrial effluent.

In addition to the EQA (1974) two new Acts, namely, Water Services Industry Act (WSI) and National Water Services Commission Act (SPAN) were introduced in 2006. The WSI Bill empowers the Federal Government to manage and regulate water and wastewater services in Malaysia, thus relieving State Government of these responsibilities. The SPAN Act calls for the establishment of a legislatively mandated body to monitor and regulate water and wastewater services industry in Malaysia.

Apart from that, standards and guidelines to regulate sludge management practices in Malaysia are issued by the sewerage industry, with the assistance of relevant Government Agencies such as Standards and Industrial Research Institute of Malaysia (SIRIM) and Sewerage Services Department (SSD).

The Malaysian Standard (MS 1228) was introduced to regulate sewerage system design. This code of practice deals with the planning, design, construction, installation and testing of sewerage system, that include the sewers and sewer appurtenances, sewage pump stations, sewage treatment works and all other works necessary to collect, convey, treat, and finally dispose domestic sewage and industrial effluents. Procedures related to sludge treatment and disposal is covered in Section 8 of MS 1228. Treatment methods applicable in this standard include preliminary treatment, sludge thickening, mechanical dewatering facilities, anaerobic sludge digestion, aerobic sludge digestion,
sludge drying beds and sludge disposal on land. The treatment methods stipulated in the NS 1228 had been adopted in the three stage strategies for sludge management formulated by IWK that was discussed earlier.

Five guidelines for developers were published by Sewerage Service Department (SSD). These guidelines provide clear policies in the development of new sewerage infrastructure. Sludge management is addressed in Guidelines for Developers: Sewage treatment plants (Volume IV). In 2009, SPAN revised and published three of five volumes of guidelines published by SSD.

In addition, six specific guidelines had been drafted by IWK and SSD to ensure proper sludge reuse and disposal. Two have been finalized while the remaining four are still under review (Abdullah et al., 2005b; Ahmad-Jani, 2010).

These Acts, Standard and Guidelines are used to regulate sludge management in Malaysia. Though these regulations are available, Malaysia still lacks regulations on the characteristics of sludge or Parameter Limits for disposal.

In considering the utilization of sludge in agriculture or any land application, several factors must be addressed. Chemical quality limits must be set for the receiving soil to prevent loss or poor crop yield by virtue uptake of chemicals. Toxic chemicals may accumulate in the food chain with serious consequences for grazing animals and human beings.

Apart from that, limit for microbiological factors must be set especially for pathogenic species. Affordable treatment is needed to prevent the spread of such pathogenic organism to human, animal or plant. Aesthetic quality, which relate to odor and visual aspect of operation must also be accounted for. Finally, factors dealing with acceptability and accountability of the farmers must also be addressed. The usefulness of sludge and the requirement of a farmer will differ depending on area and crop types. (Cecil et al., 1996).
Figure 2.2: Acts, Standard and Guidelines for Sludge Management in Malaysia (Othman et al., 2007)
Advantages and disadvantages of sludge for agriculture and health

Municipal sludge is a resource because it contains valuable nutrient to plants and improve holding capacity of poor soil. On the other hand, municipal sludge also contains toxic substances and harmful organisms for plants. The following discussion is focused on specific advantages and disadvantages of municipal sludge on agriculture.

2.1.1.1 Advantages of sludge for agriculture

Generally, there is an increasing interest in the agricultural application of sludge originating from municipal wastewater treatment plants, due to the possibility of recycling valuable components in the sludge. Sludge can provide nitrogen and phosphorus for plant growth. Several micronutrients such as zinc, copper and iron are also available in most sludge. The added organic matter enhance soil quality, making clay soils more permeable to water and air, and increasing the water and nutrient holding capacity of sandy or gravel soils. The texture of the soil can also be greatly improved (Berghlund et al., 1984; Cogliastro et al., 2001; Edson et al., 2007).

Previous studies had proven that the application of sludge to land offers several advantages (Bledsoe, 1981; Sopper et al., 1982; Berghlund et al., 1984, Cole et al., 2001; Dolgen et al., 2004). In recent years, there has been renewed interest in utilization of wastewater sludge, because of the drawback of other disposal methods (Hope, 1986; McBride, 2003). According to Hope (1986), municipal sludge can be directly applied to croplands, forest, disturbed land as soil reclamation or as composted sludge for landscaping and gardening.

Studies on the beneficial uses of sludge from several places to date have established information on sludge use. Bledsoe (1981) studied the application of sludge in relation to timber production such as Douglas fir, polar and cottonwood. The study showed dramatic improvement in tree growth over unfertilized control areas. Study on the use of sludge for soil reclaiming at coal strip in Centralia, Washington, had proven that sludge was cost effective compared to other techniques such as adding topsoil, liming, fertilizing and irrigating (Sopper et al., 1982). While Berghlund et al. (1984) reported that yield of some
were increased as much as three times compared to the use of commercial fertilizer on sandy or clay soils. In addition, composted or treated sludge is valuable for landscaping or gardening purposes. In Western Scotland, the application of sludge resulted in a better growth of *Isotomurus palustris* (Cole et al., 2001). Dolgen et al. (2004) had proven that sludge was a good soil conditioner to replace chemical fertilizer in growing lettuce. The above studies showed that utilization of sludge may represent a saving on disposal costs.

The application of sludge as fertilizer in agriculture lands represents an economic way to use the high amounts of sludge produced by the wastewater treatment plants. Currently, the agriculture sector is moving towards environmental friendly techniques with the goal of producing high quality crops. This requires the use fertiliser of high quality. Therefore limit value for heavy metals, organic micropollutants and pathogens must be established as a quality criterion.

2.1.1.2 Disadvantages of sludge for agriculture and health

Though utilization of sludge is very promising especially for soils, there are several problems which must be carefully managed to protect the safety of the environment. The most serious of these are the harmful constituents of sludge such as heavy metals, toxic organics and pathogenic microorganisms. In some situation, there may be nuisance factors such as odor on the neighboring environment, which could give impact on land values. Public acceptance is also crucial to the success of any reuse of sludge. The public is reluctant to accept the reuse of sludge especially when associated with nuisance problem and disease vector. It is noted that data on microbiological quality are almost non-existent in developing countries. Therefore, specific management practice and careful monitoring are required to mitigate these risks.

The contaminants constitute hazards for human health that may enter the food chain directly or through plant uptake. Studies from several researchers, as shown in Table 2.1, had proven that contaminants in sludge may cause significant effects towards health problem and life deterioration.
Table 2.1: Effects of pollutants in sludge on human health

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Researcher/Year</th>
<th>Specific elements</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy metals</td>
<td>Wilhelm et al., 2003</td>
<td>Mercury, Arsenic</td>
<td>Acute &amp; chronic poison of animals &amp; human.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cadmium</td>
<td>Accumulate in kidney &amp; cause irreversible kidney damage.</td>
</tr>
<tr>
<td></td>
<td>Duraibe et al., 2007</td>
<td>Zinc</td>
<td>Vomiting, diarrhea, liver &amp; kidney failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead</td>
<td>Disfunction of reproductive system</td>
</tr>
<tr>
<td>Organic contaminants</td>
<td>Hu et al., 2007</td>
<td>Phenanthrene</td>
<td>Photosensitizer of human skin &amp; mild allergen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Naphthalene</td>
<td>Hemolytic anemia &amp; nephrotoxicity</td>
</tr>
<tr>
<td>Pathogenic organisms</td>
<td>Holtby et al., 2006</td>
<td>Bacteria (Salmonella spp)</td>
<td>Food poisoning &amp; typhoid fever.</td>
</tr>
<tr>
<td></td>
<td>Hurst, 2000</td>
<td>Virus (Rotavirus)</td>
<td>Digestive problem</td>
</tr>
<tr>
<td></td>
<td>Asaolu et al., 2002</td>
<td>Ascaris lumbricoides</td>
<td>Abdominal pain, vomiting</td>
</tr>
</tbody>
</table>

Metals from the soils may also be absorbed onto root of plants. Many organic micropollutants are capable of uptake by plants. Another pathway for entering the food chain is through grazing animals. Infectious organism found in sludge can be neutralized by several treatments processes, such as, heating sludge or irradiation. However, these treatments are relatively expensive. Toxic organics on the other hand tend to concentrate in fatty tissues of animals. In all of the above situations, human health can easily be put at risk, because high concentration of pollutants accumulated in plants and animals are consumed by humans.
3.1.3 Impact of heavy metal in sludge

High concentrations of heavy metals in the environment are undesirable as they may have potentially adverse effects on the health of organisms. This problem will become more serious when they are transferred through the ecological cycle causing chronic illness due to metal accumulation in the bodies of living organisms. In the case of sludge, the problem of heavy metals is related to various purposes of sludge reuse.

In Malaysia a separate sewer networks is mainly adopted. The sewer network only caters for municipal wastewater, while industrial wastewater is channelled through other means of treatment and storm water is conveyed in open drains. As such, high concentrations of heavy metals from industries are not likely to be present in municipal wastewater sludge.

Studies related to heavy metal compounds in municipal sludge had proven that the most common heavy metals discharged into the natural environment are zinc, lead and cadmium (Hart et al., 1982; Jian-Wang, 1997; Baker et al., 2002; McBride, 2003).

Published literatures showed that zinc is one of the most common heavy metals in wastewater sludge. High concentration of zinc in the sludge can be attributed to consumables and toiletries in consumer products such as shampoo. At pH value below 7, zinc is present as divalent cation. It can readily form complex with organic and inorganic compounds. In wastewater, zinc is readily adsorbed onto suspended solid and become incorporated in sludge (Hart et al., 1982; McBride, 2003). Even though zinc is an essential element or micronutrient, excessive amounts have been known to cause gastroenteritis upon ingestion (David & Phyllis, 1993; Wang & Zhu, 2010).

Lead is a non-essential, highly toxic metal, and all known effects of lead on biological systems are deleterious. Sources of lead in the environment include atmospheric emissions, vehicular emissions, fossil fuel combustion, and industrial emissions. It exists in both inorganic (Pb(II) and Pb(IV)) and organic forms (up to four Pb-C bonds). Inorganic lead is known to be cycled globally more significantly than organically bound lead. Nevertheless, organically bound lead compounds may contribute significantly on a local scale (David & Phyllis, 1993; Gallert & Winter, 2004). The occurrence of lead in drinking water is mostly the result of contamination of the water.
source or corrosion in the distribution system where lead piping is used (Lester, 1987; Clement et al., 2000). Acute and classical lead poisoning in human adults is manifested by anemia and renal damage. Lead levels higher than 0.8 mg/L in the bloodstream have been considered to be the level for clinical lead poisoning even though symptoms are sometimes encountered with a blood lead concentration of 0.6 mg/L or less (Lester, 1987; Kwong et al., 2004). The amount of lead released from wastewater treatment plants depends on the range and nature of effluents discharged. In countries where combine sewer system are used, approximately 80-100% of lead from industrial discharges, highway run-off, and domestic wastewater end up in sewage sludge during treatment, and that remaining in the water phase is discharged into the receiving environment (Byrd & Perona, 1980; Jian-Wang, 1997; Merrington et al., 2003).

Cadmium is mainly used for electroplating, pigments, nickel-cadmium batteries or electronic equipments. Based on the separate sewer system adopted, cadmium should not be detected in Malaysian municipal sludge. However, it is possible that cadmium may be introduced through the sewer network via open access in household plumbing or directly discharged into roadside manhole as illegal discharges.

Cadmium has been established as a very toxic heavy metal. Cadmium tends to accumulate in the kidney and liver of human body (Volesky, 1990; Torra et al., 1995; Baker et al., 2002). The human body seldom excretes as much cadmium as it absorbs. Cadmium interferes with the metallothionein’s ability to regulate zinc and copper concentrations in the body. Metallothionein is a protein that binds excess essential metals to render them unavailable. When metallothionein activity is induced, the metallothionein binds to copper and zinc, thus disrupting the homeostasis levels (Kennish, 1992; Norasberg et al., 2009). Cadmium toxicity to aquatic organisms is influenced by species, size, age, hardness, pH, complexation, and their diet. Cadmium is acutely toxic to fish in the order of 1.0 to 5080 ng/l and is markedly affected by water parameters such as hardness, and dissolved oxygen concentration. (Hoffman et al., 1995)

The presence of potentially toxic heavy metals in the environment is very much a concern, primarily due to their non-biodegradability and persistence in the environment (Volesky, 1990; Alvarenga et al., 2007). It has been a cause of considerable attention for
sludge containing heavy metals due to the high desire for safe and clean sludge disposal technology.

Some metals are also known to become concentrated in food chains through bioaccumulation, notably, mercury and cadmium (Hoffman et al., 1995; McAloon & Mason, 2003). Low concentrations of certain metals are harmless and traces are considered good for nutrition; for example, cobalt, copper, iron, selenium, and zinc are considered nutrients needed for balanced growth. Higher dosages may cause toxicity that is acute, chronic, or mutagenic/teratogenic (Hoffman et al., 1995; Claxton et al., 2007). Hence, removal of heavy metal from sludge before disposal or reuse is a necessary step towards a sustainable sludge management and treatment.

2.1.1.4 Effect of heavy metals on biological systems

In most municipal wastewater treatment facilities, a biological system is employed to oxidize organics from the wastewater. In an activated sludge system, microorganisms utilize organic matter in the wastewater as substrates under aerobic conditions, thus removing them by microbial respiration and synthesis. These microorganisms include bacteria (both single and multi cellular), protozoa, fungi, rotifers, and nematodes. The principal organisms involved in bio-oxidation of organic substances in wastewaters are the single celled bacteria. Protozoa does not use the organic substances in the wastewater but instead feed on the bacterial population. Rotifers, on the other hand, feed on activated sludge fragments that are too large for protozoa. Nematodes use organic materials that are not readily oxidized by other microorganisms (Reynolds, 1995; Verlecar et al., 2006).

When the concentrations of soluble heavy metals in wastewater are increased, the activity of the microorganism will decrease. This will reduce the efficiency of a biological treatment system in degrading and may cause the effluent quality to deteriorate. If dissolved heavy metals are present in sufficient concentration in the influent to a biological treatment system, the metals tend to accumulate in the system's biological treatment operations, and this may decrease the operating efficiency in removing organic matter and suspended solids (Eysenbach, 1992; Pérez-De-Mora et al., 2006; Zhang et al.,
This decrease in efficiency may also cause the metals concentration in the effluent to increase.

In conventional wastewater treatment, metal ions are removed through primary settling and in the activated sludge process. Primary settling removes a proportion of metals that are either insoluble or adsorbed onto the suspended solids (Oliver & Cosgrove, 1974; Northcott, et al., 2007). In the activated sludge process, microorganisms biologically degrade materials (dissolved or suspended organic compounds) and convert them into carbon dioxide, water, and cellular materials (Brown & Lester, 1979; Wilen et al., 2008). The microorganisms form aggregates or flocs and then pass into a settling tank (sedimentation). Hence, any substance or metal that is adsorbed by the bacterial flocs may be partially removed from the water through a combination of microbial adsorption and sedimentation processes.

Factors which affect the settling properties of mix liquor such as loading rate, feed composition, mixing strength, and sludge volume index will also affect its capacity to remove metals (Brown & Lester, 1979; Jin et al., 2003). Over a certain range of metal loadings (<2 mg/L), there seems to be a linear relationship between the initial heavy metal loading and percentage of heavy metal removal for cadmium and chromium (Neufeld & Hermann, 1975; Jin et al., 2003). The concentration of metals in the sludge produced may be as much as twenty to thirty times greater than the concentration in the influent due to accumulation of metals in the sludge (Rysenbach et al., 1992; Chipasa, 2003).

2.2 Problem Associated to Sludge Management in Malaysia

In an attempt to improve the sewerage services in Malaysia, the government through IWK had built more centralized and permanent treatment facilities. Generally, the higher number of treatment facilities leads to the increase in the amount of sludge generated annually. The large amount of sludge is the main challenge for sewerage industry especially to assure safe treatment and disposal.
2.3.1 Sludge quantity and associated problems

The quantity of sludge produced annually in Malaysia has increased dramatically since the introduction of central sewerage system in 1990. Active research was done by IWK to establish the amount of sludge produced in all sewerage treatment facilities under its responsibilities. In 1999, Mohd Din & Velayutham had reported about 3 million cubic meters of sludge had been produced. Following that in 2000, it was reported that 4.2 million cubic meters was produced (Bradley & Dhanagunan, 2004). In 2001, it was reported that the amount had risen to 5 million cubic meters (Dhanagunan & Narendran, 2001). Until 2005, the reported value of sewage sludge generated in Malaysia was estimated at 6.5 million cubic meters. Based on the observed data over six years, the average increase in sludge production is 0.58 million cubic meters per year. The increased in sludge production observed may be due to increase in population or in number of treatment plants being put under the jurisdiction of IWK. As such this number must be treated with caution.

In Malaysia, sludge production will continue to increase as new sewerage treatment facilities are constructed to cater for the increase in population especially in urban areas. Figure 2.3 illustrates that the volume of sludge increased as the urban population increased. The correlation factor between sludge volume and urban population in Malaysia is 0.95. This shows that the sludge volume highly depends on urban population (Othman et al., 2007).

![Figure 2.3: The Volume of Sludge and urban population at different years](image-url)

(Sludge volume: Mohd Din and Velayutham, 1999; Dhanagunan and Narendran, 2001; Bradley and Dhanagunan, 2004; IWK, 2005; Population data: Dept of Statistics)
REFERENCES


