Pollutants Inflow from Btg Ai River and Delok River and Btg Ai Reservoir Outflow and Possible Impact on Aquaculture

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A dissertation submitted in partial fulfilment of the requirement for the degree of Bachelor of Science (Hons.)

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Declaration

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

___________________________
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<tr>
<td>°C</td>
<td>Degree Celcius</td>
</tr>
<tr>
<td>μg</td>
<td>Microgram</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>ANOVA</td>
<td>One-way Non-repeated Measures Analysis of Variance</td>
</tr>
<tr>
<td>BOD</td>
<td>Biological Oxygen Demand</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Calcium ion</td>
</tr>
<tr>
<td>CaCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Calcium chloride</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Chlorine</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>Cr</td>
<td>Chromium</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper (Cuprum)</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
</tr>
<tr>
<td>DOE</td>
<td>Department of Environment</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GPS</td>
<td>Global Positioning System</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Sulphuric acid</td>
</tr>
<tr>
<td>ha</td>
<td>hectare</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HClO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Perchloric acid</td>
</tr>
<tr>
<td>HDPE</td>
<td>High Density Polyethylene</td>
</tr>
<tr>
<td>HF</td>
<td>Hydrogen fluoride</td>
</tr>
<tr>
<td>HMP</td>
<td>hexametaphosphate</td>
</tr>
<tr>
<td>HNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Nitric acid</td>
</tr>
<tr>
<td>in</td>
<td>inche</td>
</tr>
<tr>
<td>K&lt;sub&gt;2&lt;/sub&gt;S&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Potassium sulphate</td>
</tr>
<tr>
<td>km</td>
<td>kilometer</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
</tbody>
</table>
L.O.I. Loss-On-Ignition

$M$ Molarity

$\text{mg} \ kg^{-1}$ Milligram per kilogram

$\text{mg}$ milligram

$\text{mg/L}$ Milligram/Litre

$\text{mL}$ Millilitre

$\text{mL/L}$ Millilitre/Litre

$\text{mm}$ Millimeter

$N$ Normality

NaOH Sodium hydroxide

$\text{NH}_3$ Ammonia

Ni Nickel

nm Nanometer

NREB Natural Resources and Environment Board

NWQSM National Water Quality Standards for Malaysia

P Phosphorus

Pb Lead (Plumbum)

pH Potential of hydrogen ion

ppm Parts per million

PSA Particle Size Analysis

SO$_3$ Sulfur trioxide

TSS Total Suspended Solids

USEPA United States Environment Protection Agency

V Vanadium

WQI Water Quality Index

wt Weight

Zn Zinc
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Abstract

Settlements and developments along the Batang Ai River and Delok River may contribute some pollutants to the water and sediment. Besides that, maintaining water quality and sediment quality are very important to sustain the cage aquaculture activity in Batang Ai Reservoir. Hence, the objective of this study was to investigate the water and sediment quality of Batang Ai River and Delok River and Batang Ai Reservoir. The results obtained from two sampling trips at 5 selected stations indicated that the water quality there was clean which fall in Class II of NWQSM classification. Results for ex-situ water quality parameters are TSS (0.58-8.33 mg/L), BOD₅ (3.4-6.61 mg/L), COD (32 – 53.3 mg/L), SRP (0-0.0141 mg/L), TP (0.0193-0.0439 mg/L), NH₃-N (0-0.0576 mg/L), NO₂-N (0.001-0.007 mg/L), NO₃-N (0.01-0.02 mg/L) and TKN (1.216-1.692 mg/L). Nonetheless, the overall water and sediment quality was classified as not possess any negative impacts and damages to the aquatic life and aquaculture activities in the river and reservoir ecosystems.

Keywords: Water quality, sediment, aquaculture, river, reservoir.

Abstrak

Perumahan dan pembangunan di sepanjang Sungai Batang Ai dan Sungai Delok mungkin menyumbang kepada sebilangan bahan pencemar terhadap air sungai dan mendapan. Selain itu, amat penting untuk mengekalkan kualiti air dan mendapan bagi mempertahankan aktiviti akuakultur sangkar di Empangan Batang Ai. Oleh itu, objektif kajian ini adalah untuk meneliti kualiti air dan mendapan di sepanjang Sungai Batang Ai, Sungai Delok dan Empangan Batang Ai. Keputusan yang diperoleh dalam dua kali pensampelan di 5 stesen menunjukkan kualiti air di situ termasuk dalam Kelas II pengelasan NWQSM, iaitu menunjukkan kualiti air adalah bersih. Keputusan ex-situ adalah TSS (0.58-8.33 mg/L), BOD₅ (3.4-6.61 mg/L), COD (32 – 203 mg/L), SRP (0-0.0141 mg/L), TP (0.0193-0.0439 mg/L), NH₃-N (0-0.0576 mg/L), NO₂-N (0.001-0.007 mg/L), NO₃-N (0.01-0.02 mg/L) dan TKN (1.216-1.692 mg/L). Walau bagaimanapun, kualiti air dan mendapan secara keseluruhan dikelaskan sebagai tidak memberi ancaman terhadap hidupan akuatik dan aktiviti akuakultur di dalam sungai dan ekosistem empangan.

Kata kunci: Kualiti air, mendapan, akuakultur, sungai, takungan.
1.0 Introduction

The ecosystems of freshwater serve as the important medium for society through provision (e.g., products and food), supporting (e.g., waster processing and supply of clean water) and enriching or cultural (e.g., aesthetic and recreational) services (Postel & Carpenter, 1997; Covich et al., 2004; Yang et al., 2007). However, the rapid development of industry and agriculture has increased human activities along the water resources which eventually deteriorate the water quality (Postel & Carpenter, 1997; Giller, 2005; Yang et al., 2007). This situation also happens in Batang Ai Reservoir, Batang Ai River and Delok River. Both Batang Ai River and Delok River are the major rivers that contribute water to the upstream of Batang Ai Reservoir are potential to increase the quantity of pollutants in the reservoir as there are longhouses situated along both rivers. Therefore, this study is inevitable to analyze the water quality in the rivers and reservoir.

Aquaculture has become a more popular fish farming method after it has been introduced by the government. In Sri Aman, cage aquaculture was initiated by Department of Agriculture Sarawak in 1993 after the construction of Batang Ai Hydroelectric Dam (ADB, 1999). Since then, it acts as an important source of freshwater fish to the local people (Paka et al., 2009). Hence, maintaining the water quality in Batang Ai Reservoir is vital to sustain the growth of aquatic organisms and to maintain the production of fish all year round without fail. By conducting this research, the possible pollutant loadings can be determined and effective measures can be figured out to control the water quality.

Sediments are defined as the mineral and organic particles layers, often fine-grained, that are present at the bottom of natural water bodies such as lakes, rivers, and oceans (Baird & Cann, 2005). These sediments play the crucial role as the ultimate sink
for heavy metals and most of the toxic organic compounds (Baird & Cann, 2005). As a result, sediments can remove part of the pollutants present in the body of water. If only the water quality is being analyzed, the results shown will not represent the actual phenomenon of the river and reservoir because the excess heavy metals, organic matter and nutrients that present in the water body have been adsorbed on the sediments.

1.1 Problem Statement

The activities in the settlement located along the Batang Ai River and Delok River are potential pollutant sources that may affect the water and sediment quality.

1.2 Objective

1. To investigate the water quality and sediment quality of inflow rivers Batang Ai River and Delok River and outflow of Batang Ai Reservoir.

2. To determine the inflow quantity of pollutants.

3. To discuss the possible impact of water quality on aquaculture.
2.0 Literature Review

The distribution of the global water is found to be approximately 96% of saline, 3% of frozen ice caps and glaciers, 1% of groundwater, 0.01% of lake water and only 0.001% of river water (Readman, 2006). Water quality indicates the fitness of water usage and the presence of pollutants. The various parameters used to measure the river water quality plays a vital role as indicative of the presence of contaminants and are measured based on in-situ and ex-situ analysis. The status of the river water quality in Malaysia is evaluated by the Department of Environment (DOE) using the Water Quality Index (WQI). WQI is used as the guideline for environment assessment of a watercourse in relation to pollution load categorization and designation of classes of beneficial uses as provided for under the National Water Quality Standards for Malaysia (NWQSM) to determine the status as clean, slightly polluted or polluted (WWF-Malaysia, n.d.). The six parameters used to determine the water quality index (WQI) are comprised of Dissolved Oxygen (DO), Total Suspended Solids (TSS), pH, Biochemical Oxygen Demand (BOD$_5$), Ammoniacal nitrogen (NH$_3$-N) and Chemical Oxygen Demand (COD).

Currently, it is about 40% of the world’s population are living in moderate to high water stress areas (WWF-Malaysia, n.d.) due to the decrease of freshwater all over the world. According to McAngus and Garrett (1986), any chemical can become a ‘pollutant’ if it is present at a high enough concentration. In other words, these chemical pollutants can be described as discharges of substances both found and not found in natural water causing it to be more than its natural levels (Turner, 1990). This means that threats to the biota will only occur when the pollutant concentration is higher than usual. Many chemicals are regarded as pollutants; such as inorganic ions, organic pollutants, organometallic compounds and radioactive isotopes. In addition, most toxicity causing
agent in the water body was found to be related to alkyl-phenols, chlorophenols, alkyl-substituted naphthalenes, alkyl-substituted fluorenes, atrazine and dimethylbenzoquinone (Readman, 2006).

2.1 Water Quality Monitoring in Malaysia

Asian rivers were reported as the “most polluted in the world, with 3 times as many bacteria from human waste as the global average and 20 times more lead than those from industrialized countries” (WWF-Malaysia, n.d.). As the population growth rate in Malaysia is continuously increasing, pollution of river water will become a never ending issue if the public is still being ignorant. In year 2006, it was reported that out of 1064 water quality monitoring stations in Malaysia, 619 (58%) were categorized as clean, 359 (34%) were categorized as slightly polluted and 86 (8%) were polluted (Water Environment Partnership in Asia, 2010). Most of the clean categorized stations reported were located upstream. Meanwhile, 80 river basins (55%) were found to be clean, 59 (40%) were slightly polluted and 7 (5%) polluted (Water Environment Partnership in Asia, 2010). Each polluted river basins reported have different types of pollutant loadings and polluted parameters. However, the river water quality was assessed according to the NWQSM where each parameter values were compared to the standard limit and designated classes.

2.1.1 Water Quality Status in Sarawak

In Sarawak, the Natural Resources and Environment Board (NREB) is holding the responsibility to monitor the river water quality status of the state since 1998. According to the Environmental Quality Report 2009 (NREB, 2009), out of 52 rivers being monitored, 13% were found to be clean, 60% slightly polluted and 27% polluted.
Most of the polluted rivers reported are situated near high density of human population and industries. Meanwhile, the slightly polluted rivers were reported located at area near the human settlement and also the plantation and other land development activities. NREB (2009) also indicated that the number of polluted rivers have increased from 9 rivers in 2008 to 14 rivers in 2009. This increment can be related to rapid population growth, land development along river basin, urbanization, agricultures, aquacultures and industrialization. These anthropogenic disturbances have generated both organic and inorganic wastes that ultimately contaminate the water bodies (Muhammad et al., 2006). Thus, NREB (2009) has been directed to ensure the water quality of every river in Sarawak to be maintained at least at Class IIB of NWQSM.

On the other hand, NREB (2009) also reported the water quality status of Batang Ai Hydroelectric Dam in year 2009 was in Class IIB of NWQSM. Each parameter’s results were reported as follows: pH 6.69, TSS – 5.75 mg/L, DO – 5.24 mg/L, BOD – 2.13 mg/L, COD – 4.75 mg/L and NH$_3$-N – 0.10 mg/L.

2.1.2 Sources of River Pollution

Pollutants are categorized into point source (domestic wastewater or industrial outfalls) and non-point sources (agrochemical run-off or deposition of atmospheric pollutants) (Readman, 2006). The untreated or partly treated effluent and sewage from manufacturing and agro-based industries can cause high BOD value in river whilst the domestic sewage and livestock farming near river contribute to high NH$_3$-N value (NREB, 2009). Besides that, Muhammad et al. (2006) reported the main pollutant sources in the surrounding of Lake Chini in Malaysia are the residential areas, illegal logging, and development and agricultural activities because the surface runoff and erosion from these activities have contributed to TSS value in water bodies (NREB, 2009). Suspended solids
that settle on the stream bed will change the nature of the substratum from riverine into lacustrine (Geraldes & Boavida, 1999; Lourantou et al., 2007) can smother many organisms living within it especially the fish communities (Mason, 1990) due to severe hydrological fluctuation. Eutrophication phenomena had occurred in Lake Chini due to nitrogen and phosphorous enrichment in wastewater runoff of oil palm plantations that contained fertilizers, pesticides, and herbicides (Muhammad et al., 2006). Furthermore, the untreated or partially treated discharges from residential areas contained high organic matter and nutrients are potential to cause an over-stimulation of primary production, eutrophication and oxygen levels depletion in the receiving water bodies (Jones et al., 2001).

2.2 Possible Impact of Water Quality on Aquaculture

Aquaculture is estimated to reduce hunger and poverty in the world’s poorest nations by half by 2015 if the aquaculture plays the important role as food producing sector (Sheriff et al., 2008). Stress caused by poor water quality especially insufficient DO in water (NREB, 2009) can caused massive fish kills, reduce growth rates and increase the susceptibility of culture organisms to diseases (Molony, 2001). This is because the presence of contaminants can reduce the richness and evenness of aquatic communities (Johnston et al., 2009). Besides that, Fisheries Research Institute (2007) reported that the decrease in salinity to less than 13 ppt with two critical parameters showing 0.66 ppm NH₃ and 0.90 ppm iron were the factors of fish mortalities in Merchang River, Malaysia. Furthermore, alteration of river and floodplain ecosystem to change the flow regime has been regarded as one of the main factors that affect the aquatic community (Bunn & Arthington, 2002; Park et al., 2010). For example, the changed in flow magnitudes and decrease in river’s variability due to the construction of
the dam in the Geum River basin in Republic of Korea was reported to cause a reduction of sensitive riffle-benthic species in the riffle-pool sequences (Park et al., 2010). In addition, Hayami et al. (2008) reported the shallow oxycline in the Cirata Reservoir (Java, Indonesia) as the main reason for the massive fish kills because the anoxic water can be easily moved up to affect the fish in cages.

Exposure to toxic or carcinogenic chemicals may cause accumulation of toxicity in aquatic organisms (Margaret & John, 2007). Studies also showed that these harmful substances are released from anthropogenic activities to cause accumulation in aquatic organisms through the food web (Agusa et al., 2005). Coastal area of Malaysia has been reported to contaminate with organochlorines, tributyltin, and heavy metals (Agusa et al., 2005). However, the presence of other matters and the characteristics of the particular water body may cause modification on these toxics (Margaret & John, 2007). For example, the toxicity of pollutants to fish is inversely related to water hardness which is due to the competition between Ca$^{2+}$ and metal ions for binding sites in biological systems (Margaret & John, 2007). Agusa et al. (2005) reported significant higher concentration of V, which is rich in crude oil, was found in liver of the bigeye scads of the west coast of Peninsular Malaysia due to the serious oil pollution in the Strait of Malacca.

2.2.1 Batang Ai Hydroelectric Dam

Reservoirs are regarded as the environmental hybrids of lotic and lentic systems (Lourantou et al., 2007). Based on a Batang Ai Case Study done by the organization of Asian Development Bank (1999), the preproject conditions of Batang Ai Dam has resulted large migratory fish species to be adapted to the fast-moving water. Consequently, these fish species become rare and nonexistent in the reservoir and the
upper reaches (ADB, 1999). However, several nonmigratory fish species have been released into the reservoir. These fish species are adapted to the reservoir condition that makes them abundant in the reservoir, which was a success for the introduction of floating cage culture into the reservoir. Furthermore, it was estimated to produce 20 tons of fish per month during the Mission. The study also predicted the long-term risk that may be posed on the fisheries there especially at the downstream and in the reservoir because the exotic tilapia will become overpopulated if they escaped from the cage aquaculture. Besides that, the study also revealed the poor living condition for aquatic life below the dam because of the production of the hydrogen sulfide is poisonous and harmful to most living things. This condition may cause the dissolved oxygen in the reservoir greatly reduced and the reservoir can possess anoxic conditions as a result of improper vegetation clearing and fish cage culture (ADB, 1999).

2.3 Sediment

Studies have identified the adsorption onto sinking particles as the major on the composition of seawater over thousands to millions of years (Whitfield & Turner, 1987; Turner, 1990). This process can affect the short term fate of metal pollutants in dynamic environments such as estuaries (Morris, 1986; Turner, 1990). Ling et al., (2009a) concluded the study of sediment is vital as sediment plays the role of sink for organic materials, nutrients and other pollutants; and eventually the contact of sediment could affect the water quality after a long time. Based on the literature (Ling et al., 2009b), organic phosphorus liberated by bacterial communities can accumulate in the upper layer of sediment, which may in turn cause eutrophication. Microorganisms utilize oxygen to break down organic matter being discharged into the watercourse to the detriment of the stream biota (Mason, 1990). During the break down of organic matter, the released
nutrients can stimulate the growth of aquatic plants and these additional nutrients in the water body are known as eutrophication (Mason, 1990). Zhang et al. (2006) reported the eutrophic waters are abundant with planktons or attached algae and contain high nutrients. Furthermore, eutrophication can cause depletion of oxygen and possess threats to aquatic organisms (Ling et al., 2009b). According to Nelson et al. (1994), the low dissolved oxygen was reported in Klang River, Malaysia, as a result of the high oxygen demand of sediment that rests on the bottom during neap tides and was resuspended during spring tides. Studies also reported that although there was a reduction of dissolved exogenous organic matters in the land-locked embayment overlying water in Hong Kong, the soft bottom sediment continued to act as sources of nutrients (Chau, 2002). Eventually, the bottom sediments of the water column becomes the potential exists for remobilization of nutrients and metals that can affect the fate and influence of pollutants through changes in redox status (Margaret & John, 2007).

Although many studies have been done on the water quality of Batang Ai Reservoir, the water and sediment quality of Batang Ai River and Delok River have never been studied. These rivers are the main rivers that flow into the reservoir where cage aquaculture is the main activity in the reservoir. The daily activities of the longhouses along the two rivers are potential to affect the water quality in the reservoir as well. Hence, this study is vital to determine how much effect has been impacted on the cage aquaculture.
3.0 Materials and Methods

3.1 Study site

Batang Ai Reservoir, Batang Ai River and Delok River are located in the Division of Sri Aman, Sarawak. It is located some 14 km from the interior town of Lubok Antu, close to the national boundary of Sarawak, Malaysia and Kalimantan, Indonesia. Batang Ai dam is 85 meters high. It floods an area of 8500 ha (21,000 acres) of forest which stretches up to Sungai Engkari and Batang Ai (Economic Planning Unit, 1996). The Batang Ai River and Delok River flow through several settlements, where domestic wastes are discharged from the Iban longhouses into the river. The development of tourism and aquaculture may result in a decline to the water quality (Economic Planning Unit, 1996). The main agricultural activities in Delok River such as shifting cultivation of rice and cash cropping of pepper (*Pipernigum L.*) and rubber can possess deterioration to the river water quality too (Mertz, 2007). There are approximately seven longhouses situated along the Batang Ai River and Delok River as informed by the boatmen during the sampling trip. Population recorded at the study in year 1999 were approximately 138 Iban natives (Itik, 1999).

Table 1: Sampling stations selected for determination of water and sediment quality.

<table>
<thead>
<tr>
<th>Station</th>
<th>GPS Reading</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N 01° 15’ 36.1” E 112° 02’ 33.3”</td>
<td>Upstream of Batang Ai River near Batang Ai National Park</td>
</tr>
<tr>
<td>2</td>
<td>N 01° 13’ 53.4” E 112° 00’ 40.1”</td>
<td>Downstream of Delok River below the longhouses</td>
</tr>
<tr>
<td>3</td>
<td>N 01° 12’ 12.4” E 111° 56’ 57.2”</td>
<td>Downstream of Batang Ai River (before the confluent of Batang Ai River and Engkari River)</td>
</tr>
<tr>
<td>4</td>
<td>N 01° 11’ 40.8” E 111° 55’ 32.6”</td>
<td>Confluent of Batang Ai River and Engkari River</td>
</tr>
<tr>
<td>5</td>
<td>N 01° 08’ 48.9” E 110° 52’ 44.1”</td>
<td>Outflow of Batang Ai Hydroelectric Dam</td>
</tr>
<tr>
<td>6 (sediment)</td>
<td>N 01° 09’ 53.1” E 111° 54’ 44.9”</td>
<td>No cage culture area near the reservoir inflow</td>
</tr>
</tbody>
</table>
Figure 1: Location of sampling stations at the study site (http://maps.google.com).
3.2 Field Sampling

Sampling was being carried out a total of two times between 25 – 26 November 2011 and 6 – 7 April 2012. Three replicates of water and soil sediments were collected from each sampling site. Then, these samples were kept in different HDPE and borosilicate glass bottles and stored in icebox filled with ice cubes to prevent oxidation. Soil sediments were wrapped in aluminium foil to exclude any light penetration. Subsequently, all the samples were transported back to the laboratory for other chemical parameters analysis within 24 hours. The entire replicates samples were composited before chemical analysis to reduce variation.

3.3 In-situ measurement of water quality

In-situ water quality parameters for each station were determined. The physical parameters such as pH, temperature, turbidity and DO were measured using YSI 6600 Multiparameter Water Monitor. The flow velocity was measured by using current meter (FLO-MATE Model 2000 Portable Flowmeter) at different depth (surface, middle and bottom) of river. The coordinates of sampling sites were measured using GPS readers. Width of water column was determined using range finder (Bushnell Laser rangefinder Elite 1500). Depth of water column at different and fixed distance from the bank at each sampling sites was determined using a depth finder (PS-7, Hondex) and water transparency was determined by using a Wildco® Secchi disc.

3.4 Ex-situ measurement of water quality

Samples for nutrient analysis were filtered at the time of sample collection, using Glass Microfibre GF/C Whatman filters (0.45 μm) (Whatman International Ltd, Kent, U.K.). Glass-free filters were used for phosphorous samples. The filtered samples were
put in acid-rinsed bottles, transported to the laboratory in an icebox, and stored at 4°C in
the dark until analysis (maximum storage time of 24 hours).

All the ex-situ measurements of water quality and sediment quality were
conducted according to the Standard Method for the Examination of Water and

3.4.1 Chemical Oxygen Demand (COD): 5220 C. Closed Reflux, Titrimetric
Method (APHA, AWWA & WEF, 1998)

Culture tubes (16 mm) and caps were washed with 20 % H₂SO₄ before first
use to prevent contamination. Then, 2.5 mL of sample and 2.5 mL of deionized water
(blank) were poured into two culture tubes respectively. After that, 1.5 mL of potassium
dichromate – mercuric sulfate digestion solution was added and 3.5 mL of sulfuric acid
reagent was run down inside the vessel. The tubes were capped and inverted several
times to mix completely. Subsequently, the tubes were placed in block digester preheated
to 150°C and refluxed for 2 hours. The tubes were cooled to room temperature after the
reflux is complete. The culture tube caps were removed and small TFE-covered magnetic
stirring bar was added. Before the titration, 2 drops of ferroin indicator were added.
Titration was run with standardized 0.10M ferrous ammonium sulphate (FAS) while
magnetic stirrer is stirring rapidly. The end point is a sharp colour change from blue-
green to reddish brown. The calculation of COD was as follows:

$$\text{COD as mg O}_2/\text{L} = \frac{(A - B) \times M \times 8000}{\text{mL sample}}$$

A: mL FAS used for blank
B: mL FAS used for sample
M: molarity of FAS
3.4.2 **Biochemical Oxygen Demand (BOD$_5$): 5210 B. 5-day BOD Test (APHA, AWWA & WEF, 1998)**

The BOD bottles were used for this measurement. Water samples were diluted with distilled water (1:1) in 1000-mL glass measuring cylinder. It was mixed thoroughly and then, was transferred into the BOD bottle until it overflows its brim. The DO of the water sample was measured and recorded using a DO meter (HANNA HI 9146 Microprocessor DO meter). A stopper was placed and no air bubble trapped in the bottle was ensured. The bottle was covered with aluminium foil to exclude light. The bottle was placed in a BOD incubator at a temperature of 20 °C. The DO value of the water in the bottle was recorded after 5 days of incubation. Subsequently, the BOD of the water sample was calculated.

$$\text{BOD}_5, \text{mg/L} = \frac{D_1 - D_5}{P}$$

$D_1$: DO of dilute sample immediately after preparation, mg/L

$D_5$: DO of dilute sample after 5 days incubation at 20°C, mg/L

$P$: Decimal volumetric fraction of sample used

3.4.3 **Total suspended solids (TSS) (APHA, AWWA & WEF, 1998)**

The filtering apparatus (vacuum filtration) was setup. The membrane filter was weighed using electronic balance. The membrane filter was placed onto the filter support and the funnel was clamp over the membrane filter. Filter paper was dried in oven and cooled in desiccators before weighed. Approximately 1 L of water sample was measured into the beaker (the water was shaken well before pouring into the measuring cylinder). Then, water in the beaker was poured into the filter funnel slowly and the vacuum pump was switched on. The water pump was stopped once all the water has