Faculty of Resource Science and Technology

**MICROBIAL AND PHYSIOCHEMICAL ANALYSIS OF WATER FROM SEMENGGOK INLAND FISHERY CENTRE, KUCHING, SARAWAK**

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Bachelor of Science with Honours
(Resource Biotechnology)
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# MICROBIAL AND PHYSIOCHEMICAL ANALYSIS OF WATER FROM SEMENGGOK INLAND FISHERY CENTRE, KUCHING, SARAWAK

**LOKE XIN YA**

A thesis submitted in partial fulfilment of the requirement of the Degree Bachelor of Science with Honours

(Resource Biotechnology)

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UNIVERSITI MALAYSIA SARAWAK

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**Microbial and Physiochemical Analysis of Water from Semenggok Inland Fishery Centre, Kuching, Sarawak**

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## ABSTRACT

Water quality is emphasized by the aquaculture sector as it affects the health and growth performance of aquaculture species. However, due to the outbreak of COVID-19 pandemic in Malaysia, various sectors including aquaculture sector, have been instructed to shut down as a lockdown alternative to control the spread of the disease. The total lockdown has made impossible to give proper management to the aquaculture ponds and indirectly led to the high mortality of aquaculture organisms. High mortality has led to rapid microbial growth, which affected the water quality and the balance in the aquaculture system. In this study, the characteristics of aquaculture environmental samples were determined. Water and soil samples were collected from three randomly selected ponds in the Semenggok Inland Fishery Centre, Kuching, Sarawak and the physiochemical and biological parameters were analysed. Bacteria were isolated from both water and soil samples and then characterised. Boiling-centrifugation method was used for DNA extraction of the bacteria. $(GTG)_5$-PCR was utilized to screen for clonal diversity among the isolates. A dendrogram was constructed using GelJ 1.0 software from the banding profile of $(GTG)_5$-PCR products. Out of all the isolates analysed, 11 representative isolates were selected for 16S rRNA sequencing based on the grouping from the dendrogram. The 11 isolates were identified as *Brevundimonas* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Escherichia* sp., *Ralstonia* sp., and *Exiguobacterium* sp.. The isolates were tested for antibiotic resistance using the disc diffusion method. Most of the isolates tested were resistant to ampicillin (10 µg), penicillin (10 µg), and streptomycin (10 µg). The MAR index of isolates were calculated, ranged from 0.143 to 0.714, indicating high possibility of culturing fish in the contaminated water. This study revealed the risk of presence of multiple antibiotic resistance (MAR) bacteria in the fishery centre. Therefore, the fishery centre should improve the aquaculture system by constantly monitoring and also provide a proper management to the wastewater to minimise the distribution of MAR bacteria.

**Keywords:** Microbial and physiochemical analysis, $(GTG)_5$-PCR, 16S rRNA sequencing, Antimicrobial susceptibility testing

## *ABSTRAK*

*Kualiti air dititikberatkan oleh sektor akuakultur kerana ia mempengaruhi kesihatan dan prestasi pertumbuhan spesies akuakultur. Berikutan dengan penularan wabak COVID-19 di Malaysia, pelbagai sektor termasuk sektor akuakultur, telah diarahkan tutup bagi mengawal penularan wabak itu. Sekatan ini telah mengakibatkan kekurangan dalam pengurusan kolam akuakultur yang betul dan secara tidak langsung membawa kepada kematian organisma akuakultur. Kematian telah menyebabkan pertumbuhan mikrob yang pesat, menjejaskan kualiti air dan keseimbangan dalam sistem akuakultur. Dalam kajian ini, ciri-ciri sampel akuakultur telah ditentukan. Sampel air dan tanah telah diambil daripada tiga kolam yang dipilih secara rawak di Pusat Perikanan Darat Semenggok, Kuching, Sarawak dan dianalisis parameter fisiokimia dan biologinya. Bakteria telah diasingkan daripada kedua-dua sampel air dan tanah dan bakteria kemudiannya dicirikan. Kaedah pendidihan-sentrifugasi digunakan untuk pengekstrakan DNA bakteria.* $(GTG)_5$*-PCR telah digunakan untuk menyaring kepelbagaian klon bakteria. Dendrogram telah dibina menggunakan GelJ 1.0 daripada profil banding produk* $(GTG)_5$*-PCR. Sebelas pencilan telah dipilih untuk penjujukan rRNA 16S. Daripada 11 pencilan tersebut, terdapat* Brevundimonas *sp.,* Staphylococcus *sp.,* Pseudomonas *sp.,* Escherichia *sp.,* Ralstonia *sp., dan* Exiguobacterium *sp.. Pencilan telah diuji untuk rintangan antibiotik menggunakan kaedah resapan cakera. Kebanyakan isolat yang diuji adalah tahan terhadap ampicillin (10 µg), penisilin (10 µg), dan streptomycin (10 µg). Indeks MAR bagi pencilan dikira, berjulat antara 0.143 hingga 0.714, menunjukkan kemungkinan tinggi untuk mengkultur ikan dalam air yang tercemar. Kajian ini mendedahkan risiko kehadiran pelbagai bakteria rintangan antibiotik (MAR) di pusat perikanan. Oleh itu, pusat perikanan harus menambah baik sistem akuakultur dengan sentiasa memantau dan juga menyediakan pengurusan yang betul kepada air sisa untuk meminimumkan taburan bakteria MAR.*

***Kata kunci:*** *Analisis mikrob dan fisiokimia,* $(GTG)_5$*-PCR, penjujukan rRNA 16S, Ujian kerentanan antimikrob*

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## List of Abbreviations

| | |
|---|---|
| $^{o}C$ | degree Celsius |
| Amp | Ampicillin |
| AST | Antimicrobial Susceptibility Testing |
| ATCC | American Type Culture Collection |
| BLAST | Basic Local Alignment Search Tool |
| BOD | Biochemical Oxygen Demand |
| bp | Base Pair |
| C | Chloramphenicol |
| CFU | Colony-Forming Unit |
| $Cl_2$ | Chlorine |
| COD | Chemical Oxygen Demand |
| COVID-19 | Coronavirus Disease 2019 |
| $CO_2$ | Carbon Dioxide |
| $dH_2O$ | Distilled Water |
| DNA | Deoxyribonucleic Acid |
| dNTP | Deoxynucleotide Triphosphate |
| DO | Dissolved Oxygen |
| E | Erythromycin |
| EDTA | Ethylenediaminetetraacetic Acid |
| EMBA | Eosin Methylene Blue Agar |
| EtBr | Ethidium Bromide |
| K | Kanamycin |
| kb | Kilobase |
| LB | Luria-Bertani |

| | |
|---|---|
| MAR | Multiple Antibiotic Resistance |
| $MgCl_2$ | Magnesium Chloride |
| MHA | Muller Hinton Agar |
| MIC | Minimum Inhibitory Concentration |
| Na | Nalidixic Acid |
| NA | Nutrient Agar |
| NC | Negative Control |
| NCBI | National Center for Biotechnology Information |
| $NH_3$ | Ammonia |
| $O_2$ | Oxygen |
| P | Penicillin |
| PCR | Polymerase Chain Reaction |
| pH | Potential of Hydrogen |
| ppm | parts per million |
| RNA | Ribonucleic Acid |
| rRNA | Ribosomal Ribonucleic Acid |
| S | Streptomycin |
| TAE | Tris-Acetate-EDTA |
| UV | Ultraviolet |
| w/v | Weight by Volume |

# CHAPTER 1

# INTRODUCTION

The aquaculture industry has constantly received the attention of the Malaysian government since it has been highlighted as a priority area in the country's economic growth (Jumatli & Ismail, 2018). Furthermore, the government and the public have always emphasized water quality in aquaculture as it is a foundation of health and well-being. Water quality is one of the most critical elements affecting the health and performance of aquaculture farms (Tower, 2015). The water quality can influence the health and growth of aquaculture species, but it also has the potential to affect people, making it zoonotic.

Water quality is described as a water body's chemical, physical, and biological qualities, typically linked to its long-term viability (Roy, 2018). It is critical in aiding scientists in predicting and determining the impact on humans and the environment. It is also significant as an assessment effort in restoration projects to maintain environmental standards. As the general population is aware, water is vital for the survival of living organisms and many activities. The decent quality of water will be free of contaminants and safe to drink for living organisms. On the other hand, poor water quality has the most significant direct influence on aquatic animals, notably fish, which is linked to food supplies.

We shall not be able to identify the quality of the water merely by looking at it with our bare eyes. To assess the water quality, specific procedures and analysis are necessary. Scientists will be able to determine the water quality using a variety of indicators. The physiochemical examination of water can provide these indicators. Not only that, but microbes are another critical factor that influences water quality. Microorganisms in the water body will enable the analysis of microbial part. Although the microorganisms in the water may not be hazardous, there is a possibility that they are pathogenic and can cause harm to humankind.

Back to the end of 2019, due to the outbreak of the COVID-19 pandemic, various sectors in Malaysia, including the aquaculture sector, have been instructed to shut down as a lockdown alternative to control the spread of the disease. The total lockdown has resulted in a shortage of manpower to give proper management to the aquaculture ponds as people were restricted from any outdoor activities. This indirectly led to the high mortality of aquaculture organisms. The dead aquaculture carcasses left in long-term unmanaged aquaculture ponds provided an opportunity for rapid microbial growth, which further affected the water quality and the balance physiochemical parameter of the aquaculture pond.

Therefore, in this research project, the microbial and physiochemical study of water and soil samples obtained from Semenggok Inland Fishery Centre was conducted. The physiochemical parameters of the water samples were examined. Following the water assessments, the collected data was compared to the Malaysia's National Water Quality Standard. Microbial analysis was performed on the water and soil samples collected to identify their microbial composition. Laboratory techniques such as $(GTG)_5$-PCR fingerprinting and agarose gel electrophoresis (AGE) were employed to aid in the investigation of the microbial content of the samples to detect their microbial profile. The bacteria species found in the water and soil samples were identified using 16S rRNA sequencing. Finally, antimicrobial susceptibility test (AST) was performed to assess the antibacterial characteristics of different isolates from water and soil samples against commonly used antibiotics in the fish farming industry. It is hypothesised that the water quality from the fishery centre is suitable to support the survival of aquatic life.

The objective of this project is to examine the water quality of selected ponds at Semenggok Inland Fishery Centre via physiochemical and microbial analysis. This project also aims to determine and identify the bacteria population in examined water samples. Lastly, this project seeks to determine the antimicrobial susceptibility patterns of identified isolates.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Water Quality

Water quality is described as the chemical, physical, and biological qualities of water, typically linked to its long-term viability (Roy, 2018). It is crucial for monitoring and analysing the impact on human being and the environment. There are three types of metrics used to evaluate water quality: physical, chemical, and biological (Omer, 2019).

Physical water body measurements included assessing the appearance of water bodies, which often included colour, turbidity, taste, odour, and temperature (Omer, 2019). The water body must be free of any contaminants that are objectionable to the senses of sight, taste, or smell to be used for a specific purpose, and the most essential physical attribute that should be addressed while assessing water quality is turbidity (Abu Shmeis, 2018). It included processes for measuring parameters such as flow conditions, substrates, and pollutants that have a direct impact on the quality of aquatic environments.

Chemical measurements are concerned with the measurement of controlled chemical compounds or combinations of chemicals that have the potential to occur in a water body at levels that are detrimental to living organisms. To assess water quality, hazardous contaminants, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, nutrients, metals, synthetic organic compounds, and radionuclides were all often collected or monitored (Omer, 2019).

The assessment of a water body's biology for its potential to give biological protection to living organisms is referred to as biological measurements of water. It is a method of determining how healthy a body of water is. The evaluation of the integrity of an aquatic ecological system that results from a biological inventory and biological potential analysis is commonly termed a biological assessment of water quality. The presence of microorganisms such as bacteria, algae, protozoa and viruses are the parameters for biological measurements (Omer, 2019).

Aside from physiological, chemical, and biological aspects, climate, vegetation, geology, and human activities all have an impact on water quality. These are known as natural and anthropogenic factors (Akhtar et al., 2021). The quality of water is frequently affected by these human and natural effects. Human activities such as mining, for example, are point sources of pollution that endanger water quality. Non-point sources, such as pollution, will also harm the water in the long and short-term owing to the pollutants that are released into the ecosystem and generate undesired impacts or squander resources. Water quality is also affected by faeces-related pollutants, agricultural pollutants, air pollutants, pathogens, and chemical contaminants.

#### *2.1.1 Water Quality in Aquaculture*

When it comes to growing the aquatic organisms, water quality is crucial. The appropriate water quality varies by species and must be evaluated to ensure the development and survival of aquatic organisms. The quality of the water used has an impact on the health and performance of aquatic organisms, as well as the cost of them in the market.

The water quality in the aquaculture industry is constantly examined by government. Temperature, dissolved oxygen (DO), pH, biochemical oxygen demand (BOD), ammonia ($NH_3$), and nitrates are all regularly measured parameters. Carbon dioxide ($CO_2$), chlorine ($Cl_2$), and salinity are occasionally monitored as well, depending on the culture system.

In Malaysia, the government established a set of water quality regulations based on the class of water and its intended use. The regulation is called "National Water Quality Standards for Malaysia" (*Appendix 1*). According to the standards, aquaculture sector can be categorized as class III water, with the uses stated as for common and economic value and tolerant species, livestock, and drinking. The parameter measured for class III water is

$NH_3$, BOD, COD, DO, pH, total suspended solid, temperature, faecal coliform, and the total coliform in the water.

## 2.2 Physiochemical Analysis

The examination of the water's physical and chemical characteristics is referred as the physiochemical analysis of the water. It is important to create an appropriate living environment for aquatic organisms in ensuring their growth quality and continuous production. There are various physiochemical parameters, such as pH, temperature, dissolved oxygen (DO), and biological oxygen demand (BOD).

### *2.2.1 Temperature*

The temperature of a water system is crucial because it affects the development and reproduction of aquatic species and the rate of all chemical reactions in a well-established water system (Patil et al., 2012). Temperature fluctuations in aquaculture system can harm aquatic species by affecting their metabolism, the degree of ammonia poisoning, and feeding rates. The rate of metabolism, biological and chemical reactions, and oxygen consumption will double for every 10°C increase in temperature. Temperature conditions, however, differ depending on the species. The temperature substantially impacts the biota respiration, such as oxygen ($O_2$) consumption rates. This is because warmer water contains less $O_2$ than cooler water.

Fishes are particularly vulnerable to rapid temperature fluctuations since the fishes can modify their body temperature to suit the environment. The water temperature can affect every element of their survival, including breathing and reproduction. Water enters the fish through the mouth and is driven out through the gills during breathing. The DO in the water will enter the blood cells of the fish. The amount of $O_2$ in the water decreases as the temperature rises, and the amount of $O_2$ given to the fish decreases as well. As a result, warmer water is incompatible with fish survival. The fish cannot breathe if the temperature rises to the dangerous levels.

Water temperature also significantly impact the reproduction system of fish since different kinds of fish have different needs for reproduction. For example, salmon and other cold-water fish spawn at low temperatures, whereas warm-water fish reproduce at a higher temperature. By these preferences, an aquatic habitat that sees a significant temperature shift that is out of character for it might lead fish to either depart or decrease in number owing to a lack of reproduction.

***2.2.2 pH***

The term pH is derived from a French phrase "*pouvoir hydrogéne*", indicating the meaning of "hydrogen power". This parameter is critical in determining whether water is corrosive as a living environment of aquatic organisms (Patil et al., 2012). Maintaining a constant pH within a healthy range is crucial since it affects the metabolism and other physiological activities of aquatic organisms. The ideal pH levels for fish farming systems should vary between 6.5 to 9.0 (Fondriest Environmental, 2013). Beyond the ideal range, stress, illness susceptibility, decreased productivity, poor growth, and even mortality may occur. Fishes are sensitive to pH shifts in the high temperature reading water body, mainly due to the amount of $CO_2$ produced in the water during the daytime that impacts the pH. The pH of the water will naturally change during the daytime as the phytoplankton will use $CO_2$ for photosynthesis. Affecting by the amount of sunlight during daytime, the pH value of the water is the lowest before sunrise and highest in the afternoon (*Figure 1*).

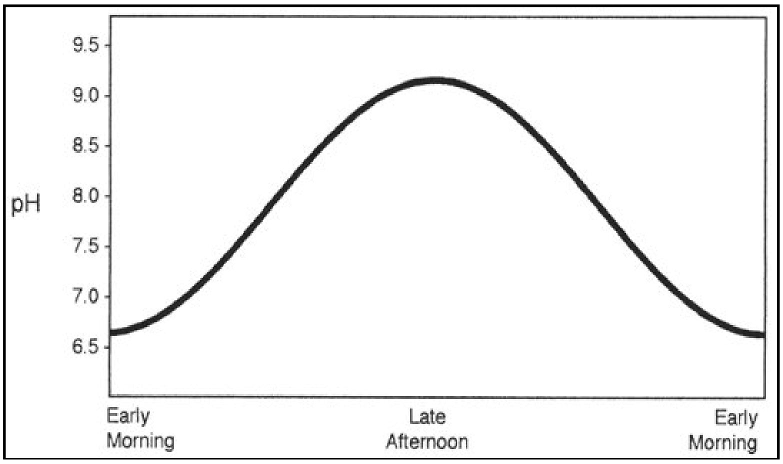


*Figure 1*. The pH changes in an aquaculture producing pond's waters throughout a 24-hour period (Fondriest Environmental, 2013).

### *2.2.3 Dissolved Oxygen*

Measurement of dissolved oxygen (DO) is also significant in aquaculture sector. It is the amount of oxygen that can be accessible by all the aquatic organisms in the water. Optimal DO level is crucial for optimum aquatic production since it directly influences their feed intake, illness resistance, and metabolism. When DO is scarce in the water, the aquatic species will become incredibly stressed. A DO level lower than 3 parts per millions (ppm or mg/L) will start to stress the growth and weaken the immune response of fish species; and levels below 1 mg/L will be lethal. A suitable DO levels for fishery aquaculture is suggested at above 4 mg/L (*Figure 2*).

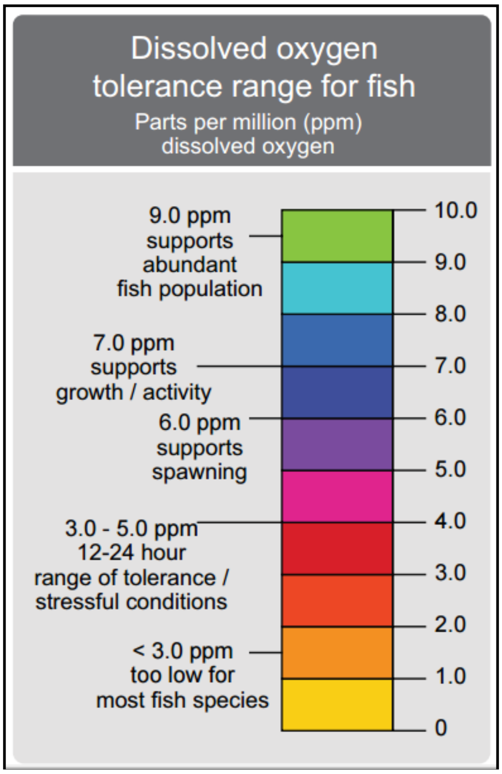


*Figure 2*. Dissolved oxygen tolerance range for fish (Leaffin, 2019).

### *2.2.4 Biochemical Oxygen Demand*

The aquaculture pond oxygen balance can also be influenced by biochemical oxygen demand (BOD). It is described as the amount of DO in a constant temperature that the aerobic organisms in the water system ought for organic substances break down. BOD is usually measured using the five-day biochemical oxygen demand ($BOD_5$). It is a significant water quality indicator that is often required to obtain with government-issued water quality permits and farm certification. The quantity of DO deplete quickly when the BOD value was high, which will affect the fish's development and production.

## 2.3 Microbial Analysis

The microbial analysis is also vital in the aquaculture farming system to ensure the survival of aquatic organisms. In an aquaculture system, microbes such as *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp., *Pseudomonas* sp., *Escherichia* sp., and *Citrobacter* sp. are often detected (Ajayi & Okoh, 2014). Microbes have beneficial role in pond system maintenance, notably in production, nutrient cycling, cultured animal feeding, water quality, and environmental run-off effect. As for pathogenic microorganisms, they may undermine the fish development, causing a myriad of problems to the industry (Nova Biologicals Teams, 2018; Sandle, 2016). As a result, microbial analysis is crucial for the early detection of the aquaculture water environment before an irreversible loss occurs.

*Escherichia* sp., *Enterobacter* sp., and *Citrobacter* sp. are the example of coliform bacteria, or known as Enterobacteriaceae. They are rod-shaped, gram-negative, and does not produce spores. Coliform bacteria do not cause disease. However, when assessing the water quality, the coliform bacteria can act as an excellent indicator of the existence of infective microbes. Excessive levels of faecal coliform indicate the failure in performing water treatment. Therefore, antibiotics are used in aquaculture sector in preventing the excessive growth of pathogenic microbes. Antimicrobial susceptibility testing (AST) is important to identify the inhibition effects of the growth of microorganisms that causing the infection to the aquatic species by using different types of antimicrobials on Muller Hinton (MH) agar.

## 2.4 Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique that often applied in the field of biotechnology. This technique will amplify the DNA segment for various laboratory applications. In the early 1980s, Mullis and associates created PCR, with the basis of Panet and Khorana's successful *in-vitro* amplification of DNA (Ghannam & Varacallo, 2022). This technique were later be awarded with a Nobel prize just a decade later. As this technique is useful due to the ability to amplify the specific regions of DNA into billion folds, it is used in many applications, such as gene cloning, infectious diseases diagnosis and genetic abnormalities screening (Ghannam & Varacallo, 2022).

To perform PCR, there are few main components required, which is the PCR primers, DNA polymerase, DNA templates and free nucleotide bases. The DNA template contains a specific region of DNA that wanted to be amplified. As for primers, it is oligonucleotides