

**STUDY ON MYCO-COAGULANT FROM *LENTINUS*
SQUARROSULUS FOR WATER TURBIDITY REDUCTION**

BY

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**A thesis submitted in fulfilment of the requirement for the
degree of Masters of Science (Biotechnology Engineering)**

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ABSTRACT

Water treatment of river water for human consumption involved the use of chemical coagulant to reduce suspended solids and turbidity. However, chemical coagulant has negative impacts thus research is carried out for bio-coagulant alternatives. In this research, basidiomycete fungi (*Lentinus squarrosulus* strain 7-4-2), which was named as RWF, was used as a source of secondary metabolites which have the potential in water treatment due to the flocculating ability. Laboratory experiments were conducted to optimize the production of the extracellular polymeric substance from *Lentinus squarrosulus*. Physical and environmental conditions important for the growth of RWF were selected using Plackett-Burman design (PBD). Physicochemical parameters such as concentration of yeast extract (1%), malt extract (1%) and NaCl (1%); as well as the initial pH (5), culture temperature (35 °C), agitation speed (250 rpm), culture period (8 days) and inoculum size (4%) were selected based on PBD, which resulted in turbidity reduction of synthetic waste water from kaolin solution up to 88 % inside 250 ml shake flask. Agitation speed and yeast extract have been identified as the main effects. The raw coagulant could reduce the turbidity from 460 NTU to 83 NTU, resulting in a reduction of about 82% in the next experiment. The myco-coagulant produced from the optimized growth condition was used to further purify the final product using cross flow filtration. After crossflow filtration using 30 kD membrane, the supernatant produce retentate and filtrate with turbidity reduction of 84% and 70%, respectively. It can be concluded that both the retentate and filtrate had good flocculation ability, although the retentate showed 14% more ability compared to the filtrate; indicating that the larger sizes of proteins were more efficient in reducing the turbidity, compared to the proteins smaller than 30 kD size. Use of various sizes of cross flow filters would help narrow down the most efficient size of the coagulant produced by the fungus RWF (*Lentinus squarrosulus* strain 7-4-2).

خلاصة البحث

تعد إزالة التعكر والمواد الصلبة العالقة مهمة بالنسبة لجماليات مياه النهر وتحسين جودة المياه المعالجة للاستهلاك البشري. تزيد المواد الصلبة العالقة والعكارة من تكلفة معالجة المياه وتقلل من المظهر الجمالي لمياه النهر. لذلك ، تستخدم مواد التخثر لجعل مياه الشرب ومياه النهر صافية. تُفضل مبيدات النفاية الكيميائية على نطاق واسع في صناعات المياه بسبب انخفاض تكلفة الإنتاج وقدرة الإنتاج السريعة. ومع ذلك ، لا ينبغي إهمال الآثار السلبية للمدقات الكيميائية للحصول على الفوائد الاقتصادية فقط. لذلك ، يعمل الباحثون على اكتشاف ندفات فعالة واقتصادية من مصادر بيولوجية. بذلت عدة محاولات وما زالت تبذل لاستخراج أو إنتاج مواد حيوية من المصادر الطبيعية مثل النباتات والبكتيريا والفطريات والخميرة والطحالب وما إلى ذلك. لقد تم القيام بقدر كبير من العمل في الماضي بحثًا عن مادة حيوية. ومع ذلك ، لم يتم تسويقها على نطاق واسع في مجال المواد العضوية الحيوية القابلة للتطبيق. في هذا البحث ، تم ، والتي سميت باسم (*Lentinus squarrosulus* 7-4-2 سلالة) استخدام الفطريات القاعدية ، كمصدر للأيضات الثانوية التي لها إمكانية في معالجة المياه بسبب القدرة على التبلد. أجريت RWF *Lentinus squarrosulus*. تم التجارب العملية لتحسين إنتاج المادة البوليمرية خارج الخلية من وقد تم Plackett–Burman باستخدام تصميم RWF اختيار الظروف المادية والبيئية المهمة لنمو تحسين المعلمات الفيزيائية والكيميائية تركيز مستخلص الخميرة (1 ٪) ، مستخلص الشعير (1 ٪) و ؛ بالإضافة إلى درجة الحموضة الأولية (5) ، درجة حرارة الاستنابت (35 درجة مئوية) ، (1 ٪) NaCl سرعة التحريض (250 دورة في الدقيقة) ، فترة الاستزراع (8 أيام) وحجم اللقاح (4 ٪) للحصول على أعلى نشاط عائم عند 88 ٪ داخل 250 مل يهز قارورة. تم تحديد سرعة التحريض وخالصة الخميرة كأثر رئيسي. يمكن أن يقلل طارد الندف من التعكر من 460 وحدة نمطية إلى 83 وحدة نمطية ، مما يؤدي إلى انخفاض بنسبة حوالي 82 ٪. تم استخدام مادة التخثر العضلي الناتجة عن حالة النمو المحسنة لزيادة تنقية المنتج النهائي باستخدام الترشيح المتداخل. بعد ترشيح التدفق المتداخل باستخدام غشاء 30 كيلو دالتون ، ينتج طاف التجويف والترشيح بنشاط التبلد بنسبة 84 ٪ و 70 ٪ على التوالي. يمكن أن نخلص إلى أن retentate كان لديه قدرة جيدة على التبلد ، على الرغم من أن filtrate و retentate كلا من مما يشير إلى أن الحجم الأكبر للبروتينات كان أكثر فعالية. filtrate أظهر 14 ٪ أكثر قدرة مقارنة مع في تقليل التعكر ، مقارنة بالبروتينات التي يقل حجمها عن 30 كيلو دالتون. سيساعد استخدام أحجام مختلفة من المرشحات المتقاطعة على تضيق الحجم الأكثر فعالية من الندف الذي ينتج عن الفطريات (*Lentinus squarrosulus* 7-4-2 سلالة) RWF.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biotechnology Engineering).

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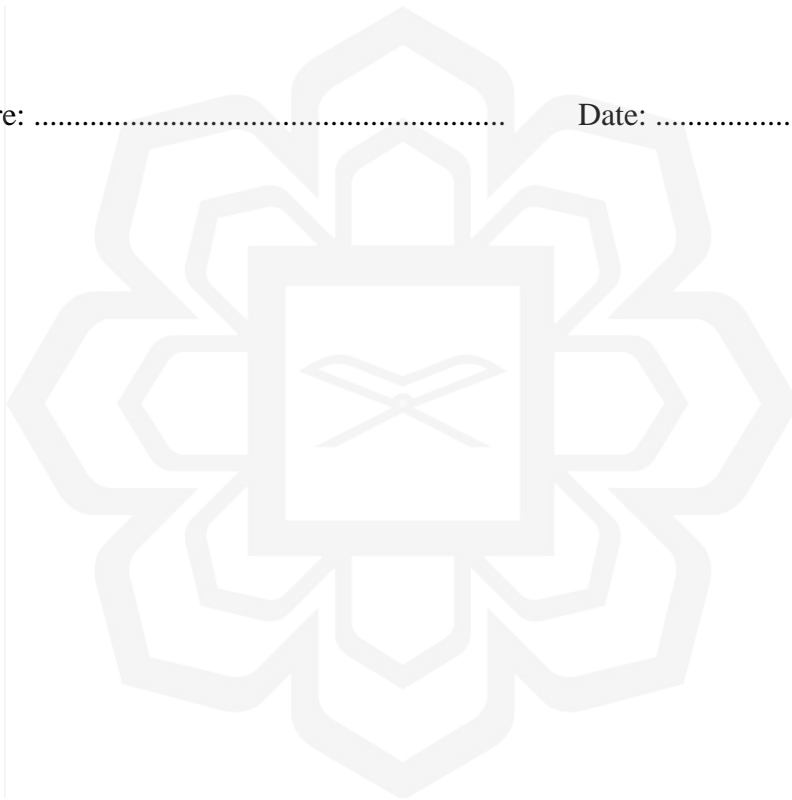
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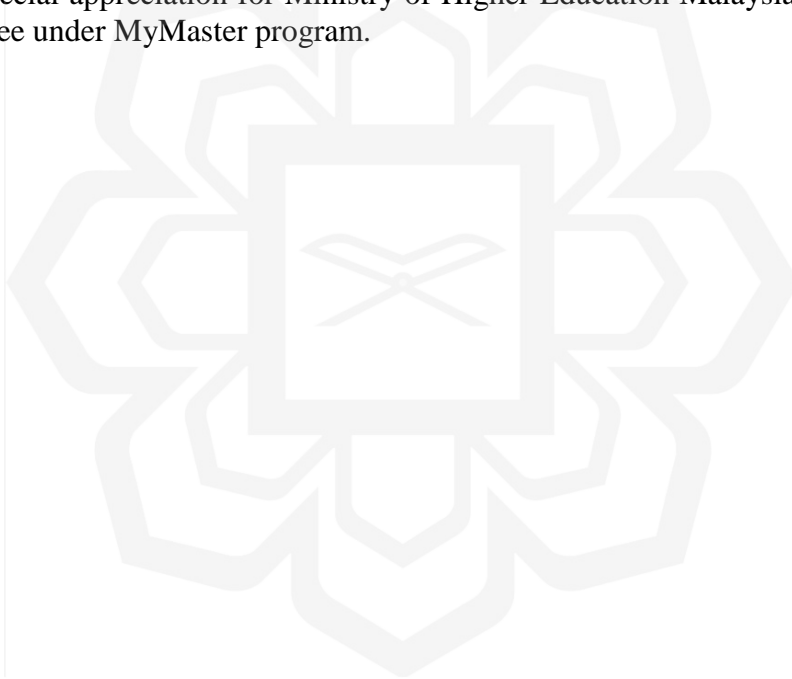


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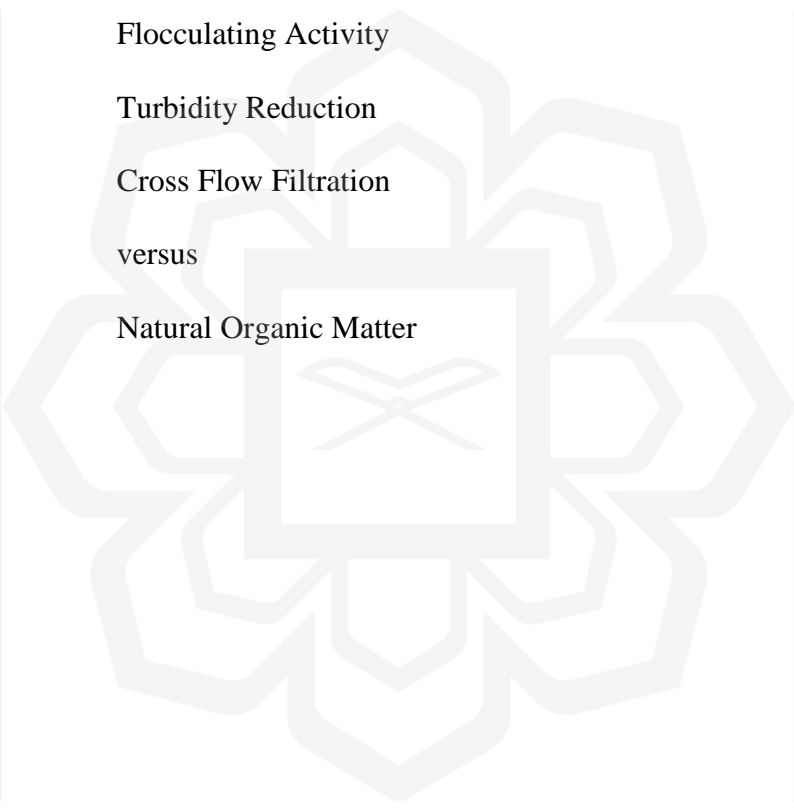
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LIST OF ABBREVIATIONS

DoE	Design of Experiment
EPS	Extracellular Polymeric Substance
FCCCD	Face Centered Central Composite Design
RSM	Response Surface Methodology
PDA	Potato Dextrose Agar
FA	Flocculating Activity
TR	Turbidity Reduction
CFF	Cross Flow Filtration
VS	versus
NOM	Natural Organic Matter



LIST OF SYMBOLS

-	Minus Sign
%	Percentage
+	Plus Sign
<	Less than Sign
>	Greater than Sign
±	Plus or Minus Sign
μ	Mu
°C	Degree Celsius
Θ	Theta
ζ	Zeta
mg	miligram
L	litre
w	weight
h	hour
v	volume
kD	kilo Dalton
P_f	Feed pressure
P_r	Retentate pressure
P_p	Permeate (or filtrate) flux
Q	Cross flow
Q_F	Feed flow rate
Q_R	Retentate flow rate
C_w	Component concentration of the membrane surface

C_b	Component concentration of the bulk solution
Q_f	Filtrate (or permeate) flow rate
C_f	Component concentration in the filtrate stream



CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Water security is one of the main issues of the modern society, which has become more challenging due to global warming and climate change. River water is the main global source of fresh water for domestic, irrigation and industrial use. However, high turbidity caused by fine colloidal particles in water is a common issue that reduces the aesthetic value of water making it unappealing for human consumption and other usages. At the same time, the cost of water treatment increases with high turbidity and other pollutants in the river water. One of the main sources of high turbidity is storm runoff from developing areas. As such, the countries in the tropical region are more susceptible to high turbidity in the rivers due to high rainfall induced soil erosion.

According to (Jebun, Al-Mamun, Alam, Karim, & Raus, 2015), *Lentinus squarrosulus* has shown better turbidity reduction which is 94% compared to commercial chemical coagulant such as polyaluminium chloride with 74% turbidity reduction only. Hence, fungus has shown promising results for water treatment alternative. Out of 6 strains isolated from Sg. Pusu water sample, RWF shows the highest turbidity reduction (Jebun, Al-Mamun, Alam, & Raus, 2016). Water turbidity is reduced by using coagulants. Most of the coagulants used till today are chemical in nature, inorganic and non-biodegradable. Such coagulants have health and environmental effect (Hierrezuelo et al., 2010; Sotero-Santos et al., 2005; Ruden, 2004). Therefore, an attempt was made to produce myco-coagulant from fungus. Several numbers of fungus species were isolated and tested for coagulant properties.

They were coded with names RWF (river water fungus). Based on the initial screening, only six species (RWF-1 – RWF-6) showed coagulant properties. Further testing revealed that RWF-5 exhibited the best results in reducing turbidity (Figure 1.1). Therefore, this RWF-5 species of river water fungus was selected for further investigation to produce efficient myco-coagulant.

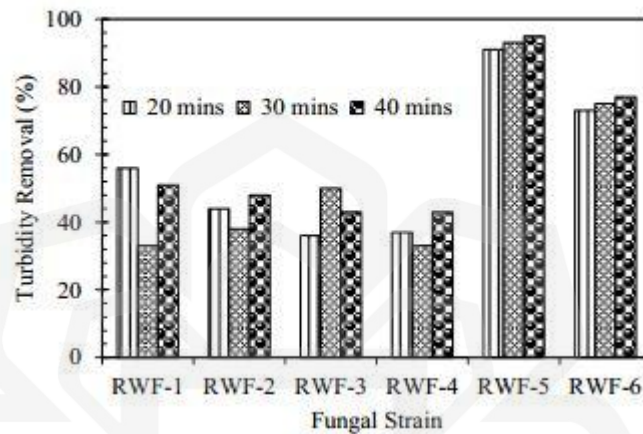


Figure 1.1 Turbidity removal of different strain (Jebun et al, 2016)

1.2 PROBLEM STATEMENT AND ITS SIGNIFICANCE

The negative effects of using chemical coagulants for water treatment are well-documented such as neuropathological diseases (Muyibi & Alfugara, 2003; Flaten, 2001) and strong carcinogenic to human body and the environment (Ruden, 2004; Dearfield et al., 1988). Despite knowing those potential impact on the human health and environmental, there is no choice but to use the existing chemical coagulants for the removal of turbidity from water. However, due to advancement in biotechnology, new hopes are emerging out to discover biodegradable and safe coagulants. This research is intended to overcome the lack of efficient bio-coagulant from renewable source, to reduce turbidity of water. Separation of active metabolite from crude

coagulant is also another challenge in producing and handling of viable amount of coagulant for commercial and application purposes.

Culture conditions affect the fungal coagulant production and the conditions must be optimized for upscaling purpose. Fungal coagulant growth and yield is directly related to few important parameters such as temperature, pH, agitation speed and dissolved oxygen concentration. Production of bulk amount of crude myco-coagulant makes the application of the coagulant inconvenient for handling and storage. In fact, higher volume of inactive compound requires more energy in harvesting and separating the final product.

1.3 RESEARCH OBJECTIVES

1. To identify significant physical and environmental culture conditions for myco-coagulant production using Plackett-Burman design (PBD) and synthetic water from kaolin solution at 500 ± 10 NTU.
2. To optimize operational conditions of shake flask fermentation for maximum turbidity reduction of myco-coagulant using Face Centered Central Composite Design (FCCCD).
3. To separate the active metabolite of the myco-coagulant by cross flow filtration (CFF) system for lab scale application to compare between the retentate and permeate effectiveness.

1.4 RESEARCH METHODOLOGY

Detail of the overall process used in the study is presented in Chapter 3. All experimental procedures in this research work were conducted according to methods

previously published in scientific journals or articles and Standard Method for the Examination of Water and Wastewater (APHA, 1999).

The fungus (*Lentinus squarrosulus*) isolated from the river water was used in this study. The fungus was cultured in PDA plate and fermented in media broth. After that, selection of physicochemical parameters was conducted to choose the best fermentation conditions in terms of efficiency in removing turbidity from kaolin solution, which was taken as turbidity reduction. Kaolin solution is used instead of real river water to ensure the consistency of initial turbidity level for each experiment.

The best media composition and culture conditions were fixed for the culture while the agitation speed and yeast extract concentration were optimized to determine the turbidity reduction. Plackett-Burman Design and Face Centered Central Composite Design (FCCCD) coupled with Response Surface Methodology (RSM) was used to determine the mathematical model of the coagulation process.

Cross flow filtration system was used with 30 kD hollow fibre membrane to separate the active metabolite. The turbidity reduction was compared between the retentate and the filtrate, using the jar test apparatus.

1.5 SCOPE OF RESEARCH

Locally isolated wild river water fungus (RWF), *Lentinus squarrosulus* was used to reduce turbidity from kaolin solution prepared with distilled water and clay powder. The main scope of the research was to optimize various conditions to maximize the production of myco-coagulant and then to separate the active metabolite from the crude coagulant. Turbidity reduction of the myco-coagulant is compared with kaolin

solution without additional flocculant as the control. The specific scopes of research for this study are stated below:

1. Identifying physical and environmental culture conditions of the fungal strain using Plackett-Burman design (PBD).
2. Optimizing the operational conditions for maximum production of myco-coagulant using Face Centered Central Composite Design (FCCCD).
3. Separating active metabolite from the myco-coagulant by a cross flow filtration (CFF) system.

1.6 THESIS ORGANIZATION

There are five chapters in this thesis. Chapter 1 consists of background of study, problem statement and its significance, research objectives, research methodology and scopes of research. Chapter 2 presents the literature review related to general coagulants and bio-coagulants, water treatment, growth conditions and factors of fungal growth. The research materials and methodology are elaborated in Chapter 3. Results for all findings are discussed in Chapter 4 separated with a few sections according to the specific objectives. Finally, Chapter 5 summarizes all the findings of this study with a number of possible recommendations for future work.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter contains details about the relevant and recent literature on surface water pollution and its treatment using coagulation and flocculation system. Main focus was given to the reduction of turbidity from surface water, coagulation - flocculation processes, types of coagulants used in the market, production of degradable bio-coagulants and their details.

2.2 WATER POLLUTION

The continuous increase in water use and polluted water sources has threatened plant and animal lives (Su et al., 2011). The rapid growth in the use of water over the last half of the century has resulted a general awareness to employ a holistic approach in examination of various options for the future. When the quality of water is altered, its domestic and industrial uses are affected thus adversely affects the optimum management of water resources (Rao & Mamatha, 2004). The physicochemical and biological nature of Surface water bodies have been affected by urbanization, industrialization and agricultural activities (Jain et al., 2007). Yuan, Zhang, Li, Wang, & Ji, (2014) reported that the sources of nutrients and sediment pollutant include emissions from agricultural activities, urbanization, industrialization, etc.

According to reports from the World Water Assessment Programme (2009), freshwater reserves have been put under high demand due to various economic and climatic drivers of change which results in a large proportion of the global population

currently living in water scarce preconditions. There has been a future projection that population growth would reach about 9 billion by 2050 and living standards would also raise. All these would result into an increase in water demand in all sectors. For agriculture (rain fed and irrigated) to blossom, it is expected that water consumption in the future will increase by 19% (approximately) by 2050. In addition, matters would only be made worse if there is no technological advancement or policy interventions. Presently, reports have shown that water withdrawal is likely to step-up by 25% in developing countries. The rapid population growth underway in Asia and the expansion of cities in the region combined to place an ever-growing demand on water resources to meet urban needs. New research shows that water stress in China is expected to become more severe for at least the next 15 years (IWA, 2015).

Many studies have been done for finding causes of river water pollution in throughout the world. Wu et al., (2013) investigated the influence of point source (PS) and non-point source (NPS) pollution of the Dongjiang River, China with NPS pollution being a dominant contribution (more than 94%) to nutrient loads. Similar works have been reported by Chen et al., (2013) on Jinjiang River, China. Water pollution of Sabarmati River in India was greatly polluted; an aftermath of municipal drainage and industrial waste discharge (Halder et al., 2014). The major factors contributing to water pollution of the Qiantang River in China were found to be sewage, agricultural runoffs, wastewater from industries, mineral weathering, carbon monoxide from vehicle exhaust and sand mining (Huang et al., 2010; Su et al., 2011).

2.3 SURFACE WATER STATUS IN MALAYSIA

Malaysian rivers have vital role on nature and human society. Major cities have been established and flourished along the rivers. Rivers are not only rich sources of life but also a major source of ecosystems, providing water supply, irrigation for agriculture, a transportation means (by sea), a major source of food as in fisheries, electric power generation (hydro-electric power) and for various industrial uses (Chan, 2002). It also serves as habitats for aquatic flora and fauna, generating a rich biodiversity of life forms which is well supported by the riparian environment (Naiman and Bilby, 2001). Nevertheless, over the years, with the amalgamation of low importance on the government agenda, public apathy, neglect and poor management have resulted in severely degraded rivers including their water quality. In the last three or four decades, the country has developed very fast, with increasing urbanization all over the major towns and cities (Weng, 2005).

In Malaysia, the rate at which rivers are been polluted is at an alarming rate. It is one of the most serious issues that have affected more than half of the rivers (Netto, 2006). Construction and deforestation have brought about the decline of water quality in the Kelang River basin. Studies have shown that it was due to high sediment loads from untreated sewage, large quantities of litter and rubbish, and majorly effluents from industries and commercial sources (Balamurugan, 1991).

In addition, it has been discovered that pollution sources of Sungai Pinang are from industries, domestic and non-classified wastewater such as hawkers, restaurants, bus stations as well as non-point sources (Saad et al., 2006). Langat River and its tributaries are polluted due to occurrence of human pharmaceuticals in wastewater effluents (Al-Odaini, Zakaria, Yaziz, Surif, & Abdulghani, 2013). The main sources

of pollution were identified as coming from poultry farms, activities of market and wastewater industries in Selangor (Fulazzaky, Seong, & Masirin, 2010).

As reported by DOE in 2010, 527 (50%) out of the 1,055 water quality monitoring stations located at 570 rivers were found to be clean. 417 (40%) was slightly polluted and 111 (10%) polluted. In 2009, the number of clean rivers was decreased from 306 rivers to 293, slightly polluted rivers decreased from 217 to 203 in 2009 while the number of polluted rivers increased from 54 to 74 in 2009. Rivers water quality trend in Malaysia are shown in Figure 2.1. The figure shows that the decrease in the number of clean rivers were attributed to an increase in the number of pollution sources such as agro-based industries and sewage treatment plants which caused to high pollution burden.

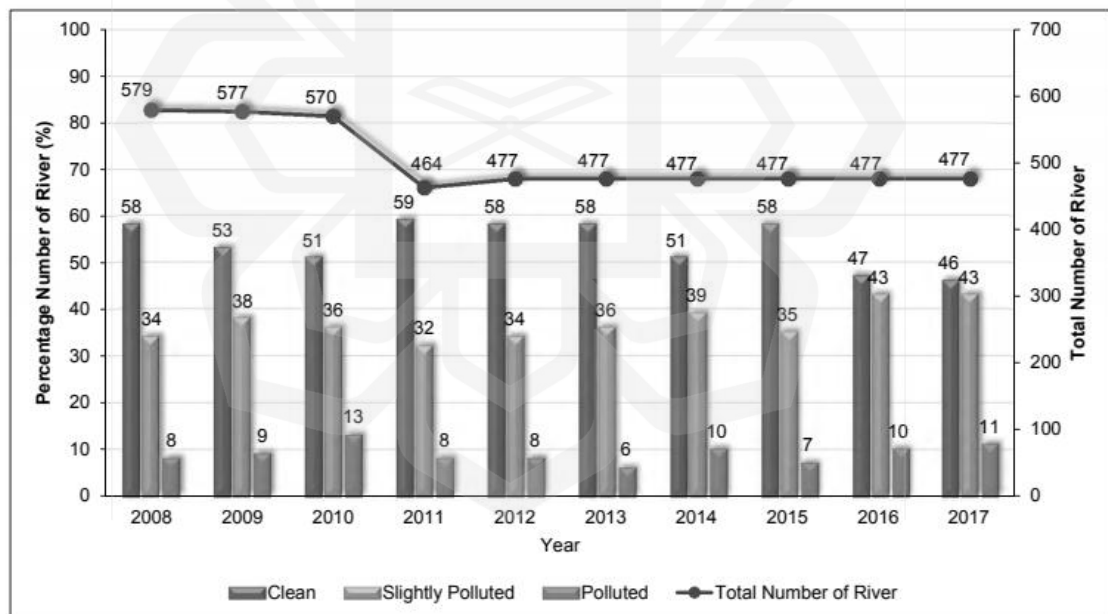


Figure 2.1 River Water Quality Trend in Malaysia (Ying, 2019)

In these rivers, major pollutants were found out to be Biochemical Oxygen Demand (BOD), Ammoniacal Nitrogen (NH₃-N) and suspended solids (TSS). The untreated sewage and discharges from agricultural and manufacturing industries were