CHAPTER I. INTRODUCTION

1.1 Background

There is a lot of waste produced by the palm oil industry. The waste generated during the production of palm oil is separated into two categories: solid waste, which is made up of wet solids, empty palm oil bunches, shells, and fibers, and liquid waste, sometimes referred to as Palm Oil Mill Effluent (POME) (Warsito *et al*., 2016). Two and a half tons of palm oil bunches yield 220-230 kg, or 22-23%, of solid waste. Sterilization and specific gravity-based product separation produce palm oil liquid waste (Silalahi and Nelvia, 2017).

Based on data from the United States Department of Agriculture (USDA), Indonesia and Malaysia are the largest producers of palm oil in the world. In 2020, Indonesia's palm oil production reached around 46 million tons, while Malaysia produced around 19 million tons. These two countries together account for more than 85% of global palm oil production. The area of oil palm plantations in Indonesia reaches around 16 million hectares, while in Malaysia it reaches around 5 million hectares (Gapki, 2022).

Indonesia is the largest exporter of palm oil in the international market, this is due to the high level of world demand for palm oil. Much of the demand for palm oil comes from the European Union, which uses vegetable oil as the main ingredient in the manufacture of renewable energy called biofuels. This phenomenon provides many opportunities for various palm oil producing

countries such as Indonesia and Malaysia to continue to trigger the expansion of the palm oil plantation sector (Gunawan, 2018).

This production comes from oil palm plantations on several islands in Indonesia, especially Sumatra, Kalimantan and Papua. The area of oil palm plantations in Indonesia is more than 14 million hectares. These farms are managed by private farmers, large plantation companies and community farms. Most of the oil palm plantations are located in Sumatra and Kalimantan, with significant plantations also in the provinces of Riau, Jambi, South Sumatra and West Kalimantan (Ximenes *et al*., 2022).

POME is palm oil liquid waste which still contains a lot of dissolved solids. Most of these dissolved solids come from lignocellulosic materials containing oil from palm fruit. Lignocellulose in POME is the largest constituent of woody plants. Lignocellulose consists of lignin, hemicellulose, and cellulose material. The chemical content of lignocellulose makes them of high value from a biotechnological perspective (Ibrahim, 2006).

Most of this lignocellulosic waste is disposed of directly by incineration, which is not prohibited in developing countries. However, problems will arise when this biomass is not treated properly and is left to rot in the planting area, where in the future there will be a buildup of organic content that is too high. Therefore, environmental management places great emphasis on reducing waste from its source or recycling processes (Ibrahim, 2006).

Currently, more and more industries are using anaerobic digestion to treat wastewater from industrial processes. Palm oil mills, sewage treatment plants

(STPs), poultry farms, and dairy farms are some of the I ndustries that have installed anaerobic reactors. The biogas produced is mostly used to generate energy (on-site use) and to power gas-powered equipment (Kumaran *et al*., 2016).

Palm oil mill effluent (POME) is one of the main wastes from the palm oil industry with the greatest potential for environmental pollution than other types of wastes (Ibe *et al*., 2014). The potential pollution of liquid waste from 1 ton of crude palm oil production requires 5-7.5 tons of water, more than 50% of which ends up as POME (Bala *et al.*, 2014). The resulting waste is divided into solid waste and liquid waste or known as Palm Oil Mill Effluent Digestate (POMED), palm oil solid waste comes from empty fruit bunches, shells and coir (*Warsito et al.*, 2016). In 1 ton of oil palm, empty fruit bunches produce 220-230% waste, shell waste 6.5%, coir 13% (Haryanti, 2014).

The palm oil industry not only produces oil but also produces waste from the oil production process. In the palm oil mill industry, AD (Anaerobic Digestate) is used to treat palm oil mill effluent (POME) derived from palm oil mill activities. In 2015, Malaysia produced approximately 60.88 million tons of POME (Choong *et al*., 2018). POME is considered as highly polluted wastewater that can pollute the environment if discharged directly into rivers or lakes due to the high concentration of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (Bala *et al*., 2014).

POMED samples from both fresh and old digestate from TDM Company will be used in this research. The liquid portion of digestate that is collected right away following the anaerobic digestate process is known as fresh digestate. It

might have high concentrations of organic acids, volatile chemicals, and pathogens, all of which, if improperly managed, can have a detrimental effect on plant development, soil health, and environmental quality. The term "old digestate" describes the mature or aged form that has been kept for six to twelve months following the process of digestion. Microbial activity, nutrient transformation, and the breakdown of organic materials are a few of the variables that might cause changes in the digestate's composition and properties during storage (David & Niculescu, 2021).

The poultry sector is among the fastest growing agro-based industries worldwide due to increasing demand for egg and meat products. Chicken manure is the waste generated in the largest quantities in the process. Chicken manure is a mixture of chicken feces, feathers, bedding materials and spilt feeds, drugs, and water (Sanchuki *et al*., 2011).

Chicken manure or organic fertilizer based on chicken manure is usually recycled into soil to improve the structure and fertility of agricultural land. As an important source of nutrients for crop production, chicken manure also contains various human pathogens that can threaten humans who consume contaminated food or water (Chen and Jiang, 2014).

Chicken manure is also a summber of human pathogens such as Salmonella, Cammpylobacter jejuni, and Listeria monocytogenes (Runge *et al*., 2007) that have the potential to contaminate fresh produce or the environment and are often associated with foodborne outbreaks (Subirats *et al*., 2020). Chicken

manure is the largest contributor of antibiotic-resistant organisms in soil compared to cow, kammbing, pig, and rabbit manure (Danilova *et al*., 2018).

Chicken manure is one of the wastes produced from the laying hens and broilers. Based on the research of Suryani *et al*. (2010), chicken manure contains several bacterias named such as Lactobacillus achidophilus, Lactobacillus reuteri, Leuconostoc mensenteroides and Streptococcus thermophilus which are lactic acid bacteria (BAL). In this study, the bacteria that will be sought is *Pseudomonas aeruginosa* which aims to determine its presence in chicken manure samples and find out how it develops when isolated.

The isolation bacteria is a microbe separation that will be tested from other microbes by using a medium agar. It is expected to obtain a pure culture. Identification bacteria is an activity for determining the carried certain types of organisms that have several steps namely observing, testing, recording, and identifying based on the test results at the fishery. There is often a problem that should be faced. It is the occurrence of disease attacks caused by pathogenic organisms such as bacteria, viruses, fungi, and parasites (Woo & Bruno, 2011). Culture media is a nutritional material used for the growth of microorganisms in the laboratory. The composition and nutrient levels of a medium for microbial growth must be balanced so that microbes can grow optimally (Haribi, 2018).

In general, to find out a type of bacteria can be done by isolation and identification. Isolation is separating one bacterium from another that comes from a mixture of various bacteria. Isolation is done because naturally, bacteria in nature are found in mixed populations. Meanwhile, bacterial identification is a

further step from the isolation results. Identification can be determined based on morphological, growth and biochemical tests (Waluyo, 2008).

According to the World Health Organization (WHO), *Pseudomonas aeruginosa* has been characterized as a species of "greatest public health concern". Although *P. aeruginosa* rarely causes serious infections in healthy people, it is associated with a variety of invasive infections in long-term hospitalized patients, intubated patients with critical conditions, patients treated with broad-spectrum antimicrobial therapy or cancer chemotherapy, and patients using ventilator pneumonia (Anonymous, 2017).

Some studies have used several media to isolate *Pseudomonas* sp. including Nutrient Agar (NA) which can be used for the growth of *Pseudomonas aeruginosa*, but NA is not a selective medium specifically designed to isolate *Pseudomonas aeruginosa* Then research Girsang *et al*. (2019), characterized the colonies of *Pseudomonas aeruginosa* on Mac Conkey (MC) media which is a medium for isolation and identification of Gram-negative bacteria, isolates that have the characteristics of round and smooth colonies and have flat colony surfaces.

Meanwhile, *Pseudomonas* Isolation Agar (PIA) media is a *Pseudomonas* selective media and also as a differential media that can distinguish *Pseudomonas aeruginosa* from other *Pseudomonas* (Liofilchem, 2014). However, PIA media has a weakness in the dependence on pyocyanin pigments as characterization markers. Many selective agents have been employed to increase selectivity in Ps. aeruginosa isolation and identification, including cetrimide, cephaloridine,

fucidin, nalidixic acid, phenanthroline, and irgasan. A relatively novel substitute that is frequently used for Ps. aeruginosa isolation and identification from food and environmental samples is cetrimide agar (Weiser et al., 2014). It has been demonstrated that Pseudomonas aeruginosa is oxidase positive, can grow in selective media containing cetrimide, and can produce pyocyanin and fluorescence when exposed to a 360 ± 20 nm wavelength UV lamp (Casanovas *et al*., 2010).

The isolation of bacteria in palm oil industry waste has been reported by several authors, but there is still little literature that reports the isolation of pathogen bacteria in Palm Oil Mill Effluent Digestate (POMED) that using Anaerobic Digestate Process and chicken manure produced from chicken farms at Edu Farm, Andalas University. Also it is necessary to know whether it contains pathogenic bacteria before using it as organic fertilizer in the surrounding environment. One of the effective ways to obtain bacteria present in liquid waste is to isolate them with specific media in testing their ability. Therefore, as an alternative to solving the problem of environmental pollution due to industry liquid waste, it is necessary to conduct research on "Isolation And Partial Characterization Of *Pseudomonas Aeruginosa* From Palm Oil Mill Effluent Digestate (POMED) And Chicken Manure". This study aims to obtain pathogen bacterial isolates of palm oil industry liquid waste and determine the characterization of pathogen bacterial isolates of industry liquid waste.

1.2 Problem Formulation

The problem formulations in this study are:

1. How the presence of *Psedomonas aeruginosa* bacteria on Palm Oil Mill Effluent Digestate (POMED) and Chicken manure on Cetrimide Agar media?
2. What is the partial character of *Pseudomonas aeruginosa* bacteria in Palm Oil Mill Effluent Digestate (POMED) and Chicken manure?

1.3 Research Objectives

The objectives of this research are:

1. To know how the presence of *Psedomonas aeruginosa* bacteria on Palm Oil Mill Effluent Digestate (POMED) and Chicken manure on Cetrimide Agar media.
2. To know what is the partial character of *Pseudomonas aeruginosa* bacteria in Palm Oil Mill Effluent Digestate (POMED) and Chicken manure.

1.4 Research benefits

The benefit of this research is to determine the growth of *Pseudomonas aeruginosa* on Cetrimide agar and it is hoped that this research can be a consideration in processing Palm Oil Mill Effluent Digestate (POMED) and Chicken manure which will be processed into a product.