I. INTRODUCTION

1.1 Research Background

The application of different microorganisms for bioremediation has been highlighted in environmental biotechnology as they are able to degrade and convert toxic chemical compounds into less toxic substances through microbial digestion which is more cost-effective and poses less disruptive effects (Baniasadi & Mousavi, 2018; Bodor et al., 2020). This approach is commonly used as an environmental clean-up technique for chemical pollutants, due to a wide range of anthropogenic activities such as accidental spills, discharge of sewage, mining, landfills, and illegal dumping, etc., which have been common pollutants found in soil including petroleum hydrocarbons, pesticides lead, aromatic hydrocarbons, and heavy metals (Shaltami et al., 2020). Through a study by Mohapatra & Phale (2021), it is considered that indigenous bacteria are more advantageous to be used as a bioremediation agent due to their relation to environmental safety, effectiveness, efficiency, cost-effectiveness, as well as promoting sustainability. This is related as well to the biological compatibility of the microorganisms being used for bioremediation and the site being remediated. This highly highlights the importance of research and the constant deepening of knowledge regarding the microorganisms to ensure the success of bioremediation which is highly dependent upon the survival and activities of the microbial community present.

For several decades, the techniques used to understand deeper regarding these microorganisms, mainly bacteria, are through traditional cultivation techniques including solid and liquid mediums for isolating and cultivating microorganisms from different samples, such as environmental samples (Mu et al., 2021). These methods are commonly optimized by manipulating the required nutrition by the bacteria and the medium formulation. However, the drawbacks of present traditional isolation methods pose a huge limitation to the study of microorganisms, especially within the isolation and cultivation of indigenous bacteria from an environmental sample. The

limitations include the limited growth for the bacteria, competition of nutrients among different bacteria grown on the same medium, negative interactions between different bacterial strain which poses risk to the growth of the bacteria, and the absence of signaling molecules that may play a significant role in the growth of certain bacterial strain (Kumar & Gosh, 2019).

Thus, to overcome the limitation of traditional methods, the newly constructed microarray device is proposed (Duran et al., 2022). This novel, gel-filled microarray device is a low-cost approach that utilizes microwells to isolate and cultivate bacteria. This device is composed of 900 gel-filling wells (600µm x 600µm x 700µm) divided into 4 subareas (225 wells in each subarea) which enable the bacteria present in the sample to grow within each well thus limiting the competition between other bacterial strains. The bacteria are expected to form colonies within each well which will be able to be picked up and transferred onto a liquid medium for upscaling. Thus, through the utilization of this device, not only bacteria with low growth rates are expected to grow independently, but microbial consortia which have positive interactions with each other are able to grow as well. Therefore, there will be a higher possibility of isolating different bacterial strains simultaneously while keeping the approach cost and time effective.

This research further focuses on isolating potential bacteria for the bioremediation of polycyclic aromatic hydrocarbons (PAHs), an example of a persistent chemical compound composed of two or more fused benzene rings (Qazi et al., 2021). These chemicals are as well considered persistent organic pollutants (POPs) due to their hydrophobicity, low water solubility, and the ability to absorb into the soil matrix which enables them to remain within the particulate for an extended period of time (Shahsavari et al., 2019; Kieta et al., 2022). PAHs could be abundantly found in the environment as they are products of anthropogenic activities such as coal gasification, coke, and aluminum production, activities related to petroleum refineries, etc., and are involved in the manufacturing processes within chemical industries such as the manufacture of pigments, dyes, pesticides, etc. (Abdel-Shafy & Mansour, 2016). Due to this, PAHs tend to accumulate in the

environment which leads to soil contamination and further would affect agricultural production. This will increase the potential of biomagnification and bioaccumulation of PAHs which will eventually risk human health (Forger et al., 2021).

One example of PAHs is naphthalene, one of the most widespread xenobiotic pollutants with high volatility properties (Sun et al., 2017). This chemical is classified as a low molecular PAH that is potentially more readily biodegradable compared to high molecular weight (HMW)-PAHs which may be challenging to be incorporated into the cell due to their large size (Shahsavari et al., 2019). Additionally, naphthalene as well serves as the simplest form of PAHs which simplifies the process of understanding the properties of these chemicals (Rabani et al., 2022).

The northwestern and southeastern region of France represents an ideal location to study bacterial diversity under PAHs contamination. This region comprises the industrialized regions within and surrounding the major cities of Lyon, Nantes, and Bordeaux, and was reported to have high contamination of PAHs according to Forger et al. (2021). Nonetheless, sediment samples were reported to have a high spatial variability of bacteria. Therefore, utilizing sediment samples retrieved from rivers could give more diversity of bacteria retrieved. Additionally, the diversity demonstrates the ability of these bacterial communities to thrive in PAH-contaminated regions, indicating a PAH degradation capability. Thus, sediment samples retrieved from the Ardour River located in southwestern France were used as a sample for this research as this river is located between the industrialized area of Nantes and Bordeaux. Moreover, no previous research utilizes sediment samples from this specific research.

1.2 Research Objectives

This research aims to assess the usage of the gel-filled microarray device for its ability to isolate and identify diverse bacteria which was further assessed for their potential naphthalene-degrading abilities.

1.3 Scope of Work

The scope of work of this study was done in a wet-lab setting with the activities including: 1) processing sediment sample to extract the bacteria, 2) utilize microarray device to isolate and culture diverse bacteria in the sample, 3) scale-up isolated bacterial strains onto 96-well plates, 4) incubate bacterial strain in growth medium and naphthalene and further analyze naphthalene concentration with UPLC, and 5) bacteria colony morphology observation.

1.4 Hypothesis

Within this research, it was hypothesized that the gel-filled microarray device was able to isolate and cultivate diverse bacteria from sediment sample where the isolated bacteria had potential naphthalene-degrading abilities.