CHAPTER I
INTRODUCTION

1.1 Background

Nitrogen (N) pollution poses significant threats to ecosystems, human health, and the economy. This issue arises from excessive reactive nitrogen due to human activities. N is essential for proteins, nucleic acids, enzymes, and certain bacteria. Fish and marine life may die from oxygen depletion and eutrophication caused by high nitrogen levels. Removing nitrogen from wastewater is a growing global priority, as domestic, industrial, and agricultural sewage often contain various nitrogen pollutants (e.g., ammoniacal, nitrite, and nitrate nitrogen) (Zhou et al., 2023). Understanding nitrogen-transforming bacteria is crucial for combating nitrogen pollution, especially in water. Anaerobic ammonium oxidation (anammox) is an established nitrogen removal method (ammonium and nitrite).

Anammox bacteria oxidize ammonium anaerobically, using nitrite as an electron acceptor. It is a shortcut method for nitrogen removal (Zhang et al., 2022). The anammox process converts ammonium to nitrogen using nitrite as an electron acceptor. It has lower operating costs than traditional nitrification-denitrification methods due to reduced aeration energy and no need for an additional carbon source (Liu et al., 2024). Anammox bacteria are found in anoxic habitats like freshwater, marine wastewater, seawater, and sludge. Currently, there are 24 Anammox species across seven genera: *Candidatus Brocadia*, *Candidatus Jettenia*, *Candidatus Kuenenia*, *Candidatus Anammoxomicrobium*, *Candidatus Anammoxoglobus*, *Candidatus Scalindua*, and *Candidatus Loosdrechtia aerotolerans*, all in the Planctomycetes phylum. (Parde et al., 2024). The only known marine anammox bacteria is *Candidatus Scalindua* (Roques et al., 2024).

Marine anammox bacteria (MAB) are a unique type of anammox bacteria that play a vital role in the marine nitrogen cycle. MAB is part of *Candidatus Scalindua*, discovered in anoxic marine habitats like saltwater or marine silt. Psychological traits

of "*Candidatus Scalindua* sp." include being halophilic, with a high affinity for nitrite, maintaining anammox activity at temperatures of 10–30 °C, optimal pH of 6.0–8.5, growth salinity of 1.5–4.0%, and a biomass yield of 0.030 mol C (mol NH_4^+)$^{-1}$, with a lower growth rate than common anammox species (Ismail et al., 2022).

Recent research on microbial interactions in anammox systems shows a correlation between reactor performance and microbial community (Ji et al., 2020). The reactor type is essential for incubating the inoculum. The bioreactor in use is a Filter Bioreactor (FtBR). FtBRs are a recent development; Zulkarnaini et al. (2018) utilized string-wound filters as an attachment medium for anammox bacteria in a two-inflow biofilm reactor. This reactor operates on activated sludge samples at startup (Zhang et al., 2015). According to Gumelar et al. (2024) Nitrogen removal performance in FtBR using intensive shrimp pond sludge shows a Nitrogen Removal Efficiency (NRE) of 63-72%. The nitrogen stoichiometric ratios are 1:1.40:0.12, similar to the anammox process. This suggests that anammox is a key part of nitrogen removal and an effective alternative for nitrogen treatment in shrimp ponds.

Previous research shows that anammox bacteria successfully grew during cultivation. Yokota et al. (2018) operated an upflow anaerobic sludge blanket (UASB) fed continuously with 30 ppt waste brine, successfully growing *Candidatus Scalindua wagneri*. Lulrahman (2021) Operate the FtBR using muaro penjalinan sludge as inoculum and artificial wastewater from seawater with a salinity of 29.7-32.4 ppt. The cultivated anammox bacteria are *Candidatus Anammoxoglobus propionicus* (2,65%), *Candidatus Brocadia sinica* (1,95%), *Candidatus Jettenia unclassified* (1.64%), *Candidatus Brocadia fulgida* (0,51%), and *Candidatus Jettenia* sp. (0,37%). Previous research on anammox bacteria has focused on freshwater species, creating a knowledge gap regarding marine processes. Thus, analyzing the microbial community of marine anammox is necessary to determine the abundance of each bacterium and its effect on maintaining anammox biomass in nitrogen during the FtBR operation removal. This study analyzes the microbial community of marine anammox in a Filter Bioreactor (FtBR) run by Indira (2024), inoculated with vaname shrimp pond sludge and fed

artificial seawater-based wastewater with 70 mg-N/L of ammonium and nitrite. Conducted with a hydraulic retention time (HRT) of 24 hours at a temperature of 25°C to 28°C and substrate salinity of 30.1 to 33.0 ppt, it achieved a nitrogen removal efficiency (NRE) of 43.797%. This study aims to enhance understanding of the microbial community involved in marine anammox cultivation and provide insights into key active microorganisms in saline Anammox systems.

1.2 Purpose and Objective

1.2.1 Purpose

This study aims to identify and analyze the microbial community of anammox bacteria, especially marine anammox bacteria, in a Filter Bioreactor (FtBR).

1.2.2 Objective

To achieve this purpose, some of the objectives in the research are to be achieved are:

1. To identify and characterize the microbial community composition within the inoculum and Filter Bioreactor (FtBR);
2. To analyze the diversity and abundance of anammox, mainly marine anammox bacteria, in the inoculum and Filter Bioreactor (FtBR);
3. To evaluate the functional roles of key microbial groups involved in the nitrogen removal process within the inoculum and FtBR.

1.3 Benefit

The results of this study are expected to provide benefits to various parties, namely:

1. Determine the identity and character of the microbial community composition within the inoculum and Filter Bioreactor (FtBR) using the Next-Generation Sequencing (NGS) method using Illumina Miseq sequencing;
2. Knowing the diversity and abundance of anammox, mainly marine anammox bacteria in the inoculum and FtBR;
3. Knowing the functional roles of key microbial groups involved in the nitrogen removal process within the inoculum and FtBR.

1.4 Scope

In order for this research to be more focused on the desired research, the authors determine the limitations of the problem regarding the following:

1. The experiment used a biomass reactor from Indira, (2024) Research using vaname shrimp pond sludge in Katapiang, Batang Anai District, Padang Pariaman Regency, as inoculum after operating in the FtBR;
2. The FtBR that Indira, (2024) Operates artificial seawater-based wastewater containing 70 mg-N/L of ammonium and nitrite, with a 24-hour HRT, an ambient temperature of 25 °C —28 °C, a substrate salinity range of 30.1-33.0 ppt, and operates for 175 days;
3. Microbiological identification was performed on biomass after completion of the FtBR operation at ambient temperature using the 16S rRNA gene sequencing, Next Generation Sequencing (NGS) method with the Illumina sequencer miseq tool at Kanazawa University, Japan.

1.5 Writing Systematics

The writing system of this final project is as follows:

CHAPTER I **INTRODUCTION**

This chapter provides the research background, purpose, aims, advantages, scope, and writing systematics.

CHAPTER II **LITERATURE REVIEW**

This chapter encompasses the theoretical foundation of nitrogen compounds, the anammox process, the FtBR reactor, DNA extraction, PCR, Next-Generation Sequencing (NGS), and a phylogenetic tree.

CHAPTER III **METHODOLOGY**

This chapter describes the research location, time, and stages, such as reactor configuration, reactor performance, bacterial

preparation, DNA extraction, PCR, Next-Generation Sequencing (NGS), and Bioinformatics.

CHAPTER IV RESULT AND DISCUSSION

This chapter contains laboratory testing results, data processing, and discussion.

CHAPTER V CONCLUSION AND SUGGESTION

This chapter contains conclusions and suggestions based on the discussion that has been described.

BIBLIOGRAPHY

APPENDIX