The Bioremediation Process Of Liquid Waste from A Fish Auction Place is Based on The Parameters of Chemical Oxygen Demand and Ammonia

ABSTRACT

Background and Objectives: The Bajomulyo fish auction place unit II, Juwana – Pati is experiencing poor sanitation due to inefficient liquid waste processing, which results in high levels of chemical oxygen demand and ammonia. Therefore, it is necessary to apply effective alternative management technology, especially bioremediation technology. The purpose of this study is the Utilization of biological agents in bioremediation technology to overcome Chemical Oxygen Demand and Ammonia. **Methods:** This study used experimental methods and was carried out during August 2023 to October 2023. The best bacteria in degradation studies require a bacterial screening strategy through isolation and selection stages. then identified using molecular genetics. **Results:** The degradation test findings indicated that Isolate 2 had the highest potential level. The effective chemical oxygen demand parameter dropped from 6,666.7 mg/l to 5,566.7 mg/l, whereas ammonia levels fell from 34.2 mg/l to 13.3 mg/l. Bacterial identification isolate 2 has a maximum similarity level of 99%. The identification of isolate 2 bacteria has a maximum similarity level of 99%. Based on the phylogenetic tree study, this isolate has a very close kinship with the *Streptomyces heilongjiangensis* strain FREP 14. **Conclusion:** Indigenous bacteria may be detected in fish auction wastewater and are capable and effective decomposers of wastewater including chemical oxygen demand and ammonia

***Keywords**:* Bioremediation, Chemical Oxygen Demand, Ammonia, Fish auction place, Molecular biology, *Streptomyces heilongjiangensis* strain FREP 14.

INTRODUCTION

Fish auction places play an important role in facilitating buying and selling transactions carried out by fishermen, so that they can encourage economic growth and improve welfare, but fish auction places also produce various types of liquid waste, especially waste from fish washing, fish cutting, and household activities at the fish auction place. This liquid waste is often discharged directly into the aquatic ecosystem, without going through a liquid waste treatment process. This condition causes pollution around the fish auction place, and increases the possibility of disease in residents around the fish auction location [1]. Given the increasingly widespread production activities of catches at the Bajomulyo Coastal Fishing Port, it is necessary to improve sanitation facilities through the construction of wastewater treatment installations [2]. Ecological balance can be disturbed due to improper management of liquid waste, which is a result of the absence of waste processing installations [3]

Based on Law Number 32 of 2009 concerning Environmental Management and Regional Regulation of Central Java Province Number 1 of 2013 concerning Management of Ports and Fisheries in the Central Java Province [4] The legislation was created largely to ensure that waste, which is a byproduct of numerous sectors and has the potential to pollute, does not harm the environment when disposed of. Referring to the Regulation of the Minister of Environment Number 68 of 2016, concerning domestic liquid waste water quality standards at fish auction

places [5]. Hendrayana *et al* in 2022, stated that the nitrate, nitrite, ammonia, and phosphate content in the waters of Munjung Agung, Tegal regency did not meet the quality standard threshold according to the Minister of Environment and Forestry Decree No. 51 of 2004, with a determination value of moderate-heavy polluted [6]. The increase in ammonia composition results from the decomposition of organic molecules, which are the primary source of total ammonia. Ammonia (NH_3), a poisonous form of total ammonia, can limit the growth of aquatic species if the tolerance threshold is exceeded [7]

Bioremediation technology can effectively manage pollution caused by ammonia and organic materials by applying a series of processes, including isolation, testing bacteria for pollutant degradation, bacterial identification, and bacterial multiplication. These bacterial isolates can come from indigenous bacteria, which is in accordance with the decree of the minister of environment No. 128 of 2003, concerning procedures and technical requirements and biological waste management (bioremediation) carried out using indigenous microorganisms [8] Indigenous bacteria isolated from wastewater have the ability to break down complex chemical molecules into simpler organic components. The enzymatic procedure uses various hydrocarbon-degrading petrophilic enzymes [9]

Nitrifying bacteria are able to oxidize ammonia (NH_3) and nitrite (NO_2^-) to nitrate (NO_3^-) aerobically. Among other things, the most common types of microorganisms are from the genus Streptomyces. [10]. The isolation and screening of Streptomyces parvus (FG-2) resulted in a 95.80% degradation of 1.0 mg/L Fluorochloridone (FLC) within 28 days at a temperature of 30 ℃, pH 6, and an inoculum of 5.0% (v/v). The FG-2 strain is composed of a circular chromosome and two plasmids, as indicated by the results of whole genome sequencing. After isolation and purification, the degradation enzymes were identified as α/β hydrolase and 2-hydroxy acid dehydrogenase [11]. *Streptomyces sp* has biological ability in reducing ammonia concentration from 1358 mg/L to 140 mg/L. and COD decreased from 427,263 to 82 mg/L. [12]. The potential and biodiversity of bioremediation bacteria in Indonesia can be introduced through the exploration and identification of selected bacteria for bioremediation activities. In addition, it has the potential to expand the collection of bioremediation bacteria that are responsible for the degradation of ammoniacal and the reduction of COD concentrations that are derived from local or indigenous isolations. Alternative technology for fish auction waste management is needed to overcome the waste produced, especially the COD (Chemical Oxygen Demand) and Ammonia (NH3) parameters [13]. *Streptomyces mediolani EM-B2*, an actinomycete, had exceptional nitrogen removal abilities. The maximal clearance rates for ammonium, nitrate, and nitrite were 3.46, 1.71, and 1.72 mg/L h. The HN-AD enzyme effectively detected them. The nitrogen balance revealed that aerobic denitrification resulted in the conversion of more than 37% of the initial ammonia into nitrogen gas. This demonstrated that *Streptomyces mediolani* EM-B2 was able to successfully decrease ammonia and several other organic pollutants that generated significant chemical oxygen demand [14])

Based on this information, it is required to do research on the processing of liquid waste generated by fish auction activities employing indigenous bacteria as biological agents in bioremediation technology.

RESEARCH METHODS

This study employed an experimental strategy to collect quantitative data in order to identify the optimum bacteria for COD and ammonia degradation. This study also included genomic analysis of selected bacteria with the highest degrading potential.

Instruments and materials

Ammonia (NH3) test [15]

Instruments: Portable UV 1800 Spectrophotometer, 190-1100 Nm, analytical balance, Erlenmeyer 50 mL, measuring flask 25 mL, volumetric pipette, measuring pipette, beaker, filter paper, and aluminium foil. **Materials:** Ammonium chloride (NaCl), phenol solution (C_6H_5OH), 0.5% sodium nitroprusda (C_5FeNa_2O), alkaline citrate solution ($C_6H_5Na_3O_7$), 5% sodium hypochlorite (NaClO), and distilled water (aquadest)

COD test [16]

Instruments: closed reflux tube, 5 ml and 10 ml measuring pipette, 1000 ml measuring flask, rubber suction cup, tube rack, thermoreactor, cuvette, Portable UV 1800 Spectrophotometer, 190-1100 N. **Materials**: The materials used are sample water, distilled water, 500 ppm Potassium Hydrogen Phthalate (PHP) solution, concentrated sulfuric acid solution, low digestion solution.

Microbiological Test (Bacterial Screening)

Instruments: Water Sampler, Test tube, Petri dish, Winkler tube, tube, Bunsen, beaker, funnel, separating funnel, Erlenmeyer, measuring cup, spatula, volume pipette, propipet, filter paper, hot plate, vortex, magnetic stirrer, autoclave, hockey stick, distillation equipment, oven, shaker, incubator, refrigerator, electric scale, analytical balance, desiccator and incubator. and Materials: Samples of liquid waste from the Fish Auction Place. **Materials :** Bromtympol Blue (indicator), Zobell agar medium and Zobell broth medium. The ingredients that make up the Zobell medium consist of NaCl (3 grams), KCl (0.7 grams), $MgCl_2.6H_2O$ (10.8 grams), $MgSO_4.7H_2O$ (5.4 grams), $CaCl_2.2H_2O$ (1 gram), distilled water, $PbNO_3$ 0.1 ppm, $Pb(CHCOO)_2$ 0.1 ppm, MR-VP Broth, methylene blue staining, 3% hydrogen peroxide, naphthol solution (1 gram per 100 ml ethyl alcohol) and phenylendiamine solution (1 gram per 100 ml distilled water), minimum yeast extract (0.25 gram) and minimum Bakto pectone (1 gram) [17]

Molecular Genetic Analysis Test

Instruments: Microcentrifuge equipped with a cooler, incubator; autoclave, laminar air flow cabinet (Esco); pH meter (Eutech), digital camera (HP Photosmart R607), analytical balance, deep freezer -20 ºC, oven, 25 µl PCR Master Mix, 0.05 U/mL Taq DNA polymerase; 0.4 mM each dNTP; 4 mM $MgCl_2$, 2 µL Primer 16E1, 2 µL Primer 16E2, 1 µL MilliQ, and 10 µL health genomic DNA template, Mini gel electrophoresis, UV transluminator, PCR Thermal Cycler, 25 µl PCR Master Mix, 2 µL Primer 16E1, 2 µL Primer 16E2, 1 µL MilliQ, 10 µL genomic DNA template 25 µl PCR Master Mix, 2 µL Primer 16E1, 2 µL Primer 16E2, 1 µL MilliQ and 10 µL minispin microcentrifuge genomic DNA template. **Materials:** Lysozyme, Sodium Dodecyl Sulfate/SDS, proteinase-K, DNAse and RNAse free aquabidest (ddH_2O), Tris base, Etylene Diamine Tetra Acetic Acid/EDTA chloroform, isoamyl alcohol, PCR master mix, Primer 16E1: GGG AGT AA GTT AAT ACC TTT GCT C, [4], Primer 16E2: TTC CCG AAG GCA CAT TCT (Biotech) [4],

Agarose ultrapure, Loading Buffer, Ethidium bromide, 1 kb plus DNA ladder, Ehrlich's reagent, methyl red, potassium hydroxide, α-naphthol. Nutrient Broth/NB media solution, Nutrient Agar/NA media , Lactose Monohydrate, Brilliant Green Lactose Bile Broth/BGLB 2% Media, Eosin Methylene Blue/EMB Agar Media, Methyl Red Voges Media- Proskauer/MRVP, Simmons Citrate Media, Tris Acetate Buffer EDTA/TAE, PVP 1%, Chloroform, Ethanol 100%, TE Buffer, Agarose, TAE1x Buffer, DNA Extraction, Forward and reverse specific primer, Green Go Taq Master Mix

Microbial Isolation Media Preparation

Method for Isolating Bacteria from Liquid Waste at Fish Auctions [18]
1) The Zobell 2216E liquid medium, which contains Bromine Thymol Blue (BTB) as an indicator, is sterilized in an autoclave. **2)** The sterilized medium is enriched with liquid waste containing ammonia/organic characteristics (COD) at 75 ppm. 3) Using a tube needle, inoculate one tube of isolated bacteria into the liquid medium. 4) Shake the sample at 150 rpm for 2x24 hours. **3)** Using a loop needle (ose), one loop (ose) of bacteria isolated from the waste water sample is injected into the liquid medium**. 4**) Shake the sample at 150 rpm for two 24 hour cycles. **5)** Pay attention to the color changes that occur; If the liquid medium changes color from blue to yellow, this indicates that the bacterial isolate can break down ammonia/COD**. 6)** The fastest color change indicates the best reduction in ammonia and COD values. The best isolate was then examined for its ability to degrade

Microbial degradation capability test
Testing the sample with a Portable UV 1800 Spectrophotometer, 190-1100 Nm: **1)** Determine the ammonia concentration.[19] The test sample is pipetted 25 ml into a 50 mL sample cell. Then, 1 mL of phenol solution, 1 mL of sodium nitropuside solution, and 2.5 mL of oxidising solution were added. Then it was homogenized and waited for one hour before the absorbance was measured with a Portable UV 1800 Spectrophotometer, 190-1100 Nm at a wavelength of 640 nm. **2)** The validation of verification results of ammonia testing methods in river water includes linearity, precision (repeatability and reproducibility), accuracy, limit of detection (LOD), limit of quantitation (LOQ), instrumental detection limit (IDL), method detection limit (MDL), and estimation measurement uncertainty using a Portable UV 1800 Spectrophotometer, 190-1100 Nm, [20]. Ammonia analysis is performed using the SNI 06.6989.30-2005 method, which meets laboratory regular testing criteria. **3)** The findings of the Ammonia and COD analyses were then used to assess the degrading ability of the selected bacteria using a regression test.

Microbial Identification using 16S-rRNA Gene Analysis [21][22]

DNA Extraction
DNA extraction using the Chelex 100 Kit, as follow:

1) Bacterial cells grown for 24 hours are placed in a 1.5 ml Eppendorf tube with 100 µl of aquabides. Then, 0.5% saponin is added and left for 24 hours at 4°C. The material was centrifuged at 12,000 rpm for 10 minutes, and the supernatant was discarded. **2)** Add 1 ml of Phosphate Buffer Saline (PBS 1x) to the Eppendorf tube. Centrifuge at 12,000 rpm for 15 minutes. Discard the supernatant. Add 100 µl of aquabides and 50 µl of Chelex 100 to the tube. **3)** The material is boiled

for 10 minutes (with a vortex for the first 5 minutes). Repeat the centrifugation at 12,000 rpm for 10 minutes. The supernatant containing DNA was transferred to a new Eppendorf tube and ready for DNA amplification.

DNA Amplification

Amplification is a molecular marker that use the 16s rDNA Polymerase Chain Reaction (PCR) method, as follows: **1)** The temperature treatment utilized in the DNA amplification process is: initial denaturation at 95 0C for 3 minutes, followed by 30 cycles (denaturation at 95 0C for 1 minute, annealing at 55 0C for 1 minute, and extension at 72 0C for 1 minute), then extension at 72 ^{0}C for 7 minutes, and lastly 4 ^{0}C. The primers for 16S rDNA PCR were the universal primer for bacteria 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and the eubacteria-specific primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). **2)** The mixture of chemicals used is the Promega kit (25 µl), primer 270 F (2.5 µl), primer 1492 R (2.5 µl), DNA template (2.5 µl), and aquabides (17.5 µl), for a total volume of 50 µl. These components were combined in a 0.2 mL PCR tube.

Visualization of DNA Amplification Results

1) Electrophoresis is used to visualize DNA amplification results. Insert 5 µl of PCR product into a well of a 1% agarose gel. To make a 1% agarose gel, dissolve 1 gram of agarose in 100 ml of 1x TAE buffer solution and heat in an oven until homogenous (clear). 5.33 µl of Ethidium Bromide was added to the gel solution and agitated until homogenous.. **2)** The gel solution is poured into the mold while the mold comb is upright, and it passes through to the required thickness. The gel is left to solidify for a while. After soaking the gel in 1x TAE buffer solution, it was electrophoresed at 100 V for ± 30 minutes. **3)** The Gel Documentation tool was used to view the amplification-generated DNA bands.

Purification of DNA amplification results

Purification is performed to acquire pure DNA from PCR amplification of 16S rDNA. 2) The PCR results are centrifuged at 12,000 rpm for seven minutes. The supernatant was collected using a micropipette to confirm that the DNA was entirely pure (no primer was left). 3) Add 50 µl of sterile distilled water to the DNA pellet and leave for 5 min. The resulting pure DNA can be sequenced to determine the base sequence.

Sekuensing DNA

DNA sequencing is the method of determining the sequence of nucleic acids (nucleotides) in DNA. As follows:

Sequencing was performed using the PCR sequencing cycle and Big Dye Terminator v.3.1. The PCR sequencing reaction requires 2 µl of large dye, 2 µl of 10x buffer, 4 µl of DNA template, 1 µl of primer at 3.2 pmol concentration, and 10 µl of ddH2O in total. **b)** DNA amplification was performed in the following order: initial denaturation (96 °C for 2 minutes), denaturation (96 °C for 10 seconds), annealing (50 °C for 5 seconds), and extension (60 °C for 4 minutes) for 25 cycles**.** **c)** The PCR findings were purified and sequenced with primer 27F. The sequences were examined automatically (ABI 3130XL, Applied Biosystem)

Phylogenetic Tree Construction [23]

A phylogenetic tree is a tree-shaped graphic that depicts the relationships between species. Phylogenetic trees are created based on the genetic structure of species expressed in DNA or

protein sequences, as follows: 1) The selected and best ammonia and organic degrading (COD) bacteria with effectively amplified 16S rRNA genes were examined in a database using their 16S-rRNA gene sequence. 2) Some sequences were modified with the Bioedit tool. 3) After getting data from the contiq nucleotide sequence based on universal amplification, the homology will be compared to other prokaryotes in the Gene Bank database. Cluster analysis was performed using the database from the RDP website (Ribosomal Database Project, http://www.rdp.com), and the phylogenetic tree was built using the MEGA 5 program.

RESULT AND DISCUSSIONS

The research period will last from August to October 2023. Wastewater sample was conducted in the Silungonggo tributary's major watershed at the Bajomulyo-Juwana Fish Auction Place in Pati Regency, Central Java, at coordinates LS 6°42'13"S 111°09'18"E. The gathered samples will subsequently be used in the screening method for specific bacteria. The experiments were carried out at Dian Nuswantoro University's Environmental Health Laboratory.

1. Bacterial Screening

Table 1 displays the outcomes of bacterial selection and screening, as follows:

Table 1. Screening results for microorganisms that degrade ammonia and Chemical Oxygen Demand

No Isolate	Discoloration COD				Discoloration Ammonia			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
2	+	++	+++	++++	+	+++	++++	+++++
3	+	+	++	++	+	+	++	++
6	+	+	++	++	+	+	++	++
8	+	++	++	+++	+	++	++++	+++++

Description: The (+) sign indicates the degree of color change in the test medium.

Based on the color change speed test using the Bromtymol blue indicator, 5 bacterial strains capable of decomposing waste fish auction sites were identified. Of the five bacterial strains obtained, bacterial strains 8 and 2 are the fastest to change color. The selected bacteria are next examined for their degrading ability using the COD (Chemical Oxygen Demand) and Ammonia (NH3) criteria.

The speed of color change on Zobell 2216 E agar medium and Zobell 2216E liquid medium with the BTB (Brom Thymol Blue) indicator was used to select bacteria that degraded Fish Auction Place garbage. The color change from blue to yellow is induced by the aerobic degradation of TPI liquid waste, which releases acid chemicals that turn the BTB indicator (Brom Thymol Blue) yellow [24]. Bromothymol blue is a member of the 2,1-benzoxathioles group, specifically 2,1-benzoxathiole 1,1-dioxide, with the two hydrogens in position 3 substituted by a 3-bromo-4-hydroxy-5-isopropyl-2-methylphenyl group. This chemical works as an acid-base indicator, dye, and two-color indicator. This chemical contains 2,1-benzoxathiole, arenesulfonate ester, organobromine compounds, polyphenols, and sultone. Bromothymol blue (BTB) is a typical acid-base indicator that appears yellow in mild acid solutions, greenish in neutral conditions, and blue in alkaline medium. In BTB samples with a neutral pH (7), the greenish color is one of the most relevant and successful approaches for detecting acidic, neutral, and alkaline contents. Bacteria

degrade protein in organic matter to create amino acids and peptides, which are identified as yellow on the bromine tymol blue indicator [25]. The speed of color change in Zobell 2216E liquid media treated with liquid waste, as well as the BTB (Brom Thymol Blue) indicator, are used to identify waste-degrading bacteria at fish auctions. The aerobic breakdown of TPI liquid waste produces acidic compounds, causing the BTB (Brom Thymol Blue) indicator to shift from blue to yellow. This demonstrates that bacteria with the best ability to digest TPI waste have a higher rate of color change.[26] Table 1 displays the findings based on direct visual observation.

Based on the bacterial screening results, 99 bacteria were successfully grown and isolated on Zobell 2216 E agar medium, but only 5 bacteria were capable of decomposing organic matter and ammonia, resulting in visible color changes (bromothymol blue indicator). Fig 1 depicts the color change of bromtymol blue in Zobell 2216 E liquid media enhanced with fish auction waste, as shown :

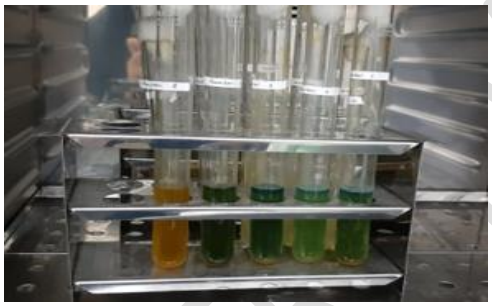


Figure 1. Screening of the selected and best bacterial strains

2. Ability of Selected Bacteria in the Bioremediation

Data from COD and Ammonia Parameter Measurement findings based on time periods are as follows:

Table 2. Test results for Chemical Oxygen Demand parameters (mg/L)

Isolate	Day 1	Day 2	Day 3	%
Isolate 2	6667	6500	5566	17%
Isolate 8	6600	6533	6366.67	4%
Concortium	7100	6900	6333.3	11%

Tabel 3. Test results for ammonia parameters (mg/L)

Isolate	Day 1	Day 2	Day 3	%
Isolate 2	34.22	21.4	13.26	61%
Isolate 8	42.86	34.83	30.94	28%
Concortium	31.18	28.55	24.69	21%

Figures 2 and 3 illustrate the analysis results as well as the capacity of indigenous bacteria to degrade ammonia and COD. Based on Fig 2, the ability of the best degrading bacteria in the COD parameter (measured on day 3) is isolate No. 2 with a degradation ability of 17%, then consortium bacteria with a degradation ability of 11%, and finally isolate No. 8 with a degradation ability of 4 %. Similarly, if an analysis is carried out based on a simple regression trend, isolate 2 has the best decreasing trend with the equation y = -550.33x +7344.9, then the consortium with the simple regression equation y = -383.35x+7544.5 and then isolate 8 with the regression equation simple y = -116.66x + 6733.2.

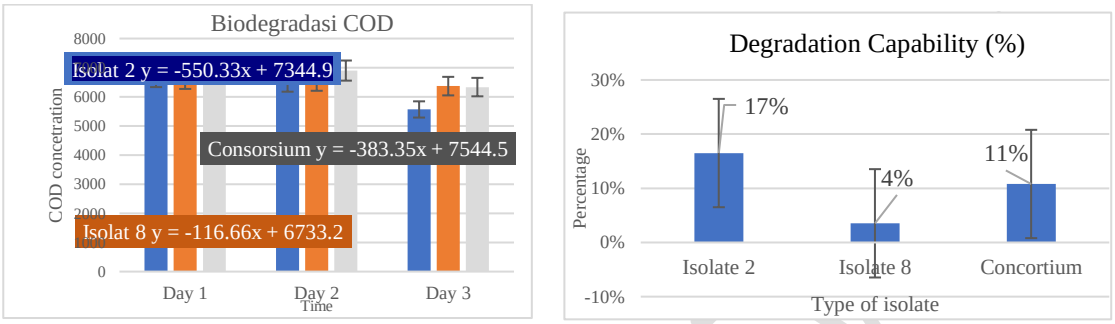


Fig 2. Data of analysis of COD degradation by selected bacteria

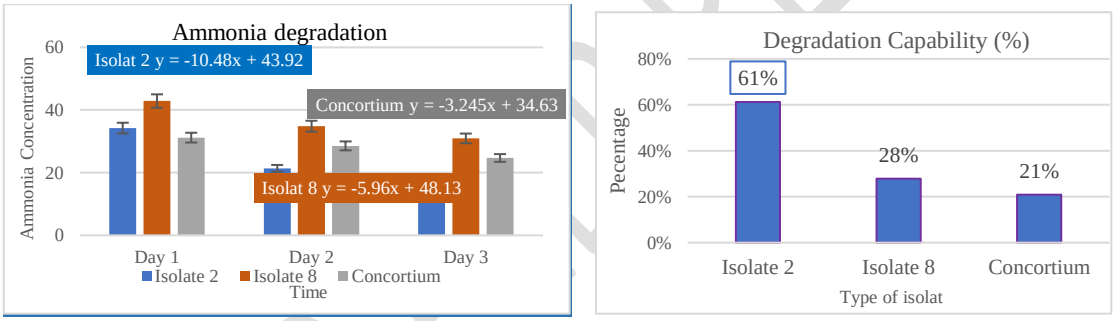


Fig 3. Data of analysis of Ammonia degradation by selected bacteria

According to Fig 3, isolate 2 has the best degradation ability based on the Ammonia parameter, with a degradation ability of 61%, followed by isolate 8 with a degradation ability of 28%, and the consortium bacteria with a degradation ability of 21%, based on measurements taken on day 3. Similarly, if analysis is performed using a simple regression trend, isolate 2 has the best reduction trend with the equation y = -10.48x +43.92, followed by isolate 8 with the equation y = -5.96 x +48.13, and finally the consortium with the regression equation y = -3.245x + 43.63. Based on this information, isolate 2 has the highest degrading capacity for ammonia parameters, thus it was identified using molecular genetic methods.

3. Identification of selected and best degrading indigenous bacteria

Indigenous bacteria that have the best ability to degrade Ammonia and Chemical Oxygen demand, then identified to find out the type of bacteria to the species level. Table 3 shows the data of selected bacterial samples at the identification stage using a molecular genetic approach.

Table 4. Information on selected and selected indigenous bacterial samples based on molecular genetic methods

Sample Name	Bacteria 2			
PCR Primer	16s (27F-1492R)			
PCR Products	Species Barcoding Bacteria (~1400bp)			
1. Genomic DNA extraction with Quick-DNA Fungal/Bacterial Miniprep Kit 2. PCR amplification with (2x) MyTaq HS Red Mix 3. Bi-directional Sequencing				
Nucleic Acid (Genomic DNA) Quantification (Nanodrop)				
Nama Sample	Conc. (ng/µl)	A260/280	A260/230	Volume (µl)
Isolate 2	13.8	1.99	2.01	35

Sample Information Isolate 2 shows the bacterial sample code that will be analyzed and identified using molecular genetics to determine the bacterial species. PCR Primer: 16s (27F-1492R) refers to a pair of primers designed to carry out a PCR (Polymerase Chain Reaction) reaction on the 16S ribosomal RNA (rRNA) gene. This primer is used to amplify DNA fragments containing the 16S rRNA gene sequence of the bacterial species to be identified. [27]. PCR Products : Species Barcoding Bacteria (~1400bp), describes a DNA fragment approximately 1400 bp long that is produced after performing PCR using primers intended for barcoding bacterial species. This fragment can then be used for further identification or analysis of the bacterial species present in the DNA sample, while PCR Products are the result of a PCR (Polymerase Chain Reaction) reaction, which is a molecular technique for amplifying (making many copies) of certain DNA fragments from a sample. which may contain DNA from various bacterial species.[28]. Species Barcoding is a short fragment of the genome (a particular gene or DNA sequence) that is used as an identifier for a particular species. In this context, PCR is performed with primers designed to amplify copies of DNA fragments of genes used for barcoding bacterial species. These DNA fragments are then used to identify bacterial species based on a comparison of their genetic sequence with a reference database. Bacteria (~1400bp): indicates that the expected length of the DNA fragment after amplification is approximately 1400 base pairs (bp). This length can vary depending on the primers used and genetic variations of the bacterial species targeted.

Nucleic Acid (Genomic DNA) Quantification (Nanodrop) is the process of measuring the concentration of genomic DNA using a Nanodrop. This method is used to determine how much DNA is present in the sample being measured (PCR (Polymerase Chain Reaction), DNA sequencing, and genetic analysis) This method utilizes the absorbance of light at specific wavelengths to determine DNA concentration, and is often performed before molecular biology experiments to ensure adequate amounts of genetic material are needed [29].

PCR amplification with 2x MyTaq HS Red Mix is a polymerase chain reaction (PCR) amplification procedure that uses a PCR reaction mixture called MyTaq HS Red Mix. PCR amplification is a process for amplifying (making many copies) of certain DNA fragments from samples in a PCR reaction. This technique is used to selectively multiply and duplicate specific DNA sequences. PCR amplification was carried out using the MyTaq HS Red Mix (2x) reaction mixture, indicating that this PCR reaction mixture had been diluted twofold or already had a concentration double the reagent concentration required for the PCR reaction, while the MyTaq HS Red Mix was the PCR master mixture which includes all the essential components needed to

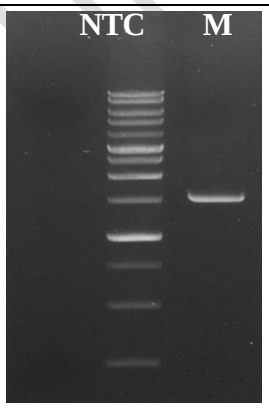
carry out a PCR reaction, including DNA polymerase, nucleotides, buffer, and red dye which helps in filling the electrophoresis gel after the PCR reaction [30][31]. Bi-directional Sequencing. Sequencing is the process of determining the sequence of nucleic acids (DNA or RNA) in a particular sample. Bi-directional sequencing refers to a sequencing method in which the sequence of nucleic acids (usually DNA) is determined from both ends of the same DNA fragment. By using bi-directional sequencing, it is possible to obtain more reliable and detailed DNA sequences, which is important for various molecular biology applications.

Nucleic Acid (Genomic DNA) Quantification (Nanodrop) is the method of quantifying genomic DNA concentrations with a device known as a nanodrop. A Nanodrop is a spectrophotometer that measures the absorption of light at certain wavelengths from DNA samples in solution. This approach is useful in molecular biology since it determines the amount of genomic DNA present in a material. The known DNA concentration is subsequently utilized to modify sample concentrations in a variety of experimental applications, including PCR and DNA sequencing. This method delivers a quick and relatively accurate quantitative estimate based on the purity and clarity of the DNA sample being evaluated. The concentration and ratio of A260/280 can provide a good knowledge of the quality and quantity of nucleic acids in test samples (table 4), as follows:

Based on the analysis results of Conc. (ng/µl) has a value of 13.8 ng/µl, indicating the concentration is 13.8 nanograms per microliter. a measure of the concentration of a substance in solution, measured in units of nanograms per microliter. Concentration (ng/µl): 13.8 ng/µl will be valid if the measurement is carried out with the right equipment and according to procedures. PCR (Polymerase Chain Reaction): For most PCR reactions, DNA concentrations between 10 to 100 ng/µl are often used. In this case, the concentration of 13.8 ng/µl that you mention is within the acceptable range for use in PCR reactions.[32] and the A260/280 ratio results in the range of 1.99 to 2.01 suggest that the protein in the nucleic acid sample is still deemed pure for molecular applications (the expected standard a260/280 value is approximately 1.8 to 2.0) [33].

Gel Photo – PCR Products

A "Gel Photo - PCR Products" is a photo or image obtained from an electrophoresis gel that is used to visualize the outcomes of a polymerase chain reaction (PCR), this approach separates DNA fragments based on their size..



1 µL PCR Products were assessed by electrophoresis with 1% TBE agarose M, 1 Kb DNA ladder (loaded 2,5 µL); samples ranged in the order of upper table NTC : Non Template Control

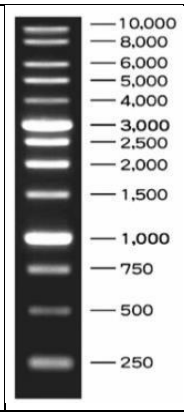


Fig 4. Gel Photo – PCR Products

Base on Fig 4. Gel Photo – PCR Products menjukan bahwa Electrophoresis of 1 µL PCR products was performed using 1% TBE agarose M and a 1 Kb DNA ladder (loaded 2.5 µL). Samples were arranged in the order shown in the table above, non-template control, This statement covers how to evaluate PCR-generated DNA fragments using electrophoresis with 1% TBE agarose. PCR-generated DNA fragments were analyzed using electrophoresis with 1% TBE agarose. Size standards are employed to compare the sizes of the resultant DNA fragments, and samples are prepared and placed into electrophoresis gels in a predetermined order. NTC was employed as a negative control to ensure the purity of the PCR reaction. This approach aids in seeing and recognizing DNA fragments produced during PCR operations.

Sequence Assembly Result – PCR Products

The term "Sequence Assembly 1395 bp" refers to the process of assembling or sequencing tiny bits of DNA or RNA that are 1395 base pairs (bp) long. This length refers to the total amount of base pairs in the DNA or RNA molecule that were constructed from short fragments to form a longer whole. [34] This process is critical in the field of molecular biology to understand the structure and function of an organism's genome, as follows:

```
1       TGCAGTCGAA CGATGAACCA CCTTCGGGTG GGGATTAGTG GCGAACGGGT GAGTAACACG
61      TGGGCAATCT GCCCTGCACT CTGGGACAAG CCCTGGAAAC GGGGTCTAAT ACCGGATACT
121     GAACCGCTTG GGCATCCGGG CGGTTCGAAA GCTCCGGCGG TGCAGGATGA GCCCGCGGCC
181     TATCAGCTTG TTGGTGAGGT AACGGCTCAC CAAGGCGACG ACGGGTAGCC GGCCTGAGAG
241     GGCGACCGGC CACACTGGGA CTGAGACACG GCCCAGACTC CTACGGGAGG CAGCAGTGGG
301     GAATATTGCA CAATGGGCGA AAGCCTGATG CAGCGACGCC GCGTGAGGGA TGACGGCCTT
361     CGGGTTGTAA ACCTCTTTCA GCAGGGAAGA AGCGAAAGTG ACGGTACCTG CAGAAGAAGC
421     GCCGGCTAAC TACGTGCCAG CAGCCGCGGT AATACGTAGG GCGCGAGCGT TGTCCGGAAT
481     TATTGGGCGT AAAGAGCTCG TAGGCGGCTT GTCGCGTCGG TTGTGAAAGC CCGGGGCTTA
541     ACCCCGGGTC TGCAGTCGAT ACGGGCAGGC TAGAGTTCGG TAGGGGAGAT CGGAATTCCT
601     GGTGTAGCGG TGAAATGCGC AGATATCAGG AGGAACACCG GTGGCGAAGG CGGATCTCTG
661     GGCCGATACT GACGCTGAGG AGCGAAAGCG TGGGGAGCGA ACAGGATTAG ATACCCTGGT
721     AGTCCACGCC GTAAACGGTG GGCACTAGGT GTGGGCGACA TTCCACGTCG TCCGTGCCGC
781     AGCTAACGCA TTAAGTGCCC CGCCTGGGGA GTACGGCCGC AAGGCTAAAA CTCAAAGGAA
841     TTGACGGGGG CCCGCACAAG CGGCGGAGCA TGTGGCTTAA TTCGACGCAA CGCGAAGAAC
901     CTTACCAAGG CTTGACATAC ACCGGAAAAC CCTGGAGACA GGGTCCCCCT TGTGGTCGGT
961     GTACAGGTGG TGCATGGCTG TCGTCAGCTC GTGTCGTGAG ATGTTGGGTT AAGTCCCGCA
1021    ACGAGCGCAA CCCTTGTCCC GTGTTGCCAG CAGGCCCTTG TGGTGCTGGG GACTCACGGG
1081    AGACCGCCGG GGTCAACTCG GAGGAAGGTG GGGACGACGT CAAGTCATCA TGCCCCTTAT
1141    GTCTTGGGCT GCACACGTGC TACAATGGCC GGTACAATGA GCTGCGATAC CGCGAGGTGG
1201    AGCGAATCTC AAAAAGCCGG TCTCAGTTCG GATTGGGGTC TGCAACTCGA CCCCATGAAG
1261    TCGGAGTCGC TAGTAATCGC AGATCAGCAT TGCTGCGGTG AATACGTTCC CGGGCCTTGT
1321    ACACACCGCC CGTCACGTCA CGAAAGTCGG TAACACCCGA AGCCGGTGGC CCAACCCCCT
1381    TGCGGGGAGG GAGCT
```

Fig 5. Sequence Assembly Result – PCR Products

Top10 Hit BLAST Results Against NCBI Database, Excluding Uncultured Sample Sequences

Top10 Hit BLAST Results. Against the NCBI Database, the results displayed are the top ten results with the highest genetic or nucleotide sequence similarity to the sequence provided as a query in the BLAST search.

Table 5. Top10 Hit BLAST Results Against NCBI Database

Description	Scientific Name	Per. ident
1. *Streptomyces heilongjiangensis strain FREP14 16S ribosomal RNA gene, partial sequence*	*Streptomyces heilongjiangensis*	99.86
2. *Streptomyces sp. Heilongjiang20134 16S ribosomal RNA gene, partial sequence*	*Streptomyces sp. Heilongjiang20134*	99.86
3. *Streptomyces sp. Heilongjiang2013B 16S ribosomal RNA gene, partial sequence*	*Streptomyces sp. Heilongjiang2013B*	99.86
4. *Streptomyces cellulosae gene for 16S rRNA, partial sequence, strain: old-30-2-1*	*Streptomyces cellulosae*	99.86
5. *Streptomyces sp. strain ZZ820 16S ribosomal RNA gene, partial sequence*	*Streptomyces sp.*	99.79
6. *Streptomyces sp. strain MJM16145 16S ribosomal RNA gene, partial sequence*	*Streptomyces sp.*	99.79
7. *Streptomyces sp. strain X3035 16S ribosomal RNA gene, partial sequence*	*Streptomyces sp.*	99.79
8. *Streptomyces cellulosae strain ROA126 16S ribosomal RNA gene, partial sequence*	*Streptomyces cellulosae*	99.79
9. *Uncultured Streptomyces sp. clone ASC810 16S ribosomal RNA gene, partial sequence*	*uncultured Streptomyces sp.*	99.79
10. *Uncultured Streptomyces sp. clone ASC786 16S ribosomal RNA gene, partial sequence*	*uncultured Streptomyces sp.*	99.71

The results of sequencing using forward and reverse primers to determine the bacterial nucleotide base sequence are as follows:

Table 6. Hasil sekuensing menggunakan primer *forward* dan *reserve*

No	Isolate code	Nucleotide Base Length (bp)	Bacteria Name	Homology (%)	*Accession Number*
1	Isolat 2	2864	*Streptomyces heilongjiangensis Strain FREP 14*	99	KY628828

The 16s-rRNA gene sequence in isolate sample 2 showed that the test bacteria were a type of nucleic acid molecule with a query length of 2864 bp. The test bacterial lineage report based on taxonomic grouping shows the following results:

1) Kingdom : *Bacteria,*
2) Filum : *Actinobacteria,*
3) Kelas : *Actinobacteria,*
4) Ordo : *Actinomycetales,*
5) Famili : *Streptomycetaceae,*
6) Genus : *Streptomyces,*
7) Spesies : *Streptomyces heilongjiangensis Strain FREP 14*

Phylogenetics of Selected Bacteria

A phylogenetic tree is a graphical representation of the evolutionary link between the test bacteria (isolate 2) and numerous bacterial species that share the closest relationship, shown as a tree that shows how bacterial species are related to one another through evolutionary lineage. Presentation A phylogenetic tree is a graphical representation of the diversity and evolutionary relationships of microbial organisms. It aids in identifying novel species and understanding how bacteria reproduce and adapt to their environments.

Phylogenetic tree analysis shows that bacterial isolate 2 has 99% identical similarity with *Streptomyces heilongjiangensis* strain FREP 14

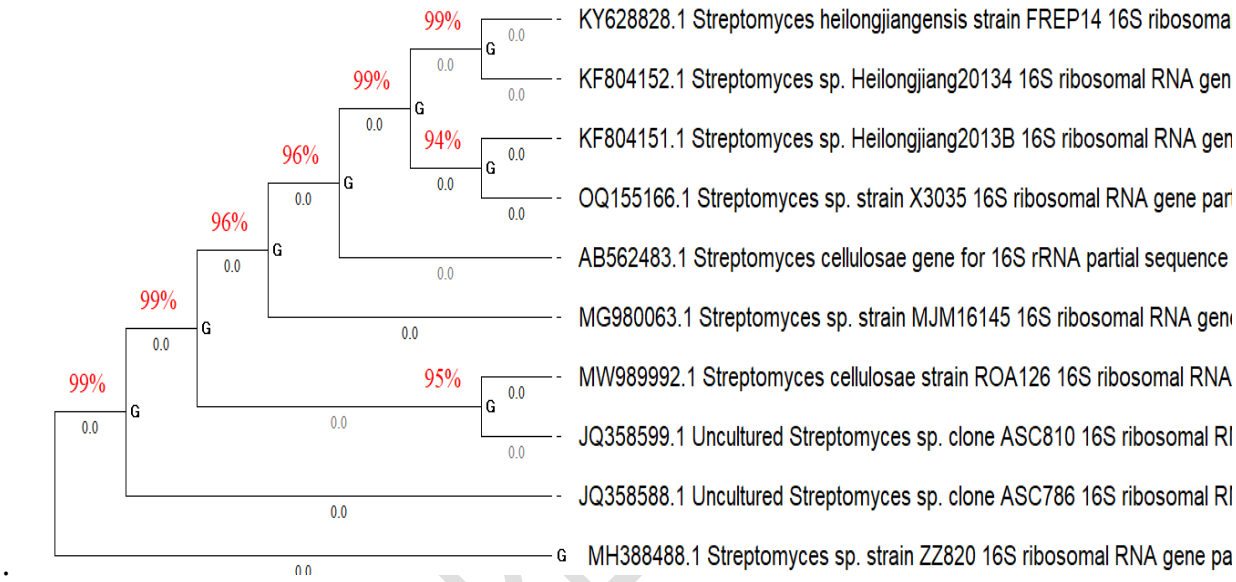


Fig 6. Phylogenetic tree analysis

CONCLUSION

Indigenous bacteria that degrade ammonia and COD can be isolated from liquid waste. Bajomulyo Juwana- Pati fish auction place. The ability of the consortium bacteria to degrade COD and Ammonia was no better than single isolate No. 2. Isolate 2 is the best choice for application in liquid waste processing Bajomulyo Juwana- Pati fish auction site with Ammonia and COD parameters. Isolate 2 was identified as having a homology level of 99% with Streptomyces heilongjiangensisstrain FREP 14

DATA AVAILABILITY

The manuscript includes all of the data sets created or analyzed during this work.

ETHICS STATEMENT

Not applicable

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

REFERENCE

1. H. Amrianah, D. Pratiwi, and M. R. Hamal, The Impact of The Existence Of Fish Auction Place (TPI) on Infrastructure Development and Socio-Economic Conditions of Communities in Sumpang Binangae Village, Barru District. *Meraja J.*, vol. 6, no. 2, pp. 153–164, 2023.
2. M. I. K. Jauhari, A. Suherman, and I. Triarso. Strategies for the development of the Bajomulyo Coastal Fishing Port, Pati, Central Java. *Depik*, vol. 10, no. 3, pp. 293–304, 2021.
3. T. Murwati. Kajian Pengaruh Aktivitas Pelabuhan Perikanan terhadap Aspek Kualitas Air Sungai Juwana dan Persepsi Masyarakat (Studi Kasus di Pelabuhan Perikanan Pantai (PPP) Bajomulyo, Kecamatan Juwana, Kabupaten Pati). Magister Ilmu Lingkungan, 2010.
4. U. Muawanah *et al.*. Review of national laws and regulation in Indonesia in relation to an ecosystem approach to fisheries management. *Mar. Policy*, vol. 91, pp. 150–160, 2018.
5. V. C. Yolanda and A. P. Heriyanti. Wastewater Quality Characteristics Test in Domestic Wastewater Treatment Plant Dinas Lingkungan Hidup Kota Semarang. *Indones. J. Earth Hum.*, vol. 1, no. 1, pp. 44–52, 2024.
6. H. Hendrayana, P. Raharjo, and S. R. Samudra.Komposisi Nitrat, Nitrit, Amonium dan Fosfat di Perairan Kabupaten Tegal. *J. Mar. Res.*, vol. 11, no. 2, pp. 277–283, 2022.
7. W. Lin *et al.*A review of the emerging risks of acute ammonia nitrogen toxicity to aquatic decapod crustaceans. *Water*, vol. 15, no. 1, p. 27, 2022.
8. A. Amin, A. T. R. Naik, M. Azhar, and H. Nayak. Bioremediation of different waste waters-a review. *Cont. J. Fish. Aquat. Sci.*, vol. 7, no. 2, p. 7, 2013.
9. [9] M. Divya, S. Aanand, A. Srinivasan, and B. Ahilan. Bioremediation–an eco-friendly tool for effluent treatment: a review. *Int. J. Appl. Res.*, vol. 1, no. 12, pp. 530–537, 2015.
10. [10] B. Sheela and S. K. K. Beebi. Bioremediation of ammonia from polluted waste

waters: a review. *Am. J. Microbiol. Res.*, vol. 26, p. 201, 2014.
11. [11] D. Zhao *et al.*. Biodegradation of Fluorochloridone in the Qinghai Plateau by Streptomyces Parvus FG-2: Performance, Pathway, Degradative Enzyme, Genetic Background, and Risk Assessment. *Pathway, Degrad. Enzym. Genet. Background, Risk Assess.*.
12. A. A. Ail, A. A. Al-Hussieny, and A. S. Taha. The Role of Filamentous Bacteria Streptomyces sp. in Reduction of Some Nutrients Concentrations in AL-Restomia Waste water Treatment Plant, Baghdad-Iraq. *Baghdad Sci. J.*, vol. 12, no. 3, pp. 445–452, 2015.
13. S. Mooralitharan *et al.* . Chemical Oxygen Demand and Ammonia Nitrogen Mycoremediation from Domestic Wastewater Effluent using Ganoderma lucidum in a Batch System. 2020.
14. T. He *et al.* New insight into the nitrogen removal capacity and mechanism of Streptomyces mediolani EM-B2. *Bioresour. Technol.*, vol. 348, p. 126819, 2022.
15. N. Thanh-Nho, L. T. Anh-Dao, L. T. Huynh-Mai, and N. Cong-Hau. Interfering ions and controlling their effects on the direct analysis of total dissolved ammonia nitrogen in aquacultural wastewater using molecular absorption spectrophotometry. *AIP Conference Proceedings*, AIP Publishing, 2022.
16. K. Velkushanova *et al.* Laboratory procedures and methods for characterisation of faecal sludge. *Methods Faecal Sludge Anal.*, 2021.
17. S. Isworo, P. S. Oetari, D. Prabowo, and H. Cerlyawati. Bioremediation Tofu Liquid Waste Based on Chemical Oxygen Demand (COD) Parameters. *Annu. Res. Rev. Biol.*, vol. 37, no. 12, pp. 94–104, 2022.
18. R. A. McPherson and M. R. Pincus. *Henry's clinical diagnosis and management by laboratory methods E-book*. Elsevier Health Sciences, 2021.
19. R. Chauhan and V. C. Srivastava. A suitable combination of electrodes for simultaneous reduction of nitrates and oxidation of ammonium ions in an explosive industry wastewater. *Ind. Eng. Chem. Res.*, vol. 60, no. 15, pp. 5482–5493, 2021.
20. T. N. Rao.Validation of analytical methods. *Calibration Valid. Anal. methods—a Sampl. Curr. approaches*, pp. 131–141, 2018.
21. D. L. Church, L. Cerutti, A. Gürtler, T. Griener, A. Zelazny, and S. Emler. Microbial Identification using 16S-rRNA Gene Analysis. *Clin. Microbiol. Rev.*, vol. 33, no. 4, pp. 10–1128, 2020.
22. N. Gupta. DNA extraction and polymerase chain reaction. *J. Cytol.*, vol. 36, no. 2, pp. 116–117, 2019.
23. [S. Challa and N. R. R. Neelapu. Phylogenetic trees: applications, construction, and assessment. *Essentials Bioinformatics, Vol. III Silico Life Sci. Agric.*, pp. 167–192, 2019.
24. O. B. Tashyrev, I. B. Sioma, G. O. Tashyreva, and V. M. Hovorukha, "Bromothymol Blue as the Universal Indicator for Determining the Stereometric Allocation of pH and Eh in the Medium in Heterophase Microorganisms Cultivation," *Microbiol J*, vol. 2, no. 81, pp. 14–24, 2019.
25. J. C. Ibarra and A. Olivares-Pérez. New holographic recording material: bromothymol blue dye with rosin. *Opt. Mater. (Amst).*, vol. 20, no. 1, pp. 73–80, 2002.
26. L. Jia *et al.* A colour indicator film based on bromothymol blue/poly-L-lactic acid/polyvinylpyrrolidone for detecting bacteria. *Packag. Technol. Sci.*, vol. 36, no. 7, pp. 549–556, 2023.
27. H. R. M. Dos Santos, C. S. Argolo, R. C. Argôlo-Filho, and L. L. Loguercio. A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. *BMC Microbiol.*, vol. 19, pp. 1–14, 2019.
28. D. Liestianty, M. Amin, S. Muliadi, and S. Sundari . Profile of 16sRNA DNA Barcode on

Basillus Licheniformis Indigenous West Halmahera Island . *Proc. B.*, 2018.
29. S. Atashpaz *et al.* A robust universal method for extraction of genomic DNA from bacterial species. *Microbiology*, vol. 79, pp. 538–542, 2010.
30. L. Neumann. Molecular detection of pathogens in southern bluefin tuna. University of Tasmania, 2017.
31. R. Afrianti, E. S. Wardi, A. H. Putri, and S. Suryani.Barcode DNA Tanaman Mengkudu (*Morinda citrifolia* L) Berdasarkan Gen ITS (Internal Transcribed Spacer). *J. Katalisator*, vol. 8, no. 1, pp. 123–136, 2023.
32. A. I. Bhat, G. P. Rao, A. I. Bhat, and G. P. Rao. Polymerase Chain Reaction DNA concentration between 10 to 100 ng/µl. Springer, 2020.
33. M. A. Davidescu, M. Ciorpac, and S. Creanga. Highly effective method of DNA extraction from blood: a first step for analysis of genetic diversity of indigenous cattle breeds. 2021.
34. B. M. Crossley *et al.* Guidelines for Sanger sequencing and molecular assay monitoring. *J. Vet. Diagnostic Investig.*, vol. 32, no. 6, pp. 767–775, 2020.

[1] H. Amrianah, D. Pratiwi, and M. R. Hamal, "THE IMPACT OF THE EXISTENCE OF FISH AUCTION PLACE (TPI) ON INFRASTRUCTURE DEVELOPMENT AND SOCIO-ECONOMIC CONDITIONS OF COMMUNITIES IN SUMPANG BINANGAE VILLAGE, BARRU DISTRICT," *Meraja J.*, vol. 6, no. 2, pp. 153–164, 2023.
[2] M. I. K. Jauhari, A. Suherman, and I. Triarso, "Strategies for the development of the Bajomulyo Coastal Fishing Port, Pati, Central Java," *Depik*, vol. 10, no. 3, pp. 293–304, 2021.
[3] T. Murwati, "Kajian Pengaruh Aktivitas Pelabuhan Perikanan terhadap Aspek Kualitas Air Sungai Juwana dan Persepsi Masyarakat (Studi Kasus di Pelabuhan Perikanan Pantai (PPP) Bajomulyo, Kecamatan Juwana, Kabupaten Pati)." Magister Ilmu Lingkungan, 2010.
[4] U. Muawanah *et al.*, "Review of national laws and regulation in Indonesia in relation to an ecosystem approach to fisheries management," *Mar. Policy*, vol. 91, pp. 150–160, 2018.
[5] V. C. Yolanda and A. P. Heriyanti, "Wastewater Quality Characteristics Test in Domestic Wastewater Treatment Plant Dinas Lingkungan Hidup Kota Semarang," *Indones. J. Earth Hum.*, vol. 1, no. 1, pp. 44–52, 2024.
[6] H. Hendrayana, P. Raharjo, and S. R. Samudra, "Komposisi Nitrat, Nitrit, Amonium dan Fosfat di Perairan Kabupaten Tegal," *J. Mar. Res.*, vol. 11, no. 2, pp. 277–283, 2022.
[7] W. Lin *et al.*, "A review of the emerging risks of acute ammonia nitrogen toxicity to aquatic decapod crustaceans," *Water*, vol. 15, no. 1, p. 27, 2022.
[8] A. Amin, A. T. R. Naik, M. Azhar, and H. Nayak, "Bioremediation of different waste waters-a review," *Cont. J. Fish. Aquat. Sci.*, vol. 7, no. 2, p. 7, 2013.
[9] M. Divya, S. Aanand, A. Srinivasan, and B. Ahilan, "Bioremediation–an eco-friendly tool for effluent treatment: a review," *Int. J. Appl. Res.*, vol. 1, no. 12, pp. 530–537, 2015.
[10] B. Sheela and S. K. K. Beebi, "``Bioremediation of ammonia from polluted waste waters: a review'','' *Am. J. Microbiol. Res.*, vol. 26, p. 201, 2014.
[11] D. Zhao *et al.*, "Biodegradation of Fluorochloridone in the Qinghai Plateau by Streptomyces Parvus FG-2: Performance, Pathway, Degradative Enzyme, Genetic Background, and Risk Assessment," *Pathway, Degrad. Enzym. Genet. Background, Risk*

Assess..
[12] A. A. Ail, A. A. Al-Hussieny, and A. S. Taha, "The Role of Filamentous Bacteria Streptomyces sp. in Reduction of Some Nutrients Concentrations in AL-Restomia Waste water Treatment Plant, Baghdad-Iraq," *Baghdad Sci. J.*, vol. 12, no. 3, pp. 445–452, 2015.
[13] S. Mooralitharan *et al.*, "Chemical Oxygen Demand and Ammonia Nitrogen Mycoremediation from Domestic Wastewater Effluent using Ganoderma lucidum in a Batch System.," 2020.
[14] T. He *et al.*, "New insight into the nitrogen removal capacity and mechanism of Streptomyces mediolani EM-B2," *Bioresour. Technol.*, vol. 348, p. 126819, 2022.
[15] N. Thanh-Nho, L. T. Anh-Dao, L. T. Huynh-Mai, and N. Cong-Hau, "Interfering ions and controlling their effects on the direct analysis of total dissolved ammonia nitrogen in aquacultural wastewater using molecular absorption spectrophotometry," in *AIP Conference Proceedings*, AIP Publishing, 2022.
[16] K. Velkushanova *et al.*, "Laboratory procedures and methods for characterisation of faecal sludge," *Methods Faecal Sludge Anal.*, 2021.
[17] S. Isworo, P. S. Oetari, D. Prabowo, and H. Cerlyawati, "Bioremediation Tofu Liquid Waste Based on Chemical Oxygen Demand (COD) Parameters," *Annu. Res. Rev. Biol.*, vol. 37, no. 12, pp. 94–104, 2022.
[18] R. A. McPherson and M. R. Pincus, *Henry's clinical diagnosis and management by laboratory methods E-book*. Elsevier Health Sciences, 2021.
[19] R. Chauhan and V. C. Srivastava, "A suitable combination of electrodes for simultaneous reduction of nitrates and oxidation of ammonium ions in an explosive industry wastewater," *Ind. Eng. Chem. Res.*, vol. 60, no. 15, pp. 5482–5493, 2021.
[20] T. N. Rao, "Validation of analytical methods," *Calibration Valid. Anal. methods—a Sampl. Curr. approaches*, pp. 131–141, 2018.
[21] D. L. Church, L. Cerutti, A. Gürtler, T. Griener, A. Zelazny, and S. Emler, "Microbial Identification using 16S-rRNA Gene Analysis," *Clin. Microbiol. Rev.*, vol. 33, no. 4, pp. 10–1128, 2020.
[22] N. Gupta, "DNA extraction and polymerase chain reaction," *J. Cytol.*, vol. 36, no. 2, pp. 116–117, 2019.
[23] S. Challa and N. R. R. Neelapu, "Phylogenetic trees: applications, construction, and assessment," *Essentials Bioinformatics, Vol. III Silico Life Sci. Agric.*, pp. 167–192, 2019.
[24] O. B. Tashyrev, I. B. Sioma, G. O. Tashyreva, and V. M. Hovorukha, "Bromothymol Blue as the Universal Indicator for Determining the Stereometric Allocation of pH and Eh in the Medium in Heterophase Microorganisms Cultivation," *Microbiol J*, vol. 2, no. 81, pp. 14–24, 2019.
[25] J. C. Ibarra and A. Olivares-Pérez, "New holographic recording material: bromothymol blue dye with rosin," *Opt. Mater. (Amst).*, vol. 20, no. 1, pp. 73–80, 2002.
[26] L. Jia *et al.*, "A colour indicator film based on bromothymol blue/poly-L-lactic acid/polyvinylpyrrolidone for detecting bacteria," *Packag. Technol. Sci.*, vol. 36, no. 7, pp. 549–556, 2023.
[27] H. R. M. Dos Santos, C. S. Argolo, R. C. Argôlo-Filho, and L. L. Loguercio, "A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information," *BMC Microbiol.*, vol. 19, pp. 1–14, 2019.
[28] D. Liestianty, M. Amin, S. Muliadi, and S. Sundari, "Profile of 16sRNA DNA Barcode on Basillus Licheniformis Indigenous West Halmahera Island," *Proc. B.*, 2018.
[29] S. Atashpaz *et al.*, "A robust universal method for extraction of genomic DNA from bacterial species," *Microbiology*, vol. 79, pp. 538–542, 2010.
[30] L. Neumann, "Molecular detection of pathogens in southern bluefin tuna." University of

Tasmania, 2017.
[31] R. Afrianti, E. S. Wardi, A. H. Putri, and S. Suryani, "Barcode DNA Tanaman Mengkudu (Morinda citrifolia L) Berdasarkan Gen ITS (Internal Transcribed Spacer)," *J. Katalisator*, vol. 8, no. 1, pp. 123–136, 2023.
[32] A. I. Bhat, G. P. Rao, A. I. Bhat, and G. P. Rao, "Polymerase Chain Reaction DNA concentration between 10 to 100 ng/µl." Springer, 2020.
[33] M. A. Davidescu, M. Ciorpac, and S. Creanga, "Highly effective method of DNA extraction from blood: a first step for analysis of genetic diversity of indigenous cattle breeds.," 2021.
[34] B. M. Crossley *et al.*, "Guidelines for Sanger sequencing and molecular assay monitoring," *J. Vet. Diagnostic Investig.*, vol. 32, no. 6, pp. 767–775, 2020.