



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

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

## **Article Information**

DOI: <https://doi.org/10.9734/mrji/2024/v34i81475>

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/121132>

**Original Research Article**

**Received: 05/06/2024**

**Accepted: 10/08/2024**

**Published: 13/08/2024**

## **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.

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**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.*

## 1. 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<sub>3</sub>), 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<sub>3</sub>) and nitrite (NO<sub>2</sub><sup>-</sup>) to nitrate (NO<sub>3</sub><sup>-</sup>) 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 °C, 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 (NH<sub>3</sub>) 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.

## 2. 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.

### 2.1 Instruments and Materials

#### 2.1.1 Ammonia (NH<sub>3</sub>) 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<sub>6</sub>H<sub>5</sub>OH), 0.5% sodium nitroprusda (C<sub>5</sub>FeNa<sub>2</sub>O), alkaline citrate solution (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub>), 5% sodium hypochlorite (NaClO), and distilled water (aquadest).

#### 2.1.2 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.

### 2.2 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<sub>2</sub>·6H<sub>2</sub>O (10.8 grams), MgSO<sub>4</sub>·7H<sub>2</sub>O (5.4 grams), CaCl<sub>2</sub>·2H<sub>2</sub>O (1 gram), distilled water, PbNO<sub>3</sub> 0.1 ppm, Pb(CHCOO)<sub>2</sub> 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].

### 2.3 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 °C, oven, 25 µl PCR Master Mix, 0.05 U/mL Taq DNA polymerase; 0.4 mM each dNTP; 4 mM MgCl<sub>2</sub>, 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<sub>2</sub>O), 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,  $\alpha$ -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.

## 2.4 Microbial Isolation Media Preparation

### 2.4.1 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

### 2.4.2 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.

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

### 2.5.1 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  $\mu$ l 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  $\mu$ l of aquabides and 50  $\mu$ l 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.

### 2.5.2 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 °C for 3 minutes, followed by 30 cycles (denaturation at 95 °C for 1 minute, annealing at 55 °C for 1 minute, and extension at 72 °C for 1 minute), then extension at 72 °C for 7 minutes, and lastly 4 °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.

### 2.5.3 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.

### 2.6 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.

### 2.7 Sekuensing DNA

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

- (a) 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 ddH<sub>2</sub>O 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)

### 2.8 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 contig 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.

## 3. RESULTS AND DISCUSSION

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

**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.*

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.

### 3.1 Bacterial Screening

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

Based on the color change speed test using the Bromthymol 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 (NH<sub>3</sub>) 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 thymol 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 bromthymol blue in Zobell 2216 E liquid media enhanced with fish auction waste, as shown Fig. 1.

### 3.2 Ability of Selected Bacteria in the Bioremediation

Data from COD and Ammonia Parameter Measurement findings based on time periods are as follows Table 2.

Figs. 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. 1. Screening of the selected and best bacterial strains

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%

Table 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%

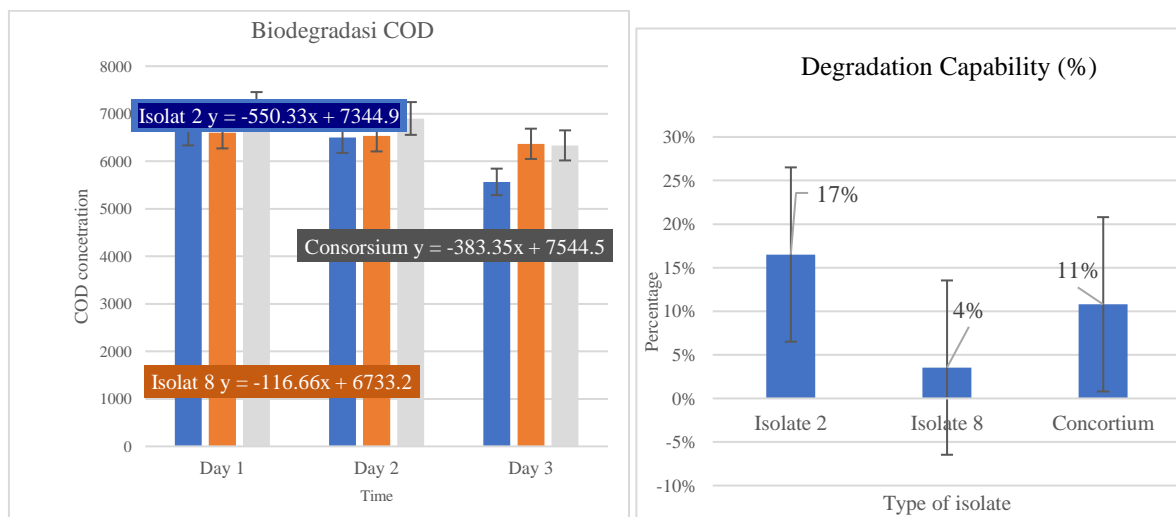
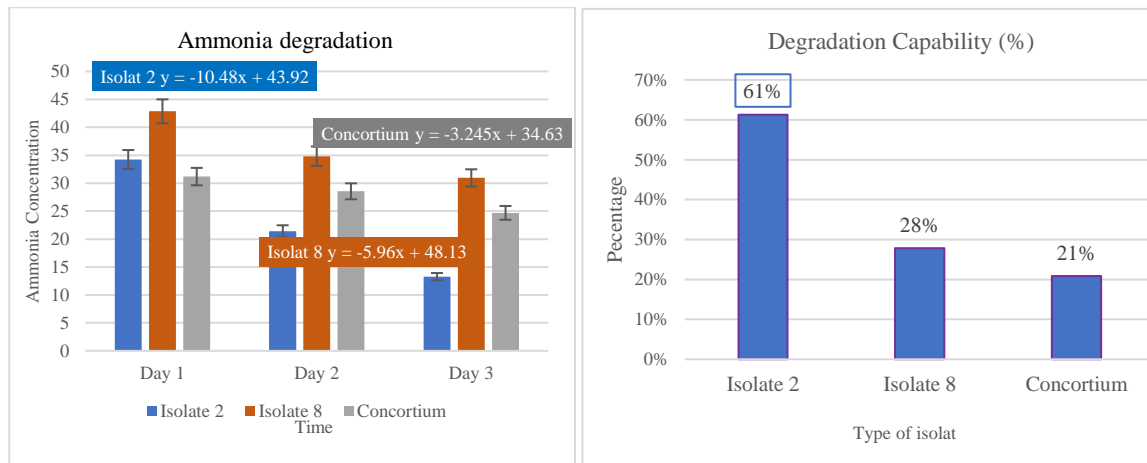


Fig. 2. Data of analysis of COD degradation by selected bacteria



**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  $y = -5.96x + 48.13$ , 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.

### 3.3 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.

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

**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)			
<ol style="list-style-type: none"> <li>1. Genomic DNA extraction with Quick-DNA Fungal/Bacterial Miniprep Kit</li> <li>2. PCR amplification with (2x) MyTaq HS Red Mix</li> <li>3. Bi-directional Sequencing</li> </ol>				
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



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].

### 3.4 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.

Base on Fig 4. Gel Photo – PCR Products menunjukan 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.

### 3.5 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 Fig.5.

### 3.6 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.

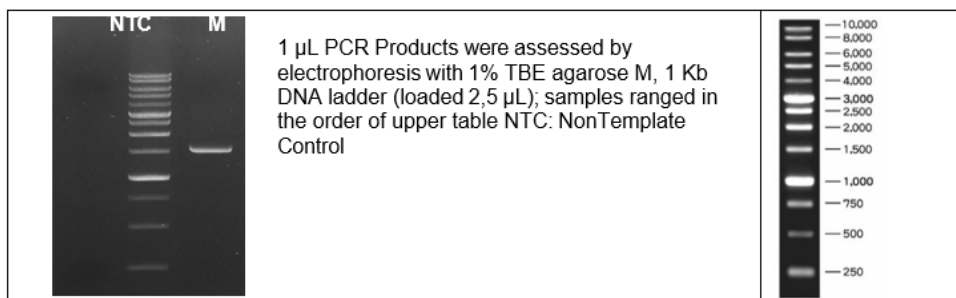


Fig. 4. Gel photo – PCR products

Table 5. Top10 hit blast results against NCBI database

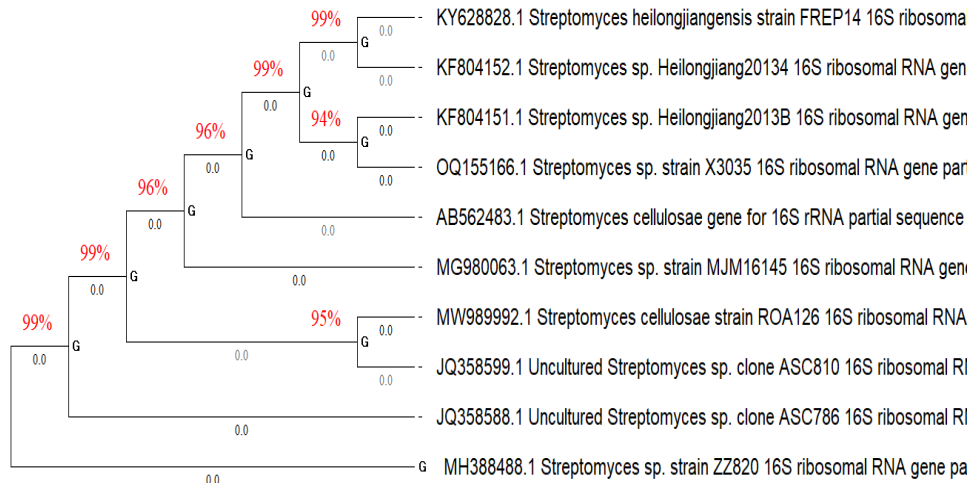
Description	Scientific Name	Per. ident
1. <i>Streptomyces heilongjiangensis</i> strain FREP14 16S ribosomal RNA gene, partial sequence	<i>Streptomyces heilongjiangensis</i>	99.86
2. <i>Streptomyces</i> sp. Heilongjiang20134 16S ribosomal RNA gene, partial sequence	<i>Streptomyces</i> sp. <i>Heilongjiang20134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

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

Fig. 5. Sequence Assembly Result – PCR Products

Table 6. Sequencing results using forward and reverse primers

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



**Fig. 6. Phylogenetic tree analysis**

The results of sequencing using forward and reverse primers to determine the bacterial nucleotide base sequence are as follows Table 6.

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

### 3.7 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.

## 4. 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 heilongjiangensis* strain FREP 14.

### DATA AVAILABILITY

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

### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that no generative AI tools, such as Large Language Models or text-to-image generators, were used during the creation or editing of the manuscript.

The authors utilized the MEGA program version 5.03 with the Neighbor-Joining Tree statistical approach, with a p-distances model of 1000 bootstrap levels, which aimed to estimate the phylogenetic tree to determine the bacterial kinship detected by molecular biology.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Amrianah H, Pratiwi D, Hamal MR. 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.* 2023;6(2):153–164.
2. Jauhari MIK, Suherman A, Triarso I. Strategies for the development of the Bajomulyo Coastal Fishing Port, Pati, Central Java. *Depik.* 2021;10(3):293–304.
3. Murwati T, 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. Muawanah U. Review of national laws and regulation in Indonesia in relation to an ecosystem approach to fisheries management. *Mar. Policy.* 2018;91:150–160.
5. Yolanda VC, Heriyanti AP. Wastewater quality characteristics test in domestic wastewater treatment plant dinas lingkungan hidup kota semarang. *Indones. J. Earth Hum.* 2024;1(1):44–52.
6. Hendrayana H, Raharjo P, Samudra SR. Komposisi nitrat, nitrit, amonium dan fosfat di perairan kabupaten tegal. *J. Mar. Res.* 2022;11(2):277–283.
7. Lin W. A review of the emerging risks of acute ammonia nitrogen toxicity to aquatic decapod crustaceans. *Water.* 2022;15(1):27.
8. Amin A, Naik ATR, Azhar M, Nayak H. Bioremediation of different waste waters-a review. *Cont. J. Fish. Aquat. Sci.* 2013;7(2):7.
9. Divya M, Aanand S, Srinivasan A, Ahilan B. Bioremediation—an eco-friendly tool for effluent treatment: A review. *Int. J. Appl. Res.* 2015;1(12):530–537.
10. Sheela B, Beebi SKK. Bioremediation of ammonia from polluted waste waters: A review. *Am. J. Microbiol. Res.* 2014;26:201.
11. Zhao D. 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. Ail AA, Al-Hussieny AA, Taha AS. 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.* 2015;12(3):445–452.
13. Mooraltharan S. Chemical oxygen demand and ammonia nitrogen mycoremediation from domestic wastewater effluent using ganoderma lucidum in a Batch System; 2020.
14. He T. New insight into the nitrogen removal capacity and mechanism of *Streptomyces mediolani* EM-B2. *Bioresour. Technol.* 2022;348:126819.
15. Thanh-Nho N, Anh-Dao LT, Huynh-Mai LT, Cong-Hau N. 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. Velkushanova K. Laboratory procedures and methods for characterisation of faecal sludge. *Methods Faecal Sludge Anal*; 2021.
17. Isworo S, Oetari PS, Prabowo D, Cerlyawati H. Bioremediation tofu liquid waste based on Chemical Oxygen Demand (COD) Parameters. *Annu. Res. Rev. Biol.* 2022;37(12):94–104.
18. McPherson RA, Pincus MR. Henry's clinical diagnosis and management by laboratory methods E-book. Elsevier Health Sciences; 2021.
19. Chauhan R, Srivastava VC. A suitable combination of electrodes for simultaneous reduction of nitrates and oxidation of ammonium ions in an explosive industry wastewater. *Ind. Eng. Chem. Res.* 2021;60(15):5482–5493.
20. Rao TN. Validation of analytical methods. Calibration Valid. *Anal. methods—A Sampl. Curr. Approaches.* 2018;131–141.
21. Church DL, Cerutti L, Gürtler A, Griener T, Zelazny A, Emler S. Microbial Identification using 16S-rRNA gene analysis. *Clin. microbiol. Rev.* 2020;33(4):10–1128.
22. Gupta N. DNA extraction and polymerase chain reaction. *J. Cytol.* 2019;36(2):116–117.
23. Challa S, Neelapu NRR. Phylogenetic trees: Applications, construction, and assessment. *Essentials Bioinformatics, Silico Life Sci. Agric.* 2019;3:167–192.

24. Tashyrev OB, Sioma IB, Tashyreva GO, Hovorukha VM. Bromothymol blue as the universal indicator for determining the stereometric allocation of pH and Eh in the medium in heterophase microorganisms cultivation," Microbiol J. 2019; 2(81):14–24.
25. Ibarra JC, A. Olivares-Pérez. New holographic recording material: Bromothymol blue dye with rosin. Opt. Mater. (Amst). 2000;20(1):73–80.
26. Jia L. A colour indicator film based on bromothymol blue/poly-L-lactic acid/polyvinylpyrrolidone for detecting bacteria. Packag. Technol. Sci. 2023;36(7):549–556.
27. Dos Santos HRM, Argolo CS, Argôlo-Filho RC, Loguercio LL. A 16S rDNA PCR-based theoretical to actual delta approach on culturable mock communities revealed severe losses of diversity information. BMC Microbiol. 2019;19:1–14.
28. Liestianty D, Amin M, Muliadi S, Sundari S. Profile of 16sRNA DNA Barcode on Basillus Licheniformis Indigenous West Halmahera Island . Proc. B; 2018.
29. Atashpaz S. A robust universal method for extraction of genomic DNA from bacterial species. Microbiology. 2010;79:538–542, 2010.
30. Neumann L. Molecular detection of pathogens in southern bluefin tuna. University of Tasmania; 2017.
31. Afrianti R, Wardi ES, Putri AH, Suryani S. Barcode DNA tanaman mengkudu (*Morinda citrifolia* L) Berdasarkan Gen ITS (Internal Transcribed Spacer). J. Katalisator. 2023;8(1):123–136.
32. Bhat AI, Rao GP, Bhat AI, Rao GP. Polymerase chain reaction DNA concentration between 10 to 100 ng/μl. Springer; 2020.
33. Davidescu MA, Ciorpac M, Creanga S. Highly effective method of DNA extraction from blood: a first step for analysis of genetic diversity of indigenous cattle breeds; 2021.
34. Crossley BM. Guidelines for sanger sequencing and molecular assay monitoring. J. Vet. Diagnostic Investig. 2020;32(6):767–775.

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