The Bioremediation Process Of Liquid Waste from A Fish Auction Place is Based on The Parameters of COD (Chemical Oxygen Demand) And Ammonia (NH_3)

ABSTRACT

The Fish Auction Place Bajomulyo Unit II, Juwana - Pati is confronted with significant sanitation issues as a result of inadequate treatment of liquid waste, leading to elevated levels of COD (Chemical Oxygen Demand) and Ammonia (NH3), In order to address this issue, it is imperative to implement effective alternative management technology, particularly bioremediation technology that harnesses indigenous microorganisms. The objective of the research is to conduct a screening procedure to identify the most efficient bacteria, assess the degradation capabilities of the chosen bacteria, define the most ideal bacteria, and do phylogenetic studies. The bacterial screening technique entails utilising the Bromo Thymol Blue indicator to identify the existence of bacteria by visually observing alterations in colour. Atomic Absorption Spectroscopy methods are utilised for conducting damage tests. Identification is accomplished by employing morphology, biochemistry, and molecular genetic tests. Results: Laboratory experiments showed that single isolate No. 2 (not consortium) had the highest capacity to degrade COD and ammonia concentration. Individual and consortium degradation tests were performed. This decrease in value shows that Isolate 2 has the highest level of potential. The effective COD parameter was reduced from 6,666.7 mg/l to 5,566.7 mg/l, while the ammonia level was reduced from 34.2 mg/l to 13.3 mg/l. Bacterial isolate 2 was determined to be Streptomyces heilongjiangensis, with a genetic composition similarity of 99%. Conclusion:, bacteria indigenous to liquid waste found at fish auction sites have the capability and effectiveness to break down liquid waste containing Chemical Oxygen Demand (COD) and Ammonia (NH3) criteria.

***Keywords**: Bioremediation, Chemical Oxygen Demand (COD), Ammonia (NH3), Fish auction place, Molecular biology, Streptomyces heilongjiangensis strain FREP14.*

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INTRODUCTION

Fish auction venues play a crucial role in facilitating the buying and selling transactions conducted by fishermen, additionally, they can also stimulate economic development and enhance the well-being of fishermen. Fish auction places also produce various kinds of liquid waste, especially those from washing fish, cutting fish, and household operations at fish auction places. (Amrianah, Pratiwi and Hamal, 2023). Fishermen's sea catches taken to the Fish Auction Place will undergo a cleansing procedure prior to being auctioned. Nevertheless, wastewater is frequently released directly into aquatic ecosystems. This situation creates an unpleasant odor around the fish auction place, and increases the possibility of disease occurring in residents around the fish auction location. (Amrianah, Pratiwi and Hamal, 2023).

The Bajomulyo fish auction place in Juwana, Pati Regency not only provides benefits to the community's economy, but also has negative impacts, especially in the form of waste which

can disrupt the ecological balance of the ecosystem. Considering the large expansion of catch production activities at the Bajomulyo Beach Fishing Port, it is necessary to improve sanitation facility services through the implementation of waste water treatment plants (Jauhari, Suherman and Triarso, 2021). The fish auction place in Bajomulyo generates both liquid and solid refuse. The ecological balance can be disrupted by the improper management of liquid waste, which is a result of the absence of waste processing installations. However, solid refuse can be processed into animal feed as a solution (Murwati, 2010)

Based on Law Number 32 of 2009 concerning Environmental Management and Central Java Province Regional Regulation Number 1 of 2013 concerning Management of Central Java Province Fishing Ports, this is a decentralized regulation from the federal government to the regional government (Muawanah *et al.*, 2018) These rules were created primarily to ensure that waste which is a by-product of various sectors does not damage the environment when dumped into it. Referring to the Minister of Environment Regulation Number 68 of 2016 concerning Domestic Wastewater Quality Standards, an inspection of the wastewater inlet at the Bajomulyo Village Fish Auction Place (TPI) was carried out to determine the level of pollutant load caused. Liquid waste produced from fish auctions results in an increase in Chemical Oxygen Demand (COD) levels and ammonia content.(Yolanda and Heriyanti, 2024). These waters are believed to be influenced by the preponderance of nutrient elements due to the presence of anthropological activities at the fish auction site. The quality standard threshold for nitrate, nitrite, ammonia, and phosphate in Munjung Agung waters is not met, as per Minister of Environment and Forestry Decree No. 51 of 2004. Ammonia concentrations range from 0.21 to 0.33 mg/l, phosphate concentrations from 0.12 to 0.22 mg/l, and nitrate concentrations from 1.21-3.80 mg/l. The moderate-heavily polluted category represents the determination value for water pollution, which ranges from -26 to -32.(Hendrayana, Raharjo and Samudra, 2022). The nitrogen cycle, which is derived from the decomposition of organic matter, is the source of TAN. The aquatic environment can be significantly impacted by uncontrolled TAN concentrations, as TAN toxicity can abruptly increase in response to fluctuations in water quality factors, including pH, temperature, ion charge, salinity, and dissolved oxygen (DO). The toxic form of TAN, ammonia (NH3), can impede the growth of aquatic organisms and even lead to their mortality if it surpasses the tolerance threshold (Lin *et al.*, 2022)

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(Amin *et al.*, 2013) Indigenous bacteria have the ability to decrease organic pollutants (COD) and ammonia (NH3) in the aquatic environment. (Divya *et al.*, 2015) Bioremediation is the biological processing of liquid waste generated from fish auction activities. Bioremediation is a method that use living organisms to convert trash into compounds that may be safely disposed of in the environment. Petrophilic bacteria has the ability to break down hydrocarbon compounds, including ammonia, to fulfil their metabolic and reproductive requirements. The nitrification process microbiologically degrades the ammonia content in waste processing. Nitrification is a sequence of biological reactions that involves the conversion of ammonia to simpler nitrates by nitrifying bacteria (Sheela and Beebi, 2014). Nitrifying bacteria

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are capable of aerobically oxidising ammonia and nitrite to nitrate. 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.(Zhao *et al.*, no date). Streptomycessp memeliki kemampuan biologis damalam menurukan konsentrasi amonia 1358 mg/L - 140 mg/L. Dan COD menurun dari 427,263 - 82 mg/L (Ail, Al-Hussieny and Taha, 2015) (Ail, Al-Hussieny and Taha, 2015). 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 (Mooralitharan *et al.*, 2020). The development of waste degradation technology (bioremediation) is an effort to reduce pollutants from polluted environments and convert toxic compounds into non-toxic compounds through the use of native bacteria. Streptomyces mediolani EM-B2, an actinomycete, exhibits exceptional nitrogen removal capabilities. The maximal removal rates of ammonium, nitrate, and nitrite were 3.46, 1.71, and 1.72 mg/L·h, respectively. The enzyme for HN-AD was efficacious in its detection. The nitrogen balance indicated that aerobic denitrification had resulted in the loss of over 37% of the initial ammonia to nitrogen gas. This demonstrates that Streptomyces mediolani EM-B2 is capable of effectively reducing ammonia and organic materials (He *et al.*, 2022))

Based on this background, it is necessary to carry out research on bioremediation of liquid waste from Fish Auction Places (TPI) based on COD and ammonia parameters using indigenous bacteria.

RESEARCH METHODS

This research employs a quantitative, true-experimental approach to identify and evaluate bacteria capable of breaking down liquid waste from fish auction sites. Additionally, it involves analysing the genomes of the selected bacteria with the highest degradation capacity.

Instruments and materials

Ammonia (NH3) test (Thanh-Nho *et al.*, 2022)

a) 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.
b) 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)

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COD test (Velkushanova *et al.*, 2021)

1. **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
2. 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

1. **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
2. **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) (Isworo *et al.*, 2022)

Molecular Genetic Analysis Test

a) **Instruments:** Microcentrifuge equipped with a cooler (Sorvall Fresco), incubator (Memmert); autoclave (Hirayama, Japan), laminar air flow cabinet (Esco); pHmeter (Eutech), digital camera (HP Photosmart R607), analytical balance (Scout and Acculab), deep freezer -20 oC (GEA), oven (Lab-line Instruments and WTB Binder); vortex mixer (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 (Mupid-ex Advance), UV transluminator (BDA Biometra TI 1), PCR Thermal Cycler (MJ Mini Biorad), 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 genomic DNA template 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 minispin microcentrifuge genomic DNA template (Eppendorf), and a glass beaker.

b) **Materials:** lysozyme (Sigma), sodium dodecyl sulfate/SDS (Sigma), proteinase-K (Usb), sodium chloride (Merck); Aquadest (Brataco), aquabidest (otsuka), DNAse and RNAse free aquabidest (ddH_2O), tris base (Merck), Etylene Diamine Tetra Acetic Acid/EDTA (Sigma); chloroform (Merck), isoamyl alcohol (Sigma), PCR master mix (Fermentas), Primer 16E1: GGG AGT AAA GTT AAT ACC TTT GCT C (Biotech) [4]; Primer 16E2: TTC CCG AAG GCA CAT TCT (Biotech) [4], Agarose ultrapure (Invitrogen), Loading Buffer, Ethidium bromide (Sentra BD); 1 kb plus DNA ladder (Invitrogen), Ehrlich's reagent, methyl red, potassium hydroxide, α-naphthol. Nutrient Broth/NB media solution (Pronadisa) pH 6.8 ± 0.2; Nutrient Agar/NA media (Difco) pH 6.8 ± 0.2; Lactose Monohydrate (Merck); Brilliant Green Lactose Bile Broth/BGLB 2% Media (Pronadisa) pH 7.2 ± 0.2; Eosin Methylene Blue/EMB Agar Media (Merck) Ph 7.3; Peptone (Difco); Methyl Red Voges Media- Proskauer/MRVP

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(Merck); Simmons Citrate Media (Difco); Tris Acetate Buffer EDTA/TAE, PVP 1%, Chloroform, I sopropanol, NaCl, Ethanol 100%, TE Buffer, Agarose, TAE1x Buffer, DNA Extraction, EtBr. Forward and reverse specific primer, Green Go Taq Master Mix

Microbial Culture Media Preparation

The media used in the isolation and identification stage are at least Zobell agar and at least Zobell broth, as follows. 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 gram), $CaCl_2.{}_2H_2O$ (1 gram), distilled water, $PbNO_3$ 0.1 ppm, $Pb(CHCOO)_2$ 0.1 ppm, MR-VP Broth, methylene blue staining, hydrogen peroxide 3% , 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)

Bacterial Isolation

a. **Method for Isolating Bacteria from Liquid Waste at Fish Auctions** (McPherson and Pincus, 2021)

1) The liquid waste sample is collected in a sample container, placed in a cool box, and transported to the laboratory for bacterial isolation. 2) A total of 1 ml of search waste sample from the fish auction site is mixed with 9 ml of sterile distilled water to a dilution of 10-1, then shaken/vortexed until homogeneous 3) 1 ml of the results of the 10^{-1} dilution was transferred into 9 ml of sterile distilled water to create a 10^{-2} dilution, and the sample was shaken/vortexed until homogeneous Step is continued until the dilution reaches 10^{-5}. 4) 35 µl of each dilution result was planted on Zobell 2216 E agar medium. 5) The isolation results were incubated at 35 ^{0}C for two 24-hour periods. 6) Growing colonies are identified by their morphology (shape, color, and texture). 7 The colonies that develop are purified using the streak plate method until pure or single colonies are obtained.

b. **Liquid waste degradation activity test method**

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

c. **Test for Ammonia and COD**

Testing the sample with a Portable UV 1800 Spectrophotometer, 190-1100 Nm: **1)** Determine the ammonia concentration.(Chauhan and Srivastava, 2021) 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-

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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, (Rao, 2018). 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 (Church *et al.*, 2020)(Gupta, 2019)

a. 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.

b. 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.

c. 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.

d. Purification of DNA amplification results

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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.

e. 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)

f. Phylogenetic Tree Construction (Challa and Neelapu, 2019)

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

Research Time: August 2023 to January 2024. Waste water samples were collected from the main drainage of the Silungonggo tributary at the Bajomulyo-Juwana Fish Auction Place in Pati Regency, Central Java. Sampling was taken out on July 15-16, 2023 at coordinates LS 6°42'13"S 111°09'18"E. The collected samples will then be used for screening procedures for selected bacteria that are most capable of degrading COD (Chemical Oxygen Demand) and Ammonia (NH3). Experimental (laboratory) research was carried out at the Environmental Health Laboratory at Dian Nuswantoro University, the genetic sciences laboratory and the Environmental Engineering laboratory at Diponegoro University.

1. Bacterial Screening

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 (Tashyrev *et al.*, 2019). 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

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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 (Ibarra and Olivares-Pérez, 2002). 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.(Jia *et al.*, 2023) 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). Figure 1 depicts the color change of bromtymol blue in Zobell 2216 E liquid media enhanced with fish auction waste, as shown :

Table 1. Screening results for microorganisms that degrade ammonia and COD

No Isolate	Discoloration			
2	+	+++	++++	+++++
3	+	+	++	++
6	+	+	++	++
8	+	++	++++	+++++

Figure 1. Screening of the selected and best bacterial strains

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.

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2. Ability of Selected Bacteria in the Bioremediation Process of Liquid Waste at Juwana Fish Auction Places

Measurement of COD and Ammonia Parameters

Table 2. COD parameter test results

Isolate	Day 1	Day 2	Day 3	%
Isolate 2	6666.67	6500	5566	17%
Isolate 8	6600	6533	6366.67	4%
Concortium	7100	6900	6333.3	11%

Tabel 3. Ammonia parameter testing results

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%

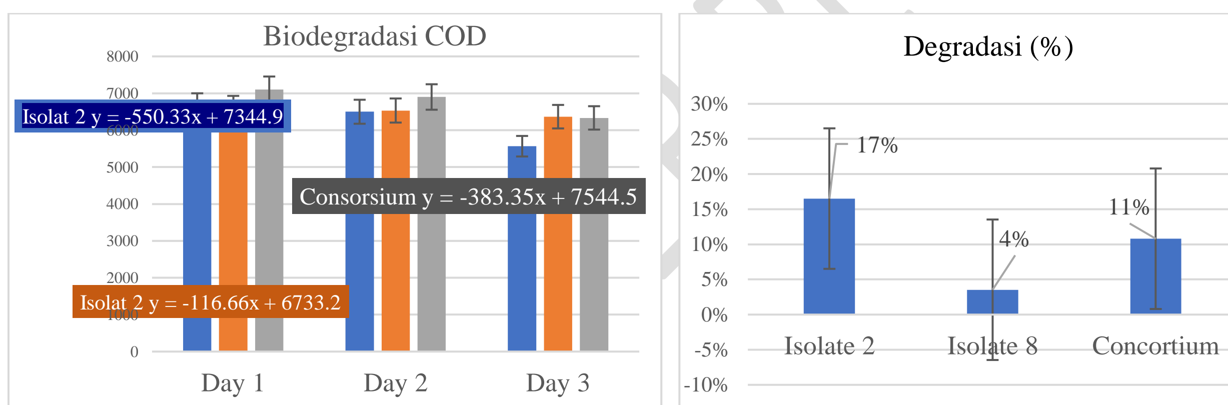

Figure 2. Data of analysis of COD degradation by selected bacteria

Based on Figure 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.

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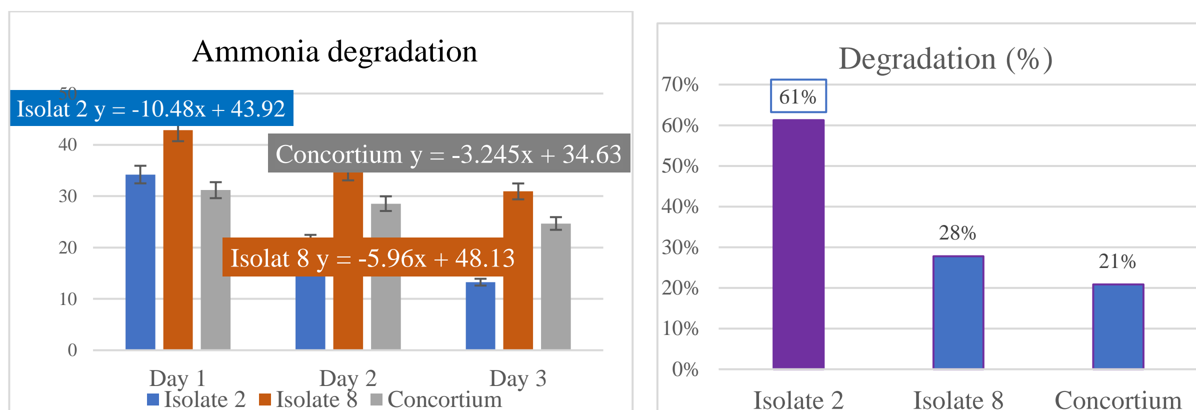

Figure 3. Data of analysis of Ammonia degradation by selected bacteria

According to Figure 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 bacteria that degrade Ammonia (NH3) and COD (Chemical Oxygen Demand)

Table 4. Information on the identification of selected bacteria using molecular genetics

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. (Dos Santos *et al.*, 2019). 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

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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.(Liestianty *et al.*, 2018). 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 (Atashpaz *et al.*, 2010). 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 (Neumann, 2017)(Afrianti *et al.*, 2023). 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:

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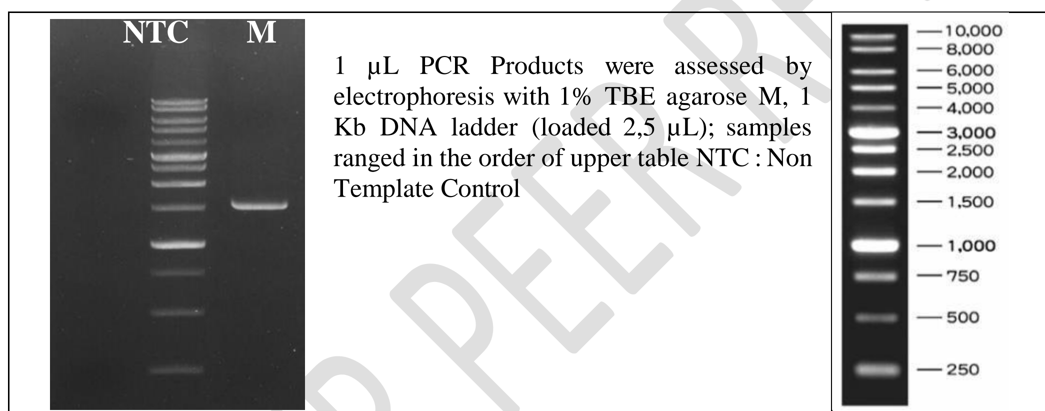
Based on the analysis results of Conc. (ng/µl) has a value of 13.8 ng/µl, indicating the concentration is 13.8 nanograms per microliter. a measure of the concentration of a substance in solution, measured in units of nanograms per microliter. Concentration (ng/µl): 13.8 ng/µl will be valid if the measurement 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.(Bhat *et al.*, 2020) 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) (Davidescu, Ciorpac and Creanga, 2021).

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

Figure 4. Gel Photo – PCR Products

Base on figure 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

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longer whole. (Crossley *et al.*, 2020) 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
```

Figure 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.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Streptomyces heilongjiangensis strain FREP14 16S ribosomal RNA gene, partial sequence	Streptomyces heilongjiangensis	2566	2566	100%	0.0	99.86%	1523	KY628828.1
Streptomyces sp. Heilongjiang20134 16S ribosomal RNA gene, partial sequence	Streptomyces sp. Heilongjiang20134	2566	2566	100%	0.0	99.86%	1523	KF804152.1
Streptomyces sp. Heilongjiang2013B 16S ribosomal RNA gene, partial sequence	Streptomyces sp. Heilongjiang2013B	2566	2566	100%	0.0	99.86%	1525	KF804151.1
Streptomyces cellulosae gene for 16S rRNA, partial sequence, strain: old-30-2-1	Streptomyces cellulosae	2566	2566	100%	0.0	99.86%	1434	AB562483.1
Streptomyces sp. strain ZZ820 16S ribosomal RNA gene, partial sequence	Streptomyces sp.	2560	2560	100%	0.0	99.79%	1514	MH388488.1
Streptomyces sp. strain MJM16145 16S ribosomal RNA gene, partial sequence	Streptomyces sp.	2560	2560	100%	0.0	99.79%	1471	MG980063.1
Streptomyces sp. strain X3035 16S ribosomal RNA gene, partial sequence	Streptomyces sp.	2560	2560	100%	0.0	99.79%	1416	OQ155166.1
Streptomyces cellulosae strain ROA126 16S ribosomal RNA gene, partial sequence	Streptomyces cellulosae	2560	2560	100%	0.0	99.79%	1435	MW989992.1
Uncultured Streptomyces sp. clone ASC810 16S ribosomal RNA gene, partial sequence	uncultured Streptomyces sp.	2560	2560	100%	0.0	99.79%	1521	JQ358599.1
Uncultured Streptomyces sp. clone ASC786 16S ribosomal RNA gene, partial sequence	uncultured Streptomyces sp.	2555	2555	100%	0.0	99.71%	1522	JQ358588.1

Figure 6. Top10 Hit BLAST Results Against NCBI Database

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

Table 5. 1 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

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

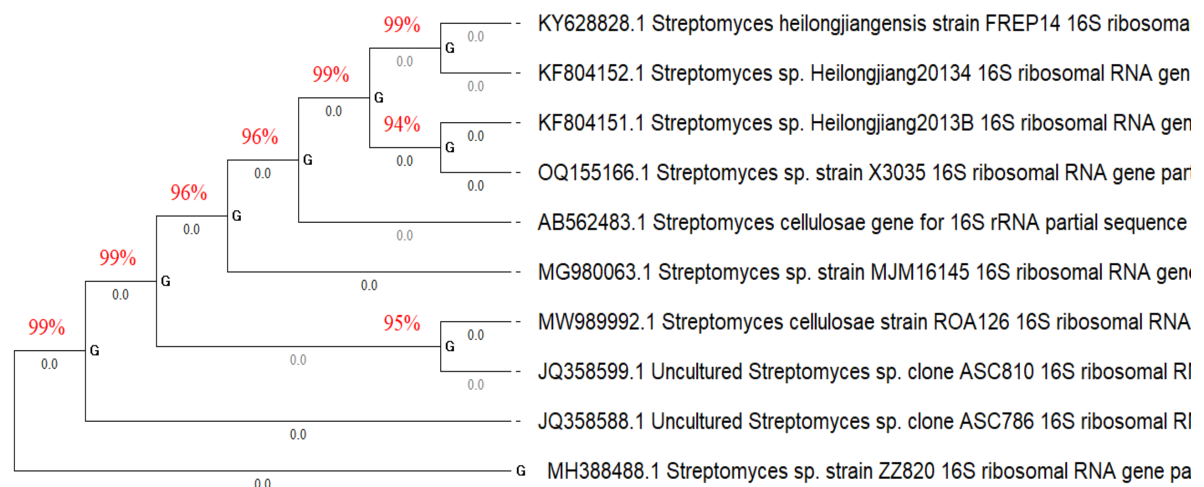

Figure 7 . Phylogenetic tree analysis

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

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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 applicabel

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