Annual Research & Review in Biology

Volume 37, Issue 12, Page 94-104, 2022; Article no.ARRB.95279
ISSN: 2347-565X, NLM ID: 101632869

Bioremediation Tofu Liquid Waste Based on Chemical Oxygen Demand (COD) Parameters

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2022/v37i1230561

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/95279

Original Research Article

Received: 25/10/2022
Accepted: 27/12/2022
Published: 29/12/2022

ABSTRACT

Background and Objective: The majority of liquid waste generated by the tofu industry in Semarang exceeds the recommended limit. This condition disturbs the ecology of the surrounding environment. so a solution is needed to manage it. The use of bacteria in bioremediation technology has been proven to reduce pollutants produced by the tofu industry. The purpose of this study was to identify specific bacteria and test their ability to bioremediate pollutants from tofu wastewater using the Chemical Oxygen Demand (COD) parameter.
Methodology: Methodology: The study was exploratory and experimental in nature, with the variable ability of degradative bacteria degrading tofu industrial wastewater based on COD parameters. Bacterial identification was performed using the 16S-rRNA Gene Analysis method, genomic DNA extraction with the Quick-DNA Bacterial Miniprep Kit, PCR amplification, and bi-directional sequencing.

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Results: The results of the identification of the selected bacteria show that they are molecules of the type nucleic acid with a query length of 1394. with a lineage report and a 93% phylogenetic tree as *Bacillus subtilis*, Bioremediation test shows that tofu waste can be degraded. The degradation percentage is 17%.
Conclusion: *Bacillus subtillis,* a bacterium that degrades tofu industrial wastewater, was found as an indigenous bacteria.

Keywords: Bioremediation; tofu liquid waste; Bacillus subtilis; COD parameters; indigenous bacteria.

1. INTRODUCTION

As a developing country, Indonesia has a variety of industries, both small and large. Small and medium industries, such as tofu production, are usually home-based. Small and medium-sized businesses have a positive impact on the people's economy [1], but they also have a negative impact, especially in the form of waste, which can disrupt the ecological balance of the environment [2]. Environmental protection regulations have been developed relating to various types of waste generated by various types of industries, namely Law No. 32 of 2009 concerning Environmental Management [3]. This regulation was created primarily to ensure that waste, a byproduct of various industries, does not harm the environment when discharged into bodies of wate [4].

Semarang is one of the cities with the fastest growth, particularly in Central Java, Indonesia; this situation has a very positive impact on the economic sector's development [5]. Among them is the emergence of various community-managed small-scale factories, such as the tofu and tempe industries, which are typical of small and medium-sized businesses in this region [6]. The tofu industry is typically run as a home-based business, with waste generated that is largely uncontrolled and dumped directly into the nearest body of water. Tofu production generates two types of waste: solid waste and liquid waste. Solid waste from the tofu manufacturing industry can be recycled as animal feed. Liquid waste, on the other hand, contains substances that can pollute the environment and clean water. Tofu industrial liquid waste pollutes the environment [7]. The majority of the quality of tofu industrial wastewater continues to exceed the recommended limit in Minister of Environment Regulation No. 5 of 2014 concerning wastewater quality standards for soybean (tofu and tempeh) processing companies [8]. The Environmental Service conducted an examination at the Tofu Small Industry Waste Management Installation in Jomblang Village, Semarang. COD values of 908.50 +/-11.0 mg/L were measured at the inlet [4]. To overcome the waste generated by the tofu industry, particularly the COD parameter, an alternative technology for tofu waste management is required. Bioremediation is an attempt to reduce pollutants in polluted environments and modify toxic compounds into non-toxic compounds. Many enzymatic, biochemical, and biotransformation processes use microorganisms as remediation agents in this process. Utilization of indigenous bacteria as pollutant degradation biological agents is advantageous because they are easily obtained and spread in nature, and indigenous bacteria will be more stable because they are bacteria that live at research sites, allowing for more controlled modification of indigenous bacteria [9]. The use of indigenous bacteria in bioremediation technology is the most efficient, cost-effective, and environmentally friendly way to address pollution issues, particularly those caused by the tofu industry. [10]. Bioremediation technology, which employs indigenous bacteria, is the most efficient, low-cost, and effective way to address pollution issues, particularly those caused by the tofu industry [11].

Based on the above background, it is necessary to conduct bioremediation research on tofu liquid waste based on cod parameters by utilizing indigenous bacteria in order to overcome the problem of organic matter pollution caused by contamination of the tofu industry in the city of Semarang.

2. METHODS

The bioremediation research was conducted exploratory and experimentally with the variable ability to degrade bacteria in tofu waste by measuring the rate at which the COD parameter decreased in the final research observations

2.1 Tools and Materials

The tools and materials used is: Water sampler, test tube, petri dish, winkler tube, ose

needle, bunsen, beaker glass, funnel, separating funnel, erlenmeyer, measuring cup, spatula, volume pipette, propipet, filter paper, hot plate, vortex, magnetic stirrer, autoclave, distillers, ovens, shakers, incubators, refrigerators, electric balances, analytical balances, desiccators, incubators and spectrophotometers [12]. Tofu waste samples, Zobell broth Medium, Bromothympol Blue, Microcentrifuges equipped with coolers (Sorvall Fresco), incubators (Memmert); autoclave (Hirayama, Japan), laminar air flow cabinet (Esco); pHmeter (Eutech), digital camera (HP Photosmart R607), analytical balance (Scout and Acculab), deep freezer -20^{o}C (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 dNTP each; 4 mM $MgCl_2$), 2 µL Primer 16E 1, 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 16E 1,2 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 glass beaker [12] 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 media solution. Broth/NB (Pronadisa) pH 6.8 ± 0.2; Nutrient Agar/NA media (Difco) pH 6.8 ± 0.2; Lactose Monohydrate (Merck); Media Brilliant Green Lactose Bile Broth/BGLB 2% (Pronadisa) pH 7.2 ± 0.2; Eosin Methylene Blue/EMB Agar Media (Merck) Ph 7.3; Peptone (Difco); Media Methyl Red Voges-Proskauer/MRVP (Merck); Simmons Citrate Media (Difco); Tris Acetate Buffer EDTA/TAE, 1% PVP, Chloroform, I sopropanol, NaCl, 100% Ethanol, TE Buffer, Agarose, TAE1x Buffer, Extracted DNA, EtBr. Forward and reverse specific primer, Green Go Taq Master Mix [12].

2.2 Data Collection Method

Culture Media : The media used in the isolation and identification stages are minimal Zobell agar and minimal Zobell broth as follows. The ingredients for 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), $CaCl_2.2H2O$ (1 gram), distilled water, 0.1 ppm $PbNO_3$, 0.1 ppm $Pb(CHCOO)_2$, MR-VP Broth, methylene blue staining, 3% hydrogen peroxide, naphtol solution (1 gram per 100 ml ethyl alcohol) and phenylenediamine solution (1 gram per 100 ml of distilled water), a minimum of yeast extract (0.25 grams) and a minimum of Bakto pectone (1 gram) (27).

2.3 Isolation of Bacteria

"Tofu waste sample is put into a sample bottle, and put into a cool box, and taken to the laboratory to isolate the bacteria, then 1 ml of Tofu waste sample is put into 9 ml of sterile distilled water, so that a 10^{-1} dilution is obtained, the sample is shaken/ vortex until homogeneous and so on to obtain a 10^{-2} dilution, sample. Divortex sample until homogeneous. Step number 6 was repeated until a 10^{-5} dilution was obtained. From each dilution, 35 µl was taken and then planted onto the surface of the Zobell 2216 E agar medium. The isolates were incubated at 32 $^{\circ}$C for 3x24 hours. Growing colonies were observed based on their morphology (shape, color and texture). Growing colonies were purified using the streak plates method to obtain pure colonies/single colonies" [13].

2.4 Identification of Bacteria based on 16S-rRNA Gene Analysis

"DNA Extraction Using the Chelex 100 Kit. Bacterial cells that had been grown for 24 hours were put into a 1.5 ml Eppendorf tube containing 100 µl of aquabides, then added 0.5% saponin and allowed to stand for 24 hours at 4 ^{0} C. The sample was centrifuged at 12,000 rpm for 10 minutes and then the supernatant was discarded. As much as 1 ml of Phospate Buffer Saline (PBS 1x) was added to the Eppendorf tube, then centrifuged again at 12,000 rpm for 15 minutes, the centrifuged supernatant was discarded, then 100 µl of aquabides and 50 µl of Chelex 100

were added to the tube. c) The sample is boiled for 10 minutes (the sample is vortexed in the first 5 minutes). Centrifuge again at 12,000 rpm for 10 minutes. The supernatant containing DNA was transferred into a new Eppendorf tube and ready for the DNA amplification process" [12].

Amplification of DNA 16s rDNA Polymerase Chain Reaction (PCR) Method: The temperature treatment used in the DNA amplification process is: initial denaturation at 95^0C for 3 minutes, then 30 cycles (denaturation at 95^0C for 1 minute, annealing at 95^0C for 1 minute, annealing at 550C for 1 minute and extension at 72^0C for 1 minute), then extension at 72^0C for 7 minutes (29) and finally 40^0 C. Universal primers for bacteria 27 F (5'-AGAGTTTTGATCMTGGCTCAG-3') and eubacteria specific primers 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were used for the 16S rDNA PCR (30). The materials used are the Promega kit (25 l), 27 F primer (2.5 l), 1492 R primer (2.5 l), DNA template (2.5 l), and aquabides (17.5 l), for a total volume of 50 l. These ingredients were combined in a 0.2 ml PCR tube.

Visualization of DNA Amplification Results: Visualization of DNA amplification results was carried out by electrophoresis by inserting 5 µl of PCR product into the wells of a 1% agarose gel. Preparation of 1% agarose gel was carried out by dissolving 1 gram of agarose in 100 ml of 1x TAE buffer solution, then heating using an oven until homogeneous (clear). As much as 5.33 µl of Ethidium Bromide was put into the gel solution, then shaken so that it was homogeneous. The gel solution is poured into the mold with the mold comb mounted in an upright position until it passes through the comb according to the desired thickness. The gel is left for a while to harden. The next step was that the gel was immersed in 1x TAE buffer solution, then the gel was electrophoresed with a voltage of 100 V for ± 30 minutes. The amplified DNA bands were observed using the Gel Documentation tool.

Purification of DNA Amplification Results: Purification was carried out to obtain pure DNA as a result of PCR 16S rDNA amplification. The method used is the conventional method. The PCR results were centrifuged at 12,000 rpm for 7 minutes. The supernatant is removed using a micropipette, making sure the DNA is completely pure (no primer is left behind. 50 µl of sterile aquabides is added to the DNA pellet, let stand for 5 minutes. The pure DNA results can be sequenced to determine the base sequence of the DNA.

Phylogenetic Tree Construction: Degradative bacteria that have successfully amplified their 16S rRNA gene can be seen for their kinship with other prokaryotes in the database based on their 16S-rRNA gene sequences. Sequences were carried out at the Dipenogoro University Integrated Laboratory. The partial sequences obtained will go through an editing process using the Bioedit program. After the contiq results of nucleotide sequences are obtained based on amplification with universals, the homology will be compared with other prokaryotes in the Gene Bank database. Cluster analysis was carried out using the database from the RDP (Ribosomal Database Project) website with the website (http://www.rdp.com). while making phylogenetic trees using the MEGA 5 program

Data Processing: In this study, data processing was carried out by preparing the data resulting from the degradation of selected bacteria on sterilized tofu waste. Bacterial degradation tests will be performed using the spectrophotometer method, in which bacteria isolated using zobell medium supplemented with Brom Thymol Blue (BTB) and selected are taken in 1 loop with a loop needle and then inserted into the sample at a predetermined concentration. The spectrophotometer test results are entered into the table and sorted by low concentrations to make it easier to read the research findings.

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1) The regression test was used to validate the results of the selected bacteria's ability test [14].

3. RESULTS AND DISCUSSION

3.1 Bacteria Identification Results

The selected bacteria's selection results are shown based on their ability to grow and change

color (BTB indicator) on media enriched with the following tofu waste.

Based on findings from the treatment of liquid zobell media, 5 bacteria strains were able to degrade tofu liquid waste using a color change indicator (BTB in liquid zobell media). Based on the speed of color change BTB indicator) of the 5 strains that were able to grow, bacterial strain no. 4 is the best bacteria.

Bacterial strain 4 has the fastest ability to change the BTB color indicator to yellow, Isolate 4 is the isolate that has the best speed in changing color, then isolate 4 is identified biochemically and molecular genetics, as shown below:

3.2 Morphology and Biochemistry

Table 2 shows the results of morphological and biochemical identification of bacteria.

Fig. 1. Selection of the best strain, strain 4

Table 1. The rate of color change in isolate observations on zobell media agar medium

Zobell Broth	Day 1	Day 2	Day 3	Day 4
Isolate 1		+	+	++
Isolate 2		+	++	++
Isolate 3		+	++	++
Isolate 4		++	+++	+++
Isolate 5		+	+	+

Table 2. Bacterial Morphological and Biochemical Analysis Results

Biochemical Test.	Bacterial Strain Isolate Code 4	Biochemical Test.	Bacterial Strain Isolate Code 4
Shape	Rod	Acid Fast	-
Grams	+	Tween 20 Hyd Tahusis	x
Motility	+	Acid Form ASS Medium	
Length 3>mm	-	Glucose	+
Position and Length of Spores	VX	Celibiose	+
Spore	+	Galactose	+
Growth on 50 ^{o}	+	Mannose	+
Growth on 37 ^{o}	+	Melibiose	+
Growth on 42 ^{o}	X	Rafinose	+
Growth on 42o	X	Sukrose	+
Growth with 10% NaCl	+	Xylose	-
Anaerob		**Acid Form Fenol Red Meduim**	
Aerobics	+	Glucose	X
ONPG	+	Celibiose	X
Utilization of citrate	+	Galactose	X

Biochemical Test.	Bacterial Strain Isolate Code 4	Biochemical Test.	Bacterial Strain Isolate Code 4
Urease	-	Mannose	X
Indole	-	Melibiose	X
VP	-	Rafinose	X
Nitrate reduced -	-	Sukrose	X
Strarch hydra tahusis	+	Xylose	X
Oxidase	+	Growth on Cetrimide Agar	X
Catalase	+	Yellow Pigmen	-

Note: V = Central/Oval, + = Facultative aerobics, x = not in the tes

The results of the Identification of Selected Bacteria show that the identified bacteria are of the genus Bacillus (Bacillus sp)

3.3 Molecular Genetics

The results of the identification of selected bacteria by molecular genetics are as follows: Primary PCR results: 16s (27F/1492R). ISW-BAC 1 isolate with Bacterial Species Barcoding (1400bp), performed as follows:

1. DNA-Fast Fungal/Bacterial Miniprep Kit for Extraction of Genomic DNA (Zymo Research, D6005)
2. PCR amplification (2x) with My Taq HS Red Mix (Bioline, BIO-25048)
3. Sequence in both directions.

The analysis' findings are as follows:

a. Nucleic Acid (Genomic DNA) Quantification (Nanodrop)

No	Sample Name	Conc. (ng/µl)	$A_{260/280}$	$A_{260/230}$	Volume (µl)
1	ISW-BAC 1	17.5	2.08	0.52	30

b. Gel Photo – PCR Products

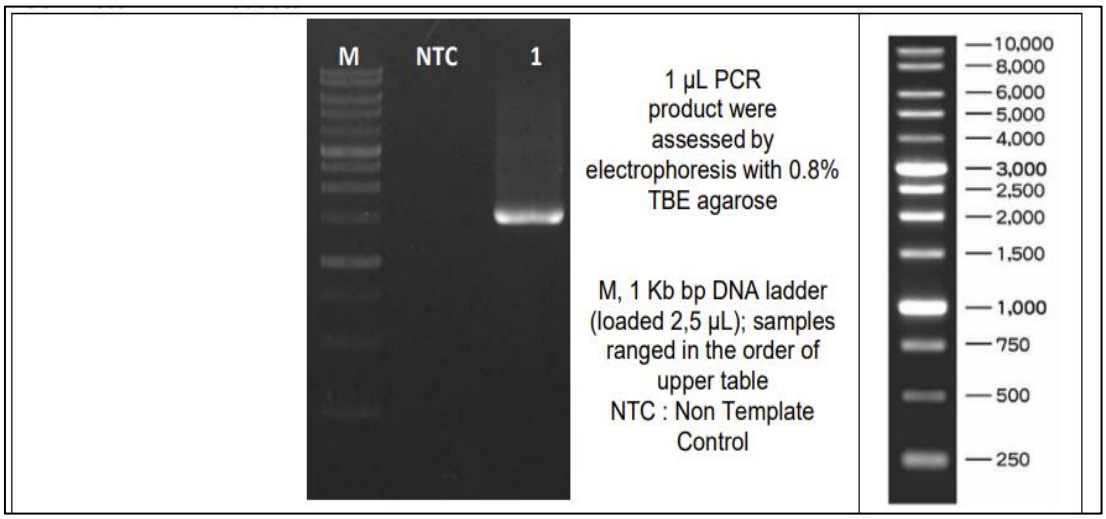

Fig. 2. Primary PCR results 16s (27F/1492R). ISW-BAC 1

c. Sequence Assembly Result – PCR Product

Sequence Assembly 1394 bp

1 TGCAGTCGAG CGGACAGATG GGAGCTTGCT CCCTGATGTT AGCGGCGGAC GGGTGAGTAA 61 CACGTGGGTA ACCTGCCTGT AAGACTGGGA TAACTCCGGG AAACCGGGGC TAATACCGGA 121 TGGTTGTTTG AACCGCATGG TTCAAACATA AAAGGTGGCT TCGGCTACCA CTTACAGATG 181 GACCCGCGGC GCATTAGCTA GTTGGTGAGG TAACGGCTCA CCAAGGCAAC GATGCGTAGC 241 CGACCTGAGA GGGTGATCGG CCACACTGGG ACTGAGACAC GGCCCAGACT CCTACGGGAG 301 GCAGCAGTAG GGAATCTTCC GCAATGGACG AAAGTCTGAC GGAGCAACGC CGCGTGAGTG 361 ATGAAGGTTT TCGGATCGTA AAGCTCTGTT GTTAGGGAAG AACAAGTACC GTTCGAATAG 421 GGCGGTACCT TGACGGTACC TAACCAGGAA GCCACGGCTA ACTACGTGCC AGCAGCCGCG 481 GTAATACGTA GGTGGCAAGC GTTGTCCGGA ATTATTGGGC GTAAAGGGCT CGCAGGCGGT 541 TTCTTAAGTC TGATGTGAAA GCCCCCGGCT CAACCGGGGA GGGTCATTGG AAACTGGGGA 601 ACTTGAGTGC AGAAGAGGAG AGTGGAATTC CACGTGTAGC GGTGAAATGC GTAGAGATGT 661 GGAGGAACAC CAGTGGCGAA GGCGACTCTC TGGTCTGTAA CTGACGCTGA GGAGCGAAAG 721 CGTGGGGAGC GAACAGGATT AGATACCCTG GTAGTCCACG CCGTAAACGA TGAGTGCTAA 781 GTGTTAGGGG GTTTCCGCCC CTTAGTGCTG CAGCTAACGC ATTAAGCACT CCGCCTGGGG 841 AGTACGGTCG CAAGACTGAA ACTCAAAGGA ATTGACGGGG GCCCGCACAA GCGGTGGAGC 901 ATGTGGTTTA ATTCGAAGCA ACGCGAAGAA CCTTACCAGG TCTTGACATC CTCTGACAAT 961 CCTAGAGATA GGACGTCCCC TTCGGGGGCA GAGTGACAGG TGGTGCATGG TTGTCGTCAG 1021 CTCGTGTCGT GAGATGTTGG GTTAAGTCCC GCAACGAGCG CAACCCTTGA TCTTAGTTGC 1081 CAGCATTCAG TTGGGCACTC TAAGGTGACT GCCGGTGACA AACCGGAGGA AGGTGGGGAT 1141 GACGTCAAAT CATCATGCCC CTTATGACCT GGGCTACACA CGTGCTACAA TGGACAGAAC 1201 AAAGGGCAGC GAAACCGCGA GGTTAAGCCA ATCCCACAAA TCTGTTCTCA GTTCGGATCG 1261 CAGTCTGCAA CTCGACTGCG TGAAGCTGGA ATCGCTAGTA ATCGCGGATC AGCATGCCGC 1321 GGTGAATACG TTCCCGGGCC TTGTACACAC CGCCCGTCAC ACCACGAGAG TTTGTAACAC 1381 CCGAAGTCGG TGAG

Table 3. Chemical Oxygen Demand reduction in Tofu Liquid Waste samples

Parameter	Unit	Test results (Avg)	Test Method
C0D	mg/L	30.798	SNI 6989-73-2019
C0D	mg/L	29.230	SNI 6989-73-2019
C0D	mg/L	25.611	SNI 6989-73-2019

3.4 Results Bacterial Bioremediation Ability (Isolates 4)

This method is used to measure spectrophotometrically the chemical oxygen demand (COD) in test media using the oxidizer Cr_2O_7-2 with closed reflux. According to the study's findings, COD decreased in the time intervals of 0 hours, 24 hours, and 48 hours.

4. DISCUSSION

4.1 Identification of Indigenous Bacteria

Identification was performed to determine the name and type of bacteria that have the best ability to degrade tofu liquid waste, namely bacteria with strain code 4 ISW, as follows:

4.2 Morphology and Biochemistry Test

The test bacteria were identified morphologically and biochemically to determine the initial screening and morphological and biochemical properties of the test bacteria. *Bacillus* is a genus of bacteria that has a rod shape, is gram (+), is motile, can produce a single spore (though some species do not), is widely distributed in nature, and is resistant to extreme conditions. *Bacillus* bacterial genera are typically catalase positive, positive oxidase capable of metabolizing carbohydrates via fermentation, unable to produce acid from mannitol, and some *Bacillus* genera are anaerobic hetero-fermentative. Bacillus bacteria can also metabolize carbohydrates, proteins, and amino acids, as well as convert nitrate to nitrite. It belongs to the

genus *Bacillus,* according to the analysis of the test bacteria.

4.3 Molecular Biology Test

The agarose gel electrophoresis method was used to identify PCR products using 16S rDNA/16S rRNA (PCR-amplified 16S rRNA) genes from bacterial species with DNA fragments ranging from 50 to 20,000 bp. This is the smallest size from which an agarose gel can separate [15]. Because the 16S rDNA/16S rRNA gene is conserved [16], "the 16S rDNA/16S rRNA gene is part of the ribosomal RNA structure, which plays an important role in protein synthesis, and the 16 rRNA gene is always present and owned by prokaryotic organisms, this 16S rRNA gene analysis is ideal for phylogenetic tree reconstruction and prokaryotic identification [17,18].

The process of isolating the genome of Strain 4 - ISW was marked by the formation of one band for each genome of the tested bacteria after being observed using a UV transluminator with a 1.5 kb 16S rRNA gene coding band and compared with a marker (1 kb DNA ladder) [19]. "The results of DNA amplification 16 rRNA were sequenced to obtain nucleotide sequences, and similarity was analyzed using Gen Bank with the BLAST-N (Basic Local Alignment Search Toll-Nucleotode) program to identify the homology and species of the test bacteria"[20].

The results of the 16S rDNA sequencing of ISW Strain 4 Isolates were compared with the 16S rDNA sequence data of several species obtained from the data bank to determine the phylogeny/kinship with other organisms. The data from the 16S rDNA sequence was then aligned using the clustalX ver 2.0 program [21]. "The Neighbor-Joining Tree statistical method with the bootstrap 1000 level p-distances model was then used to generate a phylogenetic tree. The PCR results of the 16S rDNA gene are represented on the electrophoretic gel by a single band with a size of about 1500 bp" [22].

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

The test bacteria with the code Strain 4 - ISW is a molecule type-nucleic acid with a query length of 1394. with the lineage report as follows:

1) Kingdom: Procaryotae,
2) Division : Bacteria,
3) Class : Firmicutes,
4) Family: Bacillales,
5) Order : Bacillaceae,
6) Genus: *Bacillus,*
7) Species: *Bacillus subtilis.*

Maximum identity level of 93% Stain 4 -ISW is *Bacillus subtilis*, based on phylogenetic tree analysis, has a very close kinship with *Bacillus subtilis.*

Table 4. Sequencing results using forward and reverse primers

No. Isolate Code	Nucleotide Length (bp)	Name of Bacteria	Homology %	Code
RID-HDW9DCUM01R	1394	*Bacillus subtilis*	93%	ISW

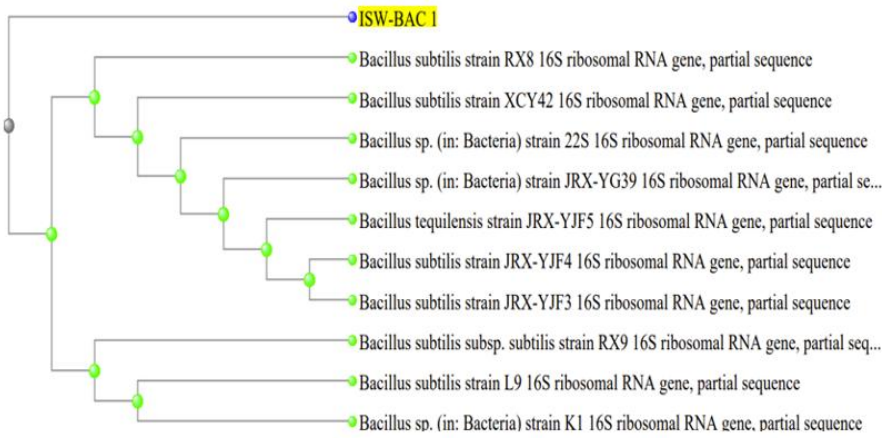

Fig. 3. Phylogenetic Tree Construction

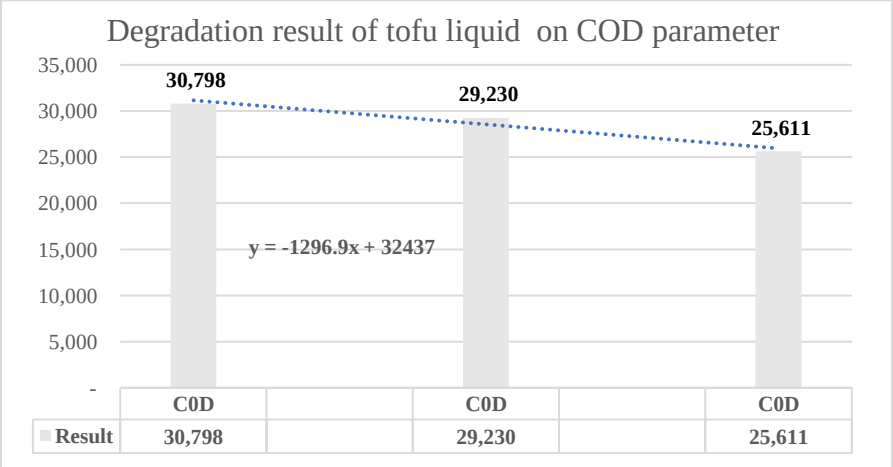

Fig. 4. Degradation Results of measuring COD concentrations based on observation time
Noted: The linear equation y = - 1296,9 x + 32437, the degradation percentage is 17 %

The use of *Bacillus sp* as a bioremediation agent is a new management effort in overcoming tofu industry wastewater pollution. This is because the identified bacterial strains are indigenous bacteria that excel in degrading organic compounds as a result of the tofu-making process.

Indigenous bacteria are bacteria that have adapted to their environment so that they do not have a negative impact on the environment in which they live. Indigenous bacteria have evolved into an ecosystem's balance system [23].

Bacillus sp bacteria are indigenous bacteria that have long adapted to an organic waste-polluted environment, so they are not pathogenic to the surrounding environment, making the use of indigenous *Bacillus sp* bacteria as a bioremediation agent quite safe [24].

The Chemical Oxygen Demand (COD) parameter is used in the bioremediation of tofu waste by selected Bacteria (Strain 4). Table 3 displays the results of measuring the COD parameter concentration based on the time of observation.

According to Fig. 2, *Bacillus subtilis* as a bioremediation agent can reduce COD content in tofu liquid waste by 17% (30.798 ppm at 0 observation time and 25.611 ppm at 48 hours observation time), using the linear regression equation y = -1296.9 x + 32437. B. subtilis can reduce protein compounds in shrimp pond wastewater. This is due to the fact that B. subtilis has extracellular enzymes capable of degrading protein compounds [25].

5. CONCLUSION

Based on the research results it can be concluded that: There are indigenous bacteria that can degrade tofu waste. The results of the identification of bacteria showed positive results for the type of *Bacillus subtilis*, this indicated that the bacterium *Bacillus subtilis* had the ability to degrade tofu waste.

It is necessary to conduct trials on a more applicable scale on the bacterial strain *Bacillus subtilis* as a bioremediation agent in degrading tofu waste (development research from laboratory scale to applied research).

DATA AVAILABILITY

The working papers and accompanying information files contain all of the necessary information. This study will aid researchers in solving environmental pollution problems, particularly in the discovery of indigenous bacteria capable of degrading tofu industry wastewater in Indonesia.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/95279