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Identification and Physiological Characteristics of Potential Indigenous Bacteria as Bioremediation Agent in the Wastewater of Sugar Factory

(Pengecaman dan Pencirian Fisiologi Potensi Bakteria Asli sebagai Agen Bio-Pemulihan dalam Air Sisa Kilang Gula)

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ABSTRACT

Wastewater is the remainder of an activity released in the liquid form. Wastewater product is feared to have negative influence on the environmental balance; therefore, it is necessary to measure the quality standards of wastewater as a reference in the disposal and treatment. Bioremediation is an environmentally friendly technology utilizing microorganisms as agents in the process of cleaning or restoring wastewater conditions. The use of microorganism services can reduce the concentration of organic waste into simpler organic compounds by converting organic compounds into CO_2, CH_4, H_2, and H_2S, as well as water and energy intended for the process of growth and production of microorganisms in the remediation process. This study aims to identify the type of dominant bacteria growing in the wastewater of the sugar factory and has a potential role as a bioremediation agent for the waste. The method used in this study is the experimental, by observing several environmental parameters as indicators, among others, BOD, COD, TSS, and pH. The results of this study found two types of dominant bacteria, i.e., Staphylococcus aureus *and* Bacillus subtilis*, then used as bioremediation agents. The bioremediation activity was able to reduce pH, BOD, and COD levels.*

Keywords: Bacillus subtilis*; bioremediation; indigenous bacteria;* Staphylococcus aureus; *wastewater*

ABSTRAK

Air sisa adalah sisa aktiviti yang dikeluarkan dalam bentuk cecair. Produk air buangan dikhuatiri mempunyai pengaruh negatif terhadap keseimbangan alam sekitar; oleh itu, adalah penting untuk mengetahui piawai kualiti air sisa sebagai rujukan dalam pembuangan dan rawatan. Biopemulihan adalah teknologi mesra alam yang memanfaatkan mikroorganisma sebagai agen dalam proses pembersihan atau pemulihan keadaan air sisa. Penggunaan perkhidmatan mikroorganisma dapat mengurangkan kepekatan sisa organik menjadi sebatian organik yang lebih sederhana dengan mengubah sebatian organik menjadi CO_2, CH_4, H_2 dan H_2S, serta air dan tenaga yang dimaksudkan untuk proses pertumbuhan dan pengeluaran mikroorganisma dalam proses pemulihan. Kajian ini bertujuan untuk mengenal pasti jenis bakteria dominan yang tumbuh di dalam air sisa kilang gula dan berpotensi berperanan sebagai agen biopemulihan bagi sisa tersebut. Kaedah yang digunakan dalam kajian ini adalah kaedah uji kaji dengan memerhatikan beberapa parameter persekitaran sebagai petunjuk, antara lain BOD, COD, TSS dan pH. Hasil kajian ini mendapati dua jenis bakteria dominan, iaitu Staphylococcus aureus *dan* Bacillus subtilis*, kemudian digunakan sebagai agen biopemulihan. Aktiviti biopemulihan dapat mengurangkan tahap pH, BOD dan COD.*

Kata kunci: Air sisa; Bacillus subtilis*; bakteria asli; biopemulihan;* Staphylococcus aureus

Introduction

Tlogo Kelang Sugar Factory is one of the sugar industries located in Malang Regency, which is located quite far from residential areas, yet very close to agricultural land, both sugar cane, rice, and plantation land with various types of plants. Tlogo Kelang is not a massive factory with a production capacity of only 1600 tons in one production (Pommiera et al. 2007). However, industrialization, like other human activities that impact on the environment, often results in pollution and degradation (Poddar & Sahu 2015). The pollution will decrease aquatic biodiversity although not impacted to non-native invasion fish such as arapaima and alligator gar (Fadjar et al. 2019; Hasan et al. 2020). Wastewater from sugar industries is one that has complex characteristics and is considered a challenge for environmental engineers in terms of treatment as well as utilization (Poddar & Sahu 2015). Wastewater from the sugar factory contributes to increasing organic matter in the water environment (Islamy et al. 2017; Kilawati &

Islamy 2020). These entire chemicals, one way or another, will increase organic matter in the water environment, dissolved solids, and suspended matter (Jadhav et al. 2013).

An alternative waste treatment that is currently use the services of microorganisms, also known as bioremediation. Bioremediation is a process in which beneficial microbiological agents, such as bacteria, are used to clean up contaminated soil and water. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the application of biological processes (Divya et al. 2015). The use of microorganism can break down organic waste into simpler organic compounds, by converting organic compounds into the form of carbon dioxide (CO_2), methane (CH_4), hydrogen (H_2), and hydrogen sulfide (H_2S), and water (H2O) and energy intended for the process of growth and production. Organic waste contains oils and fats, carbohydrates, proteins, hydrocarbons, and phenols. Generally, organic waste is easily decomposed or degraded by microorganisms (Retnosari & Shovitri 2013).

Characterization of the indigenous microbial communities, in terms of their diversity, metabolic potential, and response (change in community composition) toward bio-stimulatory agents, is essential for developing bioremediation technology (Sarkar et al. 2016). However, the success of *in situ* bioremediation using the indigenous microorganisms remains severely constrained by inappropriate nutrient level or physicochemical conditions event both of them (temperature, pH, moisture content, and nutrient availability) prevailing at the contaminated sites (Lu et al. 2014). This study aims to the identify and characterizes indigenous bacteria in the wastewater of the sugar factory, which can play as a bioremediation agent.

MATERIALS AND METHODS

The method in this study conducted by a challenge test of liquid waste given culture bacteria, control (without bacteria), and first liquid waste. The experiment was repeated three times for 3-4 weeks, carried out in a laboratory with adjusted conditions. The research began by measuring the quality of wastewater, including measurements of pH, temperature, BOD, COD, and TSS. Bacterial planting was carried out after the initial wastewater quality test. Bacterial planting is carried out using sugar factory wastewater samples so that the dominant bacterial type is obtained through the process of bacterial identification through bacterial macroscopic testing, microscopic bacterial testing, and bacterial biochemical testing. The remediation test was carried out for 4-5 test days (Mambang et al. 2014). The results of the bioremediation test are known by comparing the initial, control, and final wastewater quality values. This research was carried out in February-March 2019. Retrieval of the test material was carried out at Tlogo Kelang Factory, Malang Regency, East Java. Water quality testing was carried out at the Biotechnology and Research Laboratory, and preparations for bacterial planting were carried out at the Fishery Product Safety Laboratory. Marine Sciences and bacterial identification are carried out at the Microbiology Laboratory, Faculty of Medicine, Brawijaya University, Malang, East Java.

WASTEWATER SAMPLING

Wastewater samples collected using the modification method of a published article (Ahmed et al. 2017). The sample was collected on February-March 2019 for 20 L (2 × 20) then place in dark plastic containers. All the containers were transported using iceboxes to Brawijaya Fishery Product Safety Laboratory for further analysis. Water parameters (pH and temperature) were measured to find out the actual temperature and pH of the waste before being brought into the laboratory.

WASTEWATER QUALITY TEST

The determination of wastewater quality test (pH, temperature, BOD, COD, and TSS) was carried out twice; at the beginning of the study before and after the remediation test. The test results are used as a comparison to determine changes in water quality after being treated.

DETERMINATION OF PH AND TEMPERATURE

Determination of pH and temperature carried out using a tool, i.e. KRISBOW pH Meter.

DETERMINATION OF CHEMICAL OXYGEN DEMAND (COD)

Determination of COD conducted by $KMnO_4$ titrimetric color method based on a published article (Mohamed et al. 2017). Reagents used i.e. sulfuric acid (conc.), copper sulfate (1 g), potassium permanganate (0.025N), and sodium oxalate (0.025N). The procedure: 10 mL of the sample was taken in a 100 mL bottle, then 5 mL of concentration. $H2SO_4$ was added, and 1 g of copper sulfate ($CuSO_4$) also was added. Then 3 mL of the prepared (0.025 N $KMnO_4$) solution was added and immersed the bottle in boiling water for 30 min while keeping the surface of the boiling water at the higher level than the surface of the sample. The 3 mL of 0.025N Sodium Oxalate ($Na_2C_2O_4$) was added and immediately titrated with 0.025 N Potassium Permanganate ($KMnO_4$) violet color appeared, then repeated for the blank separately under the same condition using 10 mL of distilled water instead of 10 mL of samples. Calculation of COD using the formula:

$$COD\ (mg.L^{-1}) = \frac{(Sample\ B)(A\ 8000x0.025)}{mL\ of\ sample}$$

where A is volume of $KMnO_4$ used for sample; B is volume of $KMnO_4$ used for blank; 0.025 (1/40) is normality of $KMnO_4$; 8000 is volume equivalent weight of oxygen in 1000 $mL.L^{-1}$.

DETERMINATION OF BIOCHEMICAL OXYGEN DEMAND (BOD_5)

Winkler titrimetric method based on published articles (Mohamed et al. 2017). Reagents used, i.e. sulfuric acid ($H2SO_4$), starch indicator, sodium iodide, and manganese sulfate. The Procedure: Prepare 2 bottles (100 mL) and cleaned it well. The first bottle was covered with aluminum foil to avoid light then kept in the incubator (06287 AKyurty) at (20-22 °C) for five days. Then, 10 mL of manganese sulfate solution and 2 mL of alkali-iodide solution were added to the other bottle below the surface of the liquid by using a syringe. Then, the bottle closed and mixed by inverting it several times. When the precipitate settles, leaving a clear supernatant above the precipitate, shaken again slowly by inverting the bottle and when the setting has produced at least 50 mL supernatant 8 mL of concentration. $H2SO_4$ was added. Then, the bottle was closed and mixed by gentle inversion until dissolved. The 100 mL of the sample were titrated with 0.05M $Na_2S_2O_3$ solution until a pale-yellow solution reached. Then 2 mL of freshly prepared starch solution was added and keep titration continued until a blue color appeared. Repeat the procedure using 100 mL of distilled water (blank). Then, repeated for incubated samples after five days. Calculations of BOD5 using a formula as follows:

$$BOD_5\ mg.L^{-1} = 16\ (V1\text{-}V2)$$

where V1 is volume of $Na_2S_2O_3$ used for the sample before incubation; and V2 is volume of $Na_2S_2O_3$ used for the sample before incubation.

TOTAL SUSPENDED SOLIDS (TSS)

The determination of TSS was carried out by the filtration process according to published methods (Rahmanian et al. 2015). Therefore, the accuracy and precision of the following methods are well approved and cited in scientific literature. A fixed volume of water sample was poured on a pre-weighed glass fiber filter of a specified pore size before starting the vacuum filtration process. The filter was removed after the completion of the filtration process and placed in an aluminum dish in an oven at 100 °C for 2-3 h to dry off the remaining water completely. The filter was then weighed, and the gain in filter weight represented the TSS contents, expressed in mass per volume of sample filtered ($mg.L^{-1}$).

BACTERIAL CULTURE AND IDENTIFICATION

Bacterial culture was carried out after the initial wastewater quality test. Bacteria planting was carried out using sugar factory wastewater samples, using NA (Nutrient Agar) media with the pouring method, and incubated for 2 × 24 h at 28 °C to grow bacteria as a whole from the sugar factory liquid waste. All bacteria growing were observed through macroscopic using light microscopic identification (Tshikhudo et al. 2013). Bacterial biochemical tests conducted to obtain the type of bacteria dominant living in the sugar factory liquid waste. Then the most predominant bacterial growth was isolated, using NB (Nutrient Broth) media, and incubated for 2 × 24 h at 28 °C. These bacteria are used as bioremediation agents that are suspected to be the primary agent in the remediation process.

BIOREMEDIATION TEST

The remediation test was carried out for 4-5 test days (Mambang et al. 2014). It is considered to be sufficient time to observe the growth and development of the tested bacteria. The test is carried out aerobically based on the initial conditions of the wastewater at the location that has been given an aerator so that further treatment must be adjusted so as not to change the original bacterial composition of the wastewater.

RESULTS AND DISCUSSION

MACROSCOPIC IDENTIFICATION OF BACTERIA

In order to identify the bacteria, first we should attend to how they grow on the media to identify their cultural characteristics since different species can produce very different colonies (Christopher & Bruno 2003). Each colony has characteristics that may be unique to it, and this may be useful in the preliminary identification of a bacterial species (Tshikhudo et al. 2013). The results of macroscopic observations of cultured bacterial colonies shown in Table 1.

TABLE 1. Morphological characteristics of bacterial colonies

No	Isolat	Colony morphology characteristics				
		Form	Edge	Elevation	Color	Consistency
1.	Strain I	Rounded	Flat shaped	Convex	Yellow	Wet
2.	Strain II	Rounded	Uneven	Flat	Beige	Dry

The results of microscopic identification of bacteria obtained two strains. Strain I is known to be round, has a flat edge with convex elevation and wet consistency, and this strain is yellow, whereas Strain II is known to have the same shape as Strain 1, which is round but has uneven edges, flat elevation, and dry and creamy consistency. Bacteria have an essential role in the wastewater bioremediation process through their decomposition activity, where decomposition activity is highly related to the type of dominant bacteria in the communities (Fitriyah et al. 2013). Morphological identification techniques conducted in order to identify poorly better described, rarely isolated, or phenotypically irregular strains (Tshikhudo et al. 2013). An improved method was brought up for the bacterial cell characterization based on their different characteristics by segmenting digital bacterial cell images and extracting geometric shape features for cell morphology. The classification techniques are used to identify the bacterial cells based on their morphological characteristics (Hiremath et al. 2013).

MICROSCOPIC IDENTIFICATION

The result of the microscopic identification of bacteria is presented in Table 2.

TABLE 2. Characteristics of bacterial strains

Parameter	Strain 1	Strain 2
Colony color	Yellow	Beige
Diameter of colony (mm)	1.25	3.19
Gram reaction	Positive	Positive
Cell shape	Rounded	Stem
Motility	No Motile	Motile
Oxidase	Positive	Negative
Catalase activity	Negative	Positive
Indole production	Negative	Negative
Use of carbon from citrate	Positive	Negative
TSIA test	-	-
Voges Proskauer	Negative	Positive

The results of microscopic identification of Strain 1 are known to have a yellow color, colony diameter of 1.25 mm, a Gram-positive bacteria with round cell shape, non-motile nature, reaction to the oxidase and citric test is positive, but react negatively to the catalase test and production indole and VP test.

In Strain II, it showed microscopic identification with a cream color. The colony was 3.19 mm in diameter, was rod-shaped, and was gram-positive. In the motile test, this strain has motile properties and gives an adverse reaction to the oxidase, indole, and citrate test. In the catalase test and VP strain, 2 gave a positive reaction.

Observations of bacterial morphologies are done by light microscopy, which is aided by the use of stains (Bergmans et al. 2005). Dutch microbiologist Antoni Evan Leeuwenhoek (1632-1723) was the first person to observe bacteria under a microscope. Without staining, bacteria are colorless, transparent, and not visible, and the stain serves to distinguish cellular structure for a more detailed study. The Gram stain is a differential stain with which to categorize bacteria as either Gram-positive or Gram-negative. Observing bacterial morphologies and the Gram reaction usually constitutes the first stage of identification. It serves as a good and reliable morphological feature for identifying and classifying bacterial species (Tshikhudo et al. 2013). Light microscopy was traditionally used for identifying colonies of bacteria and morphologies of individual bacteria. However, future study we suggested to continue the research using aquatic animal test that has economical value to confirm the ability of bacteria as bioremediation agent; such as Cichlidae fish (Insani et al. 2020); Gobiidae (Hasan et al. 2021), mangrove Snail (Islamy & Hasan 2020); Snake head (Pratama et al. 2019); Genggehek fish (Valen et al. 2019). Current fish should be identify using a model of visual intelligent system for genus identification (Pramono et al. 2020).

BACTERIAL BIOCHEMICAL TEST RESULTS

Biochemical tests can also be called physiological tests on treated objects and, in this case, bacteria. The results of the bacterial biochemical tests were found in the liquid waste of the Tlogo Kelang Sugar Factory; more details shown in Table 3.

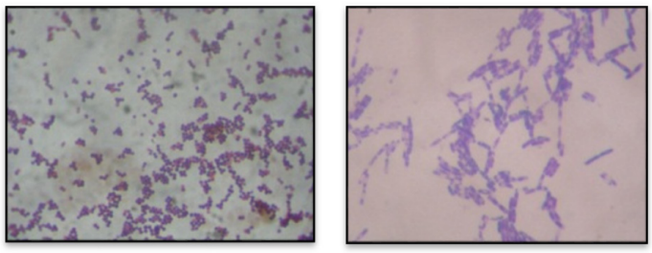
TABLE 3. Bacterial biochemical test result

No.	Biochemical Test	Strain 1	Strain 2
1	Spora	-	+
2	Oksidase	-	+
3	Motilitas	-	+
4	Nitrate	-	-
5	Lysin	-	-
6	Ornithine	-	-
7	H_2S	-	-
8	Glukosa	+	+
9	Manitol	+	+
10	Xylosa	-	+
11	ONPG	-	-
12	Indole	-	-
13	Urease	-	-
14	V-P	-	-
15	Sitrat	-	+
16	TDA	-	-
17	Gelatin	-	-
18	Malonate	-	-
19	Inositol	-	-
20	Rhamnose	-	-
21	Sucrose	-	-
22	Lactose	-	-
23	Arabinosa	-	+
24	Adonitol	-	-
25	Raffinose	-	-
26	Salicin	-	-
27	Arginine	-	-
28	Katalase	+	+
29	Coagulase	+	-

Physiological characteristics obtained from the results of biochemical tests are essential to determine and know the types of bacteria that are not known because, without adequate physiological observations of the organic content, the determination of these types of bacteria cannot be done. The importance of this test is because by doing only a morphological test alone will not be able to answer the basics of determining the type of bacteria found because the morphological characteristics of two different types of bacteria can look similar (Lehninger 1982).

The biochemical test results are a clear picture of the specifications of the bacteria being tested so that the types of bacteria can be deduced. Through these test results, we

suspect that Strain I is *Staphylococcus* sp., while Strain II is *Bacillus* sp. (Figure 1). The two bacteria found were culture bacteria, and those identified were the most dominant bacteria. These two types of bacteria are the dominant types of bacteria cultured from sugar factory liquid waste. However, poliphasic method involving morphological, biochemical, and molecular tests is required to ensure accuracy of species determination. For the next researcher of similar study, we suggest polymerase chain reaction (PCR) methods (Ethica et al. 2018), to identify bacteria so that it can infer the species of bacteria accurately.



a. b.

FIGURE 1. Two dominant bacteria that grow from sugar liquid waste; a) *Staphylococcus* sp. and b) *Bacillus* sp.

WASTEWATER QUALITY

Changes in the value of wastewater quality are presented through the graph in Figure 2 as follows:

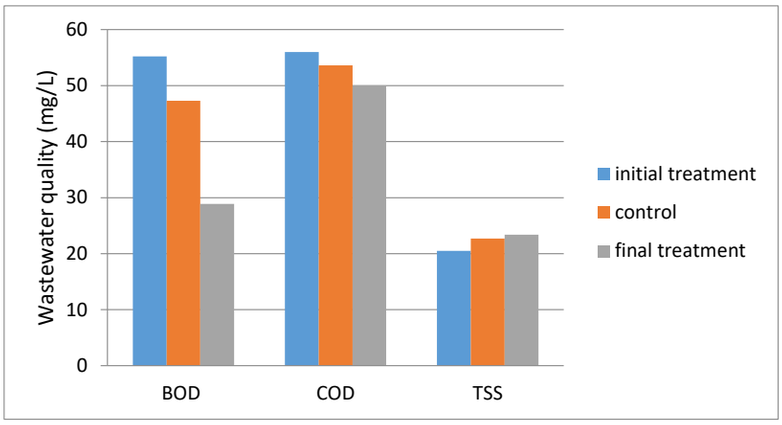


FIGURE 2. Changes in the value of the sugar factory wastewater quality Tlogo Kelang Factory

pH

The measurement results of the pH value of sugar factory wastewater are obtained pH value 4.8 for initial pH, control pH is six and the final pH is 7. Changes in the pH value indicate that there has been an overhaul of organic material in the wastewater by microorganisms. The pH value towards the base indicates that the organic material has decomposed. Degradation of organic protein and nitrogen to ammonium can cause an increase in pH to a base (Government Regulation No.82 2001). The pyruvic acid catalase process correctly occurs in the process of aerobic respiration. This process produces CO_2, H_2O, and more energy, 38 ATP. Whereas the anaerobic process only produces 2 ATP with 21 Cal (Retnosari & Shovitri 2013).

TEMPERATURE

In this study, the temperature of wastewater was 29 °C. The importance of knowing the temperature of the sugar factory wastewater is to facilitate the making of live media

for bacteria to be cultured, which is suitable for their natural habitat (Isnawati & Trimulyo 2018). The results of the identification of the pure culture of bacteria from sugar factory liquid waste found two dominant types of bacteria, namely *Staphylococcus* sp. and *Bacillus* sp. Both of these bacteria can grow in mesothermal conditions; at this temperature, bacteria range between 20-45 °C and optimum at 30-37 °C (Lehninger 1982).

BIOLOGICAL OXYGEN DEMAND (BOD_5)

BOD_5 values obtained were varied, i.e. initial BOD_5 values of 55.2 $mg.L^{-1}$, control BOD_5 obtained values of 47.3 $mg.L^{-1}$, and at the final BOD_5 values obtained of 28.9 $mg.L^{-1}$. The three tests obtained BOD_5 results in a row, which can be seen in Figure 2. The decreasing of BOD_5 shows that the *in vitro* bioremediation test using candidate bacteria can reduce BOD_5 levels from 55.2 to 28.9 $mg.L^{-1}$, the value was declared safe for released to water bodies according to that the maximum BOD_5 value that can be released in type B waters is 3 $mg.L^{-1}$ (Government Regulation No.82 2001).

CHEMICAL OXYGEN DEMAND (COD)

The COD test obtained different results, namely the initial COD value of 56 $mg.L^{-1}$, the control COD value of 53.6 $mg.L^{-1}$ and the final COD value obtained from remediation results of 50 $mg.L^{-1}$. The results of laboratory tests on the COD of the sugar factory liquid waste can be explained through Figure 2. COD value does not decrease sharply, however, the results obtained are still in good tolerance for water, it can occur because of the large mass of microorganisms contained in liquid waste so that more oxygen will be needed for the needs of oxidation of microorganisms and the overhaul of organic matter (Government Regulation No.82 2001).

TOTAL SUSPENDED SOLID (TSS)

TSS test on sugar factory wastewater obtained initial value of 20.5 $mg.L^{-1}$, TSS control test of 22.7 $mg.L^{-1}$, and obtained greater value of 23.4 $mg.L^{-1}$. The results of the TSS test are presented in Figure 2. An increase in the value of TSS can be caused by several factors, namely the continuous stirring of wastewater so that the deposition of waste is mixed into the wastewater. In addition, the increase in TSS value is also influenced by the increase in the biomass of microorganisms in wastewater so that the organic material increases (Nurhayati et al. 2014) which will be suspended on filter paper so the TSS value increases.

CONCLUSION

We found two types of bacteria, i.e., *Staphylococcus* sp. and *Bacillus* sp. that were most dominant during the bioremediation period. It was able to reduce the level of pH, BOD, and COD in 5 days aerobically, where the BOD value of wastewater had decreased from 55.2 $mg.L^{-1}$ to 28.9 $mg.L^{-1}$. The COD value had decreased from 56 to 50 $mg.L^{-1}$. It shows good results on remediation efforts. However, the value of TSS has increased from 20.5 to 23.4 $mg.L^{-1}$.

REFERENCES

Ahmed, K.M., Haroun, M.A., Mahyoub, J.A., Al-Solami, H.M. & Ghramh, H.A. 2017. Environmental impacts of the liquid waste from assalaya sugar factory in Rabek locality, White Nile State, Sudan. *International Journal of Environment, Agriculture and Biotechnology* 2(4): 238819.

Bergmans, L., Moisiadis, P., Van Meerbeek, B., Quirynen, M. & Lambrecht, P. 2005. Microscopic observation of bacteria: Review highlighting the use of environmental SEM. *Int. Endod. J.* 38: 775-788.

Christopher, K. & Bruno, E. 2003. Identification of bacterial species. In *Tested Studies for Laboratory Teaching,* edited by O'Donnell, M.A. *Proceedings of the 24th Workshop/ Conference of the Association for Biology Laboratory Education* 24: 103-130.

Dewi, A.K. 2013. Isolation, identification and sensitivity test of *Staphylococcus aureus* against amoxicillin from mastitis goat milk (PE) samples of mastitis sufferers in Yogyakarta's girimulyo kulonprogo region. *Jurnal Sain Veteriner* 31(2): 138-150.

Divya, M., Aanand, S., Srinivasan, A. & Ahilan, B. 2015. Bioremediation - An eco-friendly tool for effluent treatment: A review. *International Journal of Applied Research* 1(12): 530-537.

Ethica, S.N., Oedjijono, O., Semiarti, E., Widada, J. & Raharjo, T.J. 2018. Genotypic and phenotypic characterization of *Alcaligenes javaensis* JG3 potential as an effective biodegrader. *BIOTROPIA-The Southeast. Asian Journal of Tropical Biology* 25(1): 1-10.

Fadjar, M., Islamy, R.A. & Herawati, E.Y. 2019. Short communication: First record of Arapaima gigas (Schinz, 1822) (Teleostei: Osteoglossomorpha), in the Brantas River, Sidoarjo, East Java, Indonesia. *Biodiversitas Journal of Biological Diversity* 20(12): 3527-3531.

Fitriyah, D., Arimurti, S. & Senjarini, K. 2013. Physiological and molecular characteristics of bacterial isolates from bandealit coastal area Jember, East Java, Indonesia. *HAYATI Journal of Bioscience* 20(2): 89-93.

Government Regulation No. 82. 2001. *Management of Water Quality and Control Over Water Pollution.*

Hasan, V., Valen, F.S., Islamy, R.A., Widodo, M.S., Saptadjaja, A.M. & Islam, I. 2021. Short Communication: Presence of the vulnerable freshwater goby *Sicyopus auxilimentus* (Gobiidae, Sicydiinae) on Sangihe Island, Indonesia. *Biodiversitas* 22: 571-579.

Hasan, V., Widodo, M.S., Islamy, R.A. & Pebriani, D.A.A. 2020. New records of alligator gar, *Atractosteus spatula* (Actinopterygii: Lepisosteiformes: Lepisosteidae) from Bali and Java, Indonesia. *Acta. Ichthyol. Piscat.* 50(2): 233-236.

Hiremath, P.S., Bannigidad, P. & Yelgond, S.S. 2013. An improved automated method for identification of bacterial cell morphological characteristics. *International Journal of Advanced Trends in Computer Science and Engineering* 2(1): 11-16.

Insani, L., Hasan, V., Valen, F.S., Pratama, F.S., Widodo, M.S., Faqih, A.R., Islamy, R.A., Mukti, A.T. & Isroni, W. 2020. Presence of the invasive nile tilapia *Oreochromis niloticus* Linnaeus, 1758 (perciformes, cichlidae) in the Yamdena Island, Indonesia. *Ecol. Environ. Conserv.* 26(3): 1115-1118.

Islamy, R.A. 2019. Antibacterial activity of cuttlefish Sepia sp. (Cephalopoda,) ink extract against *Aeromonas hydrophila. Majalah Obat Tradisional* 24(3): 184.

Islamy, R.A. & Hasan, V. 2020. Checklist of mangrove snails (Mollusca: Gastropoda) in South coast of Pamekasan, Madurai Island, East Java, Indonesia. *Biodiversitas* 21: 3127-3134.

Islamy, R.A., Yanuhar, U. & Hertika, A.M.S. 2017. Assessing the genotoxic potentials of methomyl-based pesticide in Tilapia *(Oreochromis niloticus)* using micronucleus assay. *The Journal of Experimental Life Sciences* 7(2): 88-93.

Isnawati & Trimulyono, G. 2018. Temperature range and degree of acidity growth of isolate of indigenous bacteria on fermented feed "fermege". In *Journal of Physics: Conference Series.* IOP Publishing. 953(1): 012209.

Jadhav, P.G., Vaidya, N.G. & Dethe, S.B. 2013. Characterization and comparative study of cane sugar industry wastewater. *International Journal of Chemical & Physical Sciences* 2(2): 19-25.

Lehninger, A.L. 1982. *Principles of Biochemistry, Part II.* New York: Worth Pubs. Inc. p. 1011.

Kilawati, Y. & Islamy, R.A. 2019. The antigenotoxic activity of brown seaweed *(Sargassum sp.)* extract against total erythrocyte and micronuclei of Tilapia *Oreochromis niloticus* exposed by methomyl-base pesticide. *The Journal of Experimental Life Sciences* 9(3): 205-210.

Lu, L., Huggins, T., Jin, S., Zuo, Y. & Ren, Z.J. 2014. Microbial metabolism and community structure in response to bioelectrochemically enhanced remediations of petroleum hydrocarbon-contaminated soil. *Environmental Science & Technology* 48(7): 4021-4029.

Mambang, D.E.P., Rosidah & Suryanto, D. 2014. Antibacterial activity of tempe extract against *Bacillus subtilis* and *Staphylococcus aureus*. *Jurnal Teknologi dan Industri Pangan* 25(1): 115-118.

Mohamed, Y.A., Wahab, H.A., Khalifa, S.A., Yahia, D. & Mansour, F. 2017. Wastewater analysis of Assalaya Sugar Factory. *International Journal of Trend in Research and Development* 4(6): 21-24.

Nurhayati, C., Hamzah, B. & Pambayun, R. 2014. Pengaruh pH, konsentrasi isolat *Chlorella vulgaris* dan waktu pengamatan terhadap tingkat cemaran limbah cair crumb rubber. *Jurnal Dinamika Penelitian Industri* 25(2): 96-106.

Poddar, P.K. & Sahu, O. 2015. Quality and management of wastewater in the sugar industry. *Applied Water Science* 7(1): 461-468.

Pommiera, S., Chenub, D., Quintard, M. & Lefebvre, X. 2007. Modelling of moisture-dependent aerobic degradation of solid waste. *Waste Manage.* 28: 1188-1200.

Pramono, T.A., Islamy, R.A., Saprudin Putra, J.J., Suparyanto, T., Purwandari, K., Cenggoro, T.W. & Pardamean, B. 2020. A model of visual intelligent system for genus identification of fish in the Siluriformes order. 10.13140/RG.2.2.23521.12640.

Pratama, W.W., Nursyam, H., Hariati, A.M., Islamy, R.A. & Hasan, V. 2020. Short Communication: Proximate analysis, amino acid profile and albumin concentration of various weights of Giant Snakehead *(Channa micropeltes)* from Kapuas Hulu, West Kalimantan, Indonesia. *Biodiversitas* 21(3): 1196-1200.

Rahmanian, N., Ali, S.H.B., Homayoonfard, M., Ali, N.J., Rehan, M., Sadef, Y. & Nizami, A.S. 2015. Analysis of physicochemical parameters to evaluate the drinking water quality in the State of Perak, Malaysia. *Journal of Chemistry*. 2015: Article ID. 716125.

Retnosari, A.A. & Shovitri, M. 2013. Kemampuan isolat *Bacillus* sp. dalam mendegradasi limbah tangki septik. *Jurnal Sains and Seni Pomits* 2(1): 2337-3520.

Sarkar, J., Kazy, S.K., Gupta, A., Dutta, A., Mohapatra, B., Roy, A., Bera, P., Mitra, A. & Sar, P. 2016. Biostimulation of indigenous microbial community for bioremediation of petroleum refinery sludge. *Frontiers in Microbiology* 7: 1407.

Tshikhudo, P., Nnzeru, R., Ntushelo, K. & Mudau, F. 2013. Bacterial species identification getting easier. *African Journal of Biotechnology* 12(41): 5975-5982.

Valen, F.S., Sri Widodo, M., Kilawati, Y. & Islamy, R.A. 2019. Phytogenetic relationships of *Mystacoleucus marginatus* (Valenciennes 1842) based on cytochrome oxidase C subunit I (COI) gene. *Res. J. Life Sci.* 6(1): 19-28.

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