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Potential of Local Microorganisms Solution from Chicken Manure as a Bioactivator in Liquid Waste Treatment from the Fish Cracker Processing Industry

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ABSTRACT

The wastewater produced by traditional food industry is a source of problem due to its high levels of organic compounds pollutant that can increase the level of chemical oxygen demand (COD) and biological oxygen demand (BOD) values that exceed the established wastewater quality standard thresholds. The difficulty in removing high concentrations of organic material through conventional waste treatment necessitates the use of special treatment methods using local microorganisms' solution as bioactivators to accelerate the decomposition of organic compounds. This research aims to identify bacteria in local microorganisms' solution with potential applications in reducing organic compounds by its enzymatic activities. Based on the research results, among the 42 isolates examined, six isolates demonstrated the ability to hydrolyze starch, protein and fat based on qualitative tests. These isolates belong to the genus *Bacillus* based on partial sequencing of 16S rRNA gene. The qualitative tests confirmed the potential of these isolates as they exhibited enzymatic activities that showed potential to reduce organic compounds.

Keywords: amylase, lipase, molecular identification, organic compounds, protease

INTRODUCTION

Waste from the food industry can pose issues as it contains substantial amounts of carbohydrates, proteins, fats, oils, mineral salts and chemical residues. The high content of organic compounds in liquid waste from the food industry can serve as nutrients for microbial growth, potentially leading to a reduction in dissolved oxygen in water. If liquid waste is released into water without undergoing proper processing, it can pollute the water, damage the aquatic ecosystem and threaten the availability of clean water (Gameissa *et al*., 2012). Furthermore, the presence of organic waste in liquid waste from the food industry can lead to human health problems. The difficulty in removing high concentrations of organic material through conventional waste treatment necessitates the use of special treatment methods (Pervez *et al*., 2021).

To prevent these negative impacts, it is crucial to process liquid waste before discharging it into the environment. Wastewater treatment technology can be implemented through various methods, including physical, chemical and biological approaches. One of the most effective strategies involves utilizing biological methods with microorganisms (Zaman *et al*., 2020). The addition of microorganisms as bioactivators, accelerates the decomposition of organic compounds. Importantly, bioactivators are environmentally friendly as they lack dangerous or toxic ingredients. In wastewater treatment, bioactivators play a crucial role in preventing odors, significantly increasing the metabolic rate of bacteria, and enhancing the decomposition of organic compounds. These bioactivators can be derived from local microorganisms solution (Yunilas *et al*., 2022).

Local microorganisms solution is a fermented liquid that contains micro and macro nutrients, along with bacteria that have the potential to decompose organic matter (Hadi, 2019). Local microorganisms' solution contains rice washing water as its source of carbohydrates, palm sugar and coconut water as a source of glucose, and chicken manure as a source of microorganisms (Indasah & Muhith, 2020). Carbohydrates are a source of energy for microorganisms, including glucose which is a simple sugar that is easily metabolized by microorganisms (Sandle, 2016). Chicken manure is one of the organic materials that can be used in producing local microorganisms' solution. The presence of organic compounds in liquid waste can be significantly reduced because these compounds serve as an energy source for microorganisms, leading to the production of enzymes that break down the organic material. Enzymes such as amylase break down carbohydrates, protease breaks down proteins and lipase breaks down fats and oils. While local microorganisms solution is commonly used as a bioactivator in organic compost production, its potential in processing liquid waste has not been thoroughly explored. This research aimed to identify bacteria in local microorganisms solution with potential applications in liquid waste treatment, allowing these bacteria to be utilized as bioactivators.

MATERIALS AND METHODS

Local Microorganisms Solution Production

Local microorganisms' solution production from chicken manure was carried out by aerobic and anaerobic fermentation. The chicken manure was crushed and mixed with palm sugar, water from rice rinsing, and coconut water. Fermentation was carried out for 14 days aerobically and anaerobically. The aerobic fermentation was carried out by incubating the local microorganisms mixture in jerry cans and the oxygen was supplied from a hose. The anaerobic fermentation was carried out by local microorganisms fermentation in tightly closed jerry cans with a hose attached on top of the jerry can that connected to a bottle which contains clean water so that the air from outside did not enter the jerry can to keep the anaerobic incubation.

Isolating Bacteria from Local Microorganisms Solution

The fermented local microorganism solution samples were serially diluted up to 10^{-5} dilutions. A total of 100 µl aliquots from the dilution 10^{-3} to 10^{-5} were taken and spread onto a nutrient agar plate and incubated for 24 hours at 37 °C.

Characterization of Isolated Bacteria

Isolated colonies from the samples were characterized by identifying the morphology of the colonies in terms of shape, edges, elevation, colour and Gram staining procedure were conducted for microscopic identification.

Qualitative Screening of Potential Bacteria

The starch agar and Lugol's solution were used for screening amylolytic bacteria, skim milk agar for screening proteolytic bacteria, while olive oil or fish oil and rhodamin B medium for screening lipolytic bacteria. Bacteria that showed a clear zone around its colony on starch agar and skim milk agar were selected as potential strains. Bacteria that grew on oil medium and showed orange luminescence under UV light were also selected as potential strains.

Molecular Identification of Potential Isolates

The potential isolates were chosen for molecular identification using the 16S rRNA genes based on the outcome of enzymatic screening. The selected bacterial isolates were cultured in nutrient broth (NB) at 37 °C for 24 h. The supernatant was extracted from the cultures after they were centrifuged for 5 min at 10,000 rpm. A 10% Chelex solution in ddH2O (500 µl) was added. The cultures were then heated for 5 min at 95 °C, vortexed, and then heated for an additional 5 min at 95 °C. The culture was centrifuged once again for 15 min at 10,000 rpm, and the DNA-containing supernatant was then moved to a new microtube (Gautam, 2022). The extracted DNA was amplified using My Taq HS Red DNA Polymerase (Bioline) by following the manufacturer's instructions. The amplified products were shipped to Genetika Science, Indonesia for further DNA sequencing process. The retrieved sequences were aligned and compared in order to determine the closest evolutionary relatives using the Basic Local Alignment Search Tool (BLAST) in the National Centre for Biotechnology Information (NCBI) GenBank. The software Molecular Evolutionary Genetics Analysis (MEGA) X was utilized for both sequence alignment and phylogenetic identification.

RESULTS AND DISCUSSION

There were 42 isolated bacteria from chicken manure, as shown in Table 1. All of the isolates were further tested for its enzymatic activity by a qualitative test. The result showed various enzymatic activity results. There are 22 isolates showing positive results on the amylase screening, 38 isolates showing positive results on the protease screening and 20 isolates showing positive results on the lipase screening. The 10 isolates that show positive results on the 3 enzymatic screening were selected for lipase screening with fish oil and it all showed positive results (Table 1). Based on the Gram staining procedure, all isolates are rod-shaped and belong to the Gram-positive group.

Table 1. Qualitative enzymatic screening of bacteria from local microorganisms from chicken manure

Bacteria isolate code	Amylase screening	Protease screening	Lipase screening using olive oil	Lipase screening using fish oil*
A1	-	+	-	
A2	-	+	-	
A3	-	+	+	
A4	+	-	+	
A5	-	+	+	
A6	+	+	-	
A7	-	+	+	
A8	+	+	-	
A9	-	-	-	
A10	-	+	+	
A11	+	+	+	+
A12	-	+	+	
A13	-	+	+	
A14	-	+	+	
A15	-	+	-	
A16	+	+	+	+
A17	+	+	-	
A18	+	+	-	
A19	+	+	-	
A20	+	+	-	
A21	+	+	+	+
A22	+	+	+	+
A23	+	+	+	+
A24	+	+	+	+
B1	+	+	-	
B2	+	+	-	
B3	-	+	-	
B4	-	-	-	
B5	-	+	+	
B6	-	+	+	
B7	-	+	-	
B8	+	+	+	+
B9	-	+	-	
B10	+	+	+	+
B11	+	+	+	+
B12	+	+	-	
B13	+	+	-	
B14	-	+	-	
B15	+	+	-	
B16	-	+	-	
B17	-	-	-	
B18	+	+	+	+

Notes:
- (+) shows positive result, (-) shows negative result
- The lipase screening using fish oil was only conducted to the isolates that have shown positive results on amylase, protease and lipase screening.

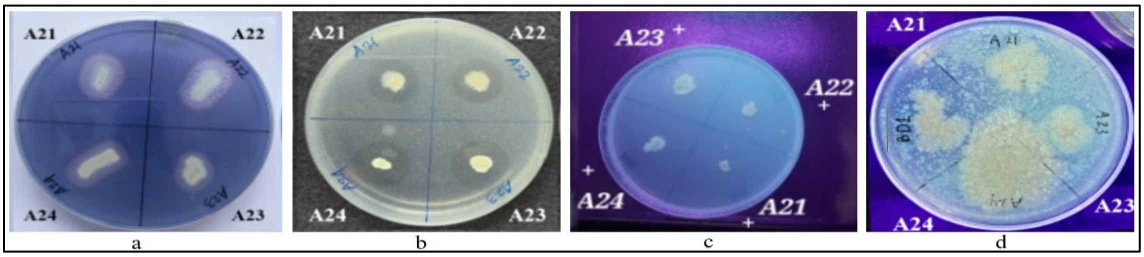

Figure 1. a) Presence of a clear zone around the microbial colony on the starch agar after addition of Lugol's solution indicates that the isolate secretes amylolytic enzymes; b) Presence of a clear zone around the microbial colony on the skim milk agar indicates that the isolate secretes proteolytic enzymes; c) Microbial colony that shows orange fluorescence under UV light indicates that the isolate secretes lipolytic enzymes on olive oil media; d) Microbial colony that showed orange fluorescence under UV light indicates that the isolate secretes lipolytic enzymes on fish oil media.

Screening of Amylolytic Bacteria

Amylase is an enzyme that hydrolyzes starch into simpler sugars, such as glucose and maltose (Sundarram & Murthy, 2014). Among the 42 isolates, 23 isolates showed the ability to hydrolyze starch (Table 1) by the formation of a clear zone around the bacterial colony after 24 h of incubation after the addition of Lugol's solution to the Starch Agar medium (Figure 1a). Brust *et al.* (2020) explained that iodine binding to amylose results in a dark blue color, whereas binding with amylopectin results in a reddish-brown color. The presence of a clear zone indicates the decomposition of starch in the medium, preventing iodine from binding to the starch.

Screening of Proteolytic Bacteria

Protease is an enzyme that can hydrolyze proteins or peptides by breaking the peptide bonds that links amino acid residues in proteins, resulting in shorter peptides and amino acids (Razzaq *et al.*, 2019). Based on research conducted, there were 40 bacterial isolates that could hydrolyze protein (Table 1) by the formation of a clear zone around the bacterial colony after incubation for 24 h on Skim Milk Agar medium (Figure 1b). Pratika *et al.* (2021) stated that a clear zone can form in media containing protein if the inoculated bacteria have proteolytic ability.

Screening of Lipolytic Bacteria

Lipase is an enzyme that can hydrolyze ester bonds in water-insoluble substrates, such as fat compounds (triglycerides), into diglycerides, monoglycerides and fatty acids (Lim *et al.*, 2022). Among the 42 isolates, there were 21 bacterial isolates that could hydrolyze fat (Table 1). It is indicated by the color of bacterial colonies that glow orange when observed under UV light after being incubated for 24 h in media containing olive oil and rhodamine B (Figure 1c). Bartasun *et al.* (2013) stated that rhodamine B binds to free fatty acids that have been decomposed from olive oil by the lipase enzyme, so that it can produce orange fluorescent light when exposed to UV light.

Further screening on 10 bacteria on media containing fish oil showed that the isolated bacteria also have the ability to break down the fat compounds that present in fish oil (Figure 1d). The composition in plant oil and animal oil are different. Olive oil has high content of fatty acids with high level of monounsaturated fatty acids (MUFA), containing up to 85% unsaturated fatty acid with high oleic acid content, linoleic acid and palmitoleic acid and 14% of saturated fatty acid such as palmitic acid and stearic acid (Jimenez-Lopez *et al.*, 2020). Meanwhile fish oil contains saturated fatty acids, MUFAs and PUFAs (Huang *et al*., 2018). In crude fish oil, the dominant saturated fatty acid is palmitic acid in 21.21 – 26.63% the dominant unsaturated fatty acid is oleic acid in 19.78 – 27.11% and dominant PUFA are eicosapentaenoic acid (EPA) in 6.02 – 9.97%, and docosahexaenoic acid (DHA) in 17.00 – 20.83% (Mgbechidinma *et al.*, 2023). Based on the enzymatic screening results of the isolates that have been conducted, six isolates with the potential to degrade amylase, protein and fat were selected for identification due to their distinct morphological characteristics from each isolate.

Molecular Identification

The phylogenetic tree generated from the 16S rDNA gene sequences of the six isolates showed that the isolates came from the same genus, namely *Bacillus*, with isolate A11 having the highest homology with *Bacillus tequilensis* strain 10b (NR 104919.1), A23 having the highest homology with *Bacillus velezensis* strain ES1-02 (OQ566944.1), A24 having highest homology with *Bacillus amyloliquefaciens* strain NBRC 15535 (NR 041455.1), B8 has the highest homology with *Bacillus amyloliquefaciens* strain W36 (MN922613.1), B10 has the highest homology with *Bacillus subtilis* strain IAM 12118 (NR 112116.2) and B18 has the highest homology with *Bacillus siamensis* KCTC 13613 strain PD-A10 (NR 117274.1). Homology data for isolates and bacteria in GenBank can be seen in Table 2.

The dominance of the *Bacillus* in local microorganisms from chicken manure can be attributed to its prolific growth in the intestines, playing a crucial role in maintaining digestive ecological balance (Su *et al*., 2020). Previous research by Abinaya *et al.* (2015) has shown that cow dung contains a consortium which has species that were used in wastewater treatment, such as *Pseudomonas aeruginosa*, *P. putida*, *Aspergillus niger*, *Bacillus cereus* and *B. subtilis.* Local microorganisms of chicken manure are representatives of all five species (Figure 2 and Figure 3).

Bacillus is a gram-positive bacteria that are rod-shaped and endospore-forming. It has the capability to ferment glucose and exploit a wide variety of organic and inorganic substances as nutritional sources. *Bacillus* can be either obligate aerobes which are dependent on oxygen, or facultative anaerobes which can survive in the lack of oxygen (Logan & Vos, 2015). The optimal temperature for its growth is 25 – 40 °C (Hlordzi *et al*., 2020), and for the pH, it varies between species and varieties, e.g. pH of *B. subtilis* ranges in 5.0 – 9.0 (Gauvry *et al*., 2021), *B. amyloliquefaciens* ranges in 5.0 – 9.0 (Gotor-Vila *et al*., 2017), *B. siamensis* ranges in 4.5 – 9.0 (Sumpavapol *et al.*, 2010), *B. tequilensis* ranges in 5.5 – 8.0 (Gatson *et al.*, 2006) and *B. velezensis* ranges in 5.0 – 10.0 (Ji *et al*., 2021). In response to nutritional or environmental stresses, *Bacillus* produces highly resilient dormant endospores that exhibit resistance to cold, heat, disinfectants, radiation and desiccation. *Bacillus* plays a role in regulating various water quality parameters, including physical aspects like total dissolved solids, and chemical factors such as pH, chemical oxygen demand and biological oxygen demand. They also contribute to mitigating oil spills and maintaining microbial balance (Hlordzi *et al*., 2020).

Table 2. Molecular identification of potential strain

Bacteria isolate code	Strain	Max score	E-value	Percent identity	GenBank accession
A11	*Bacillus tequilensis* strain 10b	1544	0.0	98.52%	NR 104919.1
A23	*Bacillus velezensis* strain ES1-02	2233	0.0	97.77%	OQ566944.1
A24	*Bacillus amyloliquefaciens* strain NBRC 15535	1770	0.0	98.41%	NR 041455.1
B8	*Bacillus amyloliquefaciens* strain W36	2123	0.0	97.75%	MN922613.1
B10	*Bacillus subtilis* strain IAM 12118	1812	0.0	99.30%	NR 112116.2
B18	*Bacillus siamensis* KCTC 13613 strain PD-A10	1517	0.0	97.42%	NR 117274.1

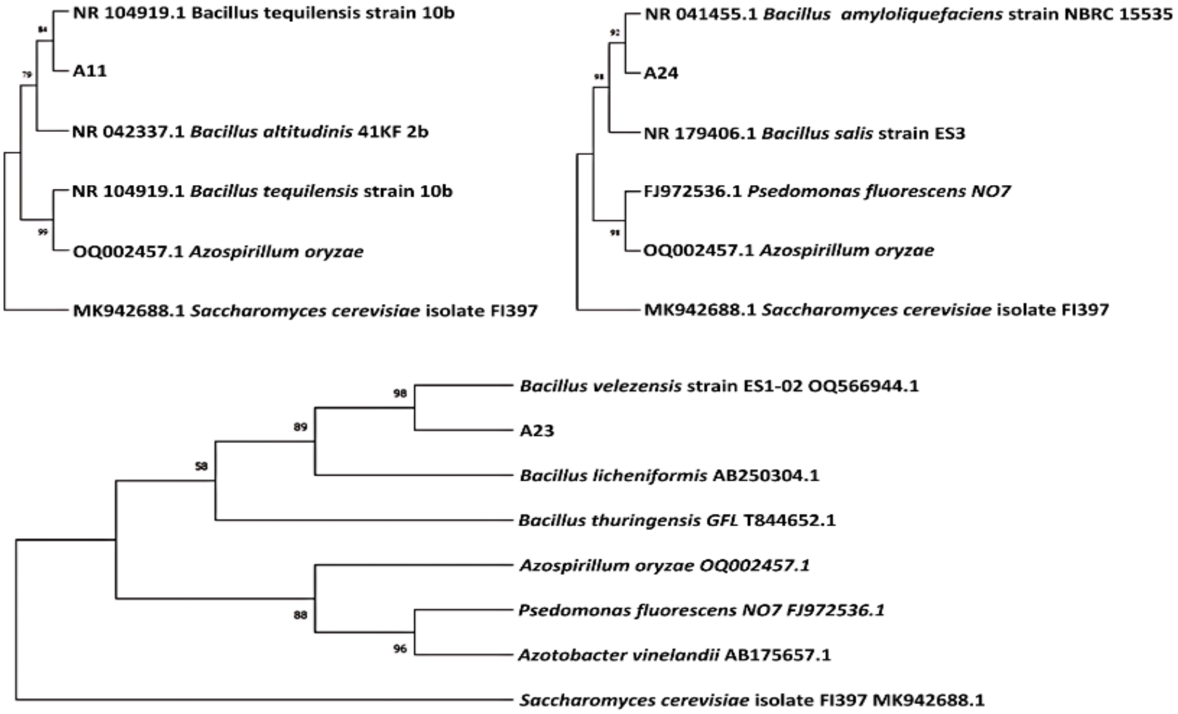

Figure 2. Phylogenetic analysis of identified isolates A11, A23, A24

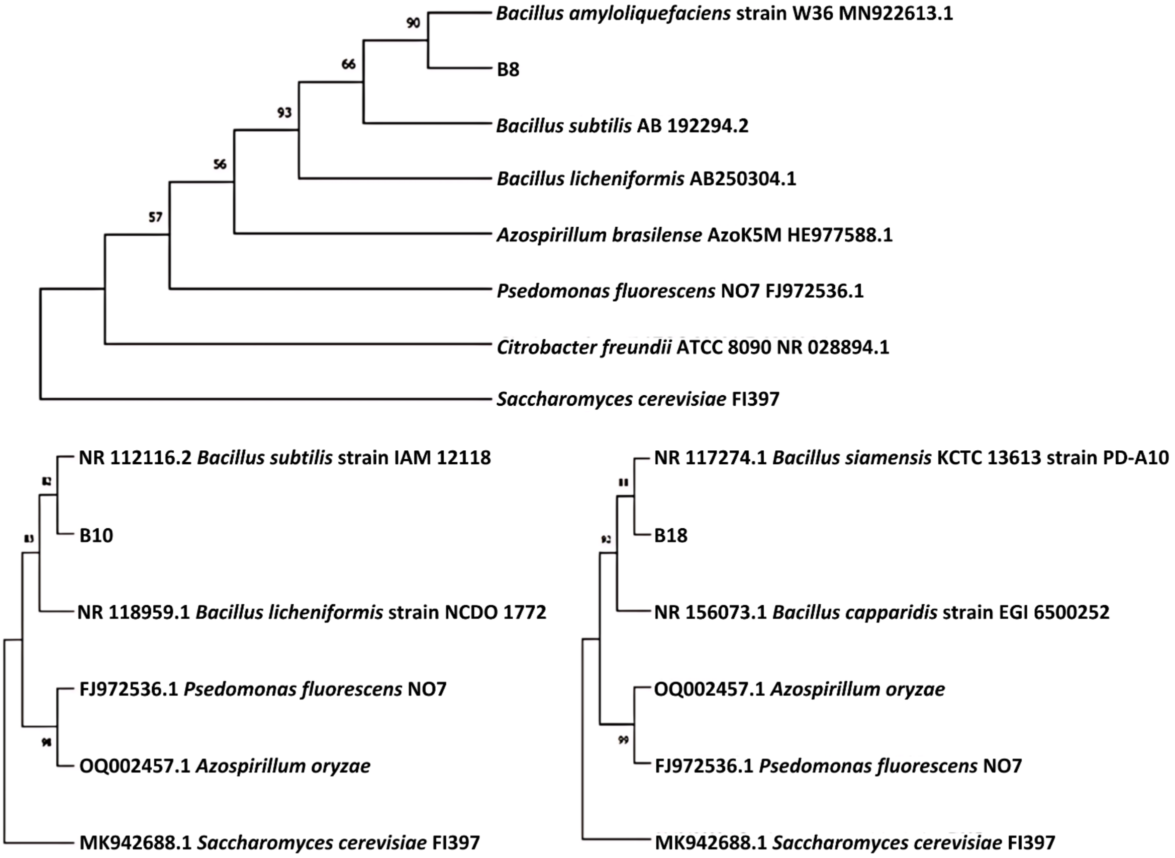

Figure 3. Phylogenetic analysis of identified isolates B8, B10 and B18

Based on previous research, various species of bacteria from the genus *Bacillus* have potential in waste treatment applications. In a study by Othman *et al*. (2023), *B. B. tequilensis*, for instance, shows the ability to degrade ammonia in aquaculture liquid waste with a degradation rate of 89.9% on 100 - 500 mg/L of ammonia concentration (Wang *et al*., 2023). *B. velezensis* has demonstrated potential in treating various types of wastewater, such as slaughter wastewater (Deng *et al.*, 2022), brewery wastewater (Agunbiade *et al.,* 2022), and pulp and paper wastewater (Nair et al., 2020).*velezensis* was utilized for processing domestic liquid waste from various human activities, encompassing residential, industrial, commercial, livestock and agricultural sectors. The common contaminants found in this liquid waste include organic compounds, nitrogen, phosphorus, heavy metals and other chemical compounds. The analysis conducted by Othman *et al*. (2023) on chemical oxygen demand (COD) levels in the treated domestic liquid waste revealed a decrease after 216 h, from 24 mg/L up to 16.90 mg/L. Additionally, total suspended solid (TSS) analysis showed a significant reduction in the amount of suspended solids from 7.50 mg/L up to 6.41 mg/L. *B. amyloliquefaciens* has shown the ability to treat domestic wastewater by decreasing the concentration of TSS from 2300 mg/L to 1400 mg/L and COD from 925 mg/L up to 133 mg/L (El-Bestawy *et al*., 2014). *B. subtilis* has demonstrated its efficacy in treating domestic liquid waste with a reduction in biochemical oxygen demand (BOD) values by over 85% until lower than 20 mg/L within two weeks (Meekwamdee *et al.*, 2023). Furthermore, its application has been found to decrease the total value of organic compounds decreased from 86.58 mg/L to 12.74 mg/L, with an efficiency of 85.29% (Anggraini *et al*., 2019) and significantly increase the denitrification rate with a nitrate decomposition percentage of up to 96%, reducing nitrate concentration to 3.2 mg/L (Rahimi *et al*., 2020). In a study conducted by Prasad & Manjunath (2011), the utilization of a bacterial consortium including *B. subtilis, B. licheniformis, B. amyloliquefaciens, Serratia marcescens, Pseudomonas aeruginosa* and *Staphylococcus aureus*, was examined for the treatment of liquid waste generated by various industrial processes, decreasing BOD from 3500 mg/L to less than 20 mg/L in 12 days. The application of the *B. siamensis* strain ZF1-1 to livestock waste with high levels of nitrogen and ammonia resulted in a significant reduction from 1277.41 mg/L, with total nitrogen concentration decreased to 22.22% and ammonia to 37.50% (Chen *et al*., 2020). Additionally, Mehetre *et al*. (2019) found that *B. siamensis* possesses the ability to synthesize bio-surfactant agents, aiding in the binding of waste containing fats and oils.

Additionally, previous research by Sahendra *et al.* (2021) showed that cow dung can be used as an adsorbent in liquid waste processing with COD values of 12 mg/L, BOD 28.72 mg/L, TSS 26.67 mg/L and pH of 6, but there were no mentions of possible microorganisms that were present in the cow dung. Another research by Safitri *et al.* (2015) shows that the use of bacterial consortium including *Bacillus coagulans, B. licheniformis, B. pumilus, B. subtilis, Nitrosomonas* sp. and *Pseudomonas putida* were able to reduce the amount of pollutant from industrial wastewater up to 71.93% of BOD, 64.30% of COD, 94.85% of TSS, and 88.58% of ammonia. Research from John *et al*. (2020) has also used bacterial consortium consisting of *B. cereus, B. amyloliquefaciens* and *P. stutzeri* to maintain ammonia and nitrite below toxic level in aquaculture wastewater. Biswas *et al*. (2022) has also utilized bacterial consortium consisting of various genera dominated by *Bacillus, Vibrio* and *Staphylococcus* species for petrochemical wastewater treatment to reduce up to 88% of BOD content.

CONCLUSION

Based on the research results, among the 42 isolated bacteria from local microorganisms of chicken manure, six bacteria isolates demonstrated the ability to hydrolyze starch, protein and fat based on qualitative tests. These isolates, A11, A23, A24, B8, B10 and B18, all belong to the *Bacillus* genus. The qualitative tests confirmed the potential of these isolates as they exhibited enzymatic activities. Considering their ability, these isolates show promising potential as a bioactivator in the liquid waste treatment.

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REFERENCES

Abd-Elhalim, B.T., Gamal, R.F., El-Sayed, S.M. & Abu-Hussien, S.H. (2023). Optimizing alpha-amylase from Bacillus amyloliquefaciens on bread waste for effective industrial wastewater treatment and textile desizing through response surface methodology. *Scientific Reports*, 13(1): p.19216. DOI: 10.1038/s41598-023-46384-6.

Agunbiade, M., Oladipo, B., Ademakinwa, A.N., Awolusi, O., Adesiyan, I.M., Oyekola, O., Ololade, O. & Ojo, A. (2022). Bioflocculant produced by *Bacillus velezensis* and its potential application in brewery wastewater treatment. *Scientific Reports*, 12(1). DOI: 10.1038/s41598-022-15193-8.

Anggraini, S. I., Arfiati, D. & Nursyam, H. (2019). Effectiveness of *Bacillus subtilis* bacteria as a total organic matter reducer in catfish pond (Clariasgariepinus) cultivation. *International Journal of Biotech Trends and Technology*, 9(2): 7–10. DOI: 10.14445/22490183/ijbtt-v9i2p602.

Bartasun, P., Cieśliński, H., Bujacz, A., Wierzbicka-Woś, A. & Kur, J. (2013). A study on the interaction of rhodamine B with methylthioadenosine phosphorylase protein sourced from an Antarctic soil metagenomic library. *PLoS One*, 8(1): p.e55697. DOI: 10.1371/journal.pone.0055697.

Beric, T., Urdaci, M., Stankovic, S. & Knezevic-Vukcevic, J. (2009). RAPD analysis of genetic diversity and qualitative assessment of hydrolytic activities in a collection of *Bacillus* sp. isolate. *Archives of Biological Sciences*, 61(4): 645–652. DOI: 10.2298/abs0904645b.

Biswas, T., Banerjee, S., Saha, A., Bhattacharya, A., Chanda, C., Gantayet, L.M., Bhadury, P. & Chaudhuri, S.R. (2022). Bacterial consortium based petrochemical wastewater treatment: from strain isolation to industrial effluent treatment. *Environmental Advances*, 7: 100132. DOI: 10.1016/j.envadv.2021.100132.

Brust, H., Orzechowski, S., & Fettke, J. (2020). Starch and glycogen analyses: methods and techniques. *Biomolecules*, 10(7): 1020. DOI: 10.3390/biom10071020.

Chen, M., Li, A., Yang, M., Zhang, M., Zhou, L. & Zhu, H. (2020). Analysis of a heterotrophic nitrification bacteria isolated from livestock and poultry wastewater and its nitrogen removal characteristics. *Modern Food Science and Technology*, 10: 50–58.

Deng, J., Jia, M., Zeng, Y. Q., Li, W., He, J., Ren, J., Bai, J., Zhang, L., Li, J. & Yang, S. (2022). Enhanced treatment of organic matter in slaughter wastewater through live *Bacillus velezensis* strain using nano zinc oxide microsphere. *Environmental Pollution*, 292: 118306. DOI: 10.1016/j.envpol.2021.118306.

Earl, A.M., Losick, R. and Kolter, R. (2008). Ecology and genomics of Bacillus subtilis. *Trends in microbiology*, 16(6): pp.269-275. DOI: 10.1016/j.tim.2008.03.004.

El-Bestawy, E. (2014). Decontamination of Domestic Wastewater Using Suspended Individual and Mixed Bacteria in Batch System. *Journal of Bioremediation & Biodegradation*, 05(05). DOI: 10.4172/2155-6199.1000231.

Gameissa, M.W. & Suprihatin, N.S.I. (2012). Pengolahan tersier Limbah Cair Industri Pangan dengan Teknik Elektrokoagulasi Menggunakan Elektroda Stainless Steel. *Agro Industri Indonesia*, 1(1): 31–37.

Gatson, J.W., Benz, B.F., Chandrasekaran, C., Satomi, M., Venkateswaran, K. & Hart, M.E. (2006). *Bacillus tequilensis* sp. nov., isolated from a 2000-year-old Mexican shaft-tomb, is closely related to *Bacillus subtilis*. *International Journal of Systematic and Evolutionary Microbiology*, 56 (7): 1475–1484. DOI: 10.1099/ijs.0.63946-0.

Gautam, A. (2022). DNA *Isolation by Chelex method in DNA and RNA isolation techniques for non-experts*. Cham: Springer International Publishing. pp. 79–84.

Gauvry, E., Mathot, A. G., Couvert, O., Leguérinel, I. & Coroller, L. (2021). Effects of temperature, pH and water activity on the growth and the sporulation abilities of *Bacillus subtilis* BSB1. *International Journal of Food Microbiology, 337*: 108915. DOI: 10.1016/j.ijfoodmicro.2020.108915.

Gotor-Vila, A., Teixidó, N., Sisquella, M., Torres, R. & Usall, J. (2017). Biological characterization of the biocontrol agent *Bacillus amyloliquefaciens* CPA-8: The effect of temperature, pH and water activity on growth, susceptibility to antibiotics and detection of enterotoxic genes. *Current Microbiology*, 74(9): 1089–1099. DOI: 10.1007/s00284-017-1289-8.

Hadi, R.A. (2019). Pemanfaatan MOL (Mikroorganisme Lokal) dari Materi yang Tersedia di Sekitar Lingkungan. *Agroscience (AGSCI)*, 9(1): 93. DOI:

10.35194/agsci.v9i1.637.

Hlordzi, V., Kuebutornye, F.K.A., Afriyie, G., Abarike, E.D., Lu, Y., Chi, S. & Anokyewaa, M.A. (2020). The use of *Bacillus* species in maintenance of water quality in aquaculture: A review. *Aquaculture Reports*, 18: 100503. DOI: 10.1016/j.aqrep.2020.100503.

Huang, T.H., Wang, P.W., Yang, S.C., Chou, W.L. & Fang, J.Y. (2018). Cosmetic and therapeutic applications of fish oil's fatty acids on the skin. *Marine Drugs*, 16(8): 256. DOI: 10.3390/md16080256.

Hui, C., Guo, X., Sun, P., Khan, R.A., Zhang, Q., Liang, Y. & Zhao, Y.H. (2018). Removal of nitrite from aqueous solution by *Bacillus amyloliquefaciens* biofilm adsorption. *Bioresource Technology*, 248: 146–152. DOI: 10.1016/j.biortech.2017.06.176.

Indasah, I. & Muhith, A. (2020). Local Microorganism From "Tape" (Fermented Cassava) In composition and its effect on physical, chemical and biological quality in environmental. IOP Conference Series: *Earth and Environmental Science*, 519: 012003. DOI: 10.1088/1755-1315/519/1/012003.

Ji, C., Wang, X., Song, X., Zhou, Q., Li, C., Chen, Z., Gao, Q., Li, H., Li, J., Zhang, P. & Cao, H. (2021). Effect of *Bacillus velezensis* JC-K3 on endophytic bacterial and fungal diversity in wheat under salt stress. *Frontiers in Microbiology*, 12. DOI: 10.3389/fmicb.2021.802054.

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., Lorenzo, J.M., Barba, F.J., Prieto, M.A. & Simal-Gandara, J. (2020). Bioactive compounds and quality of extra virgin olive oil. *Foods*, 9(8): 1014. DOI: 10.3390/foods9081014.

John, E.M. Krishnapriya, K. & Sankar, T. (2020). Treatment of ammonia and nitrite in aquaculture wastewater by an assembled bacterial consortium. *Aquaculture*, 526: 735390. DOI: 10.1016/j.aquaculture.2020.735390.

Lim, S.Y., Steiner, J.M. & Cridge, H. (2022). Lipases: it's not just pancreatic lipase! *American Journal of Veterinary Research*, 83(8). DOI: 10.2460/ajvr.22.03.0048.

Logan, N.A. & Vos, P.D. (2015). Bacillus. *Bergey's manual of systematics of archaea and bacteria*, pp.1-163. DOI: 10.1002/9781118960608.gbm00 530.

Meekwamdee, B., Suebsaiprom, W. & Chunhachart, O. (2023). Study on effective of *Bacillus subtilis* for domestic wastewater treatment in the royal thai army chemical department area. *Journal of Namibian Studies: History Politics Culture*, 33. DOI: 10.59670/jns.v33i.1014.

Mehetre, G. T., Dastager, S. G. & Dharne, M. S. (2019). Biodegradation of mixed polycyclic aromatic hydrocarbons by pure and mixed cultures of biosurfactant producing thermophilic and thermo-tolerant bacteria. *Science of the Total Environment*, 679: 52–60. DOI: 10.1016/j.scitotenv.2019.04.376.

Mgbechidinma, C.L., Zheng, G., Baguya, E.B., Zhou, H., Okon, S.U. & Zhang, C. (2023). Fatty acid composition and nutritional analysis of waste crude fish oil obtained by optimized milder extraction methods. *Environmental Engineering Research*, 28(2). DOI: 10.4491/eer.2022.034.

Nair, A.S., Al-Bahry, S. & Sivakumar, N. (2020). Co-production of microbial lipids and biosurfactant from waste office paper hydrolysate using a novel strain Bacillus velezensis ASN1. *Biomass Conversion and Biorefinery*, 10(2): pp.383-391. DOI: 10.1007/s13399-019-00420-6.

Othman, M.F., Abu Hasan, H., Muhamad, M.H. & S. Babaqi, B. (2023). Biopolishing of Domestic Wastewater Using Polyvinyl Alcohol – Supported Biofilm of Bacterial Strain *Bacillus velezensis* Isolate JB7. *Journal of Ecological Engineering.* 24(8): 33–42. DOI: 10.12911/22998993/165780.

Pervez, M.N., Mishu, M.R., Stylios, G.K., Hasan, S.W., Zhao, Y., Cai, Y., Zarra, T., Belgiorno, V. & Naddeo, V. (2021). Sustainable treatment of food industry wastewater using membrane technology: A short review. *Water*, 13(23): p.3450. DOI: 10.3390/w13233450.

Prasad, M.P. & Manjunath, K. (2011). Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacterial species. *Indian Journal of Biotechnology*, 10: 121–124.

Pratika, M., Ananda, M. & Suwastika, I.N. (2021). Protease activity from bacterial isolates of Nepenthes maxima reinw. ex nees. *Journal of Physics: Conference Series*, 1763(1): 012092. DOI: 10.1088/1742-6596/1763/1/012092

Rahimi, S., Modin, O., Roshanzamir, F., Neissi, A., Saheb Alam, S., Seelbinder, B., Pandit, S., Shi, L. & Mijakovic, I. (2020). Co-culturing *Bacillus subtilis* and wastewater microbial community in a bio-electrochemical system enhances denitrification and butyrate formation. *Chemical Engineering Journal*, 397: 125437. DOI: 10.1016/j.cej.2020.125437.

Ravel J. & Fraser C.M. (2005). Genomics at the genus scale. *Trends Microbiol*, 13(3): 95-97. DOI: 10.1016/j.tim.2005.01.004.

Razzaq, A., Shamsi, S., Ali, A., Ali, Q., Sajjad, M., Malik, A. & Ashraf, M. (2019). Microbial proteases applications. *Frontiers in Bioengineering and Biotechnology*, 7. DOI: 10.3389/fbioe.2019.00110.

Safitri, R., Priadie, B., Miranti, M. and Astuti, A.W. (2015). Ability of bacterial consortium Bacillus coagulans, Bacilus licheniformis, Bacillus pumilus, Bacillus subtilis, Nitrosomonas sp., and Pseudomonas putida in bioremediation of Waste Water in Cisirung Waste Water Treatment Plant. *AgroLife Scientific Journal*, 4(1): 146-152.

Sahendra, S.L., Hamsyah, R.A. & Sa'diyah, K., (2021). Pengolahan Limbah Cair Pabrik Gula Menggunakan Adsorben dari Kotoran Sapi dan Ampas Tebu. *Chemical Engineering Research Articles*, 4(1): pp.31-38. DOI: 10.25273/cheesa.v4i1.8416.31-38.

Sandle, T. (2016). Pharmaceutical Microbiology. *Woodhead Publishing.* DOI: 10.1016/c2014-0-00532-1.

Sondhi, S., Kumar, D., Angural, S., Sharma, P. & Gupta, N. (2018). Enzymatic approach for bioremediation of effluent from pulp and paper industry by thermo alkali stable laccase from *Bacillus tequilensis* SN4. *Journal of Commercial Biotechnology*, 23(4). DOI: 10.5912/jcb799.

Su, Y., Liu, C., Fang, H. & Zhang, D. (2020). Bacillus subtilis: a universal cell factory for industry, agriculture, biomaterials and medicine. *Microbial Cell Factories*, 19 (1). DOI: 10.1186/s12934-020-01436-8.

Sundarram, A. & Murthy, T.P.K. (2014). α-amylase production and applications: a review. *Journal of Applied & Environmental Microbiology*, 2(4): pp.166-175. DOI: 10.12691/jaem-2-4-10.

Sumpavapol, P., Tongyonk, L., Tanasupawat, S., Chokesajjawatee, N., Luxananil, P., and Visessanguan, W. (2010). *Bacillus siamensis* sp. nov., isolated from salted crab (poo-khem) in Thailand. *International Journal of Systematic and Evolutionary Microbiology*, 60(10):2364–2370. DOI: 10.1099/ijs.0.018879-0.

Turnbull PCB. (1996). *Bacillus*. In: Baron S, editor. Medical microbiology. 4th ed. galveston (TX): University of Texas Medical Branch at Galveston. Chapter 15. PMID: 21413260.

Wang, Z., Liu, H. & Cui, T. (2023). Identification of a strain degrading ammonia nitrogen, optimization of ammonia nitrogen degradation conditions, and gene expression of key degrading enzyme nitrite reductase. *Fermentation*, 9(4): 397. DOI: 10.3390/fermentation9040397.

Yunilas, Y., Siregar, A.Z., Mirwhandhono, E., Purba, A., Fati, N., & Malvin, T. (2022). Potensi dan Karakteristik Larutan Mikroorganisme Lokal (MOL) Berbasis Limbah Sayur sebagai Bioaktivator dalam Fermentasi. *Journal of Livestock and Animal Health*, 5(2), 53–59. DOI: 10.32530/jlah.v5i2.540.

Zaman, B., Sutrisno, E., Sudarno, S., Simanjutak, M.N. & Krisnanda, E. (2020). Natural Soil as Bio-activator for Wastewater Treatment System. *IOP Conference Series: Earth and Environmental Science*, 448(1): 012032. DOI: 10.1088/1755-1315/448/1/012032.