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Effect of Sidoarjo Mud Inoculant and *Bacillus subtilis* on The Degradation of Tofu Liquid Waste in Microbial Fuel Cell Continuous Series Reactors

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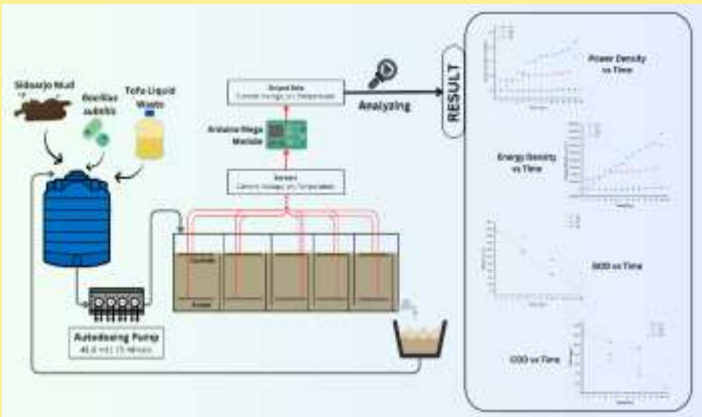
***Abstract*—** The tofu industry in Indonesia generates large amounts of liquid waste with high BOD and COD, causing significant environmental impact. Microbial Fuel Cells (MFC) offer a sustainable solution for treating organic waste while generating electricity. Effective treatment of tofu liquid waste is essential. This study explores the use of Sidoarjo mud and *Bacillus subtilis* as inoculants in MFC systems, integrated with an advanced reactor design for improved degradation of pollutants. A Continuous Series Reactor was employed, equipped with an auto dosing pump for precise substrate feeding, optimizing microbial activity. The system was monitored using an Arduino Mega data logger and an ESP01 module for remote data transmission. The effectiveness of Sidoarjo mud and *Bacillus subtilis* was tested by measuring BOD, COD, and protein removal, with different inoculant combinations compared to a control group. The control group achieved BOD and COD removal rates of 70.43% and 47.92%, respectively. Sidoarjo mud alone improved these to 94.30% and 91.84%, while combining Sidoarjo mud with *Bacillus subtilis* increased removal rates to 94.70% and 92.55%. Protein degradation also improved by 79.79% with *Bacillus subtilis*. *Bacillus subtilis*, used as a pure laboratory strain, was obtained from the Microbiology Laboratory of the Department of Chemical Engineering, Sepuluh Nopember Institute of Technology and selected for its strong proteolytic activity. During the operation, the system achieved a power density of 214,179.571 mW/m^2, confirming its electrogenic potential. Sidoarjo mud and *Bacillus subtilis* effectively enhance waste degradation in MFC systems, providing a sustainable solution for tofu waste treatment.



***Keywords*—** *Bacillus subtilis*, Sidoarjo mud, Tofu liquid waste

I. INTRODUCTION

The tofu industry in Indonesia produces a very large amount of liquid waste, with a volume of 3,065.4 liters per day for every 150 kg of soybeans processed, or around 20.4 m^3/ton per day [1]. This liquid waste contains significant organic compounds, consisting of proteins (40-60%), carbohydrates (25-50%), and fats (10%) [2]. The high organic content results in a BOD value of 6,000-8,000 mg/l and a COD value of 7,500-8,400 mg/l [3]. This value far exceeds the standard for wastewater quality regulated in the Regulation of the Minister of Environment of the Republic of Indonesia Number 5 of 2014, which requires BOD and COD limits of 150 mg/l and 300 mg/l respectively [4]. If this waste is not managed properly, it can cause significant environmental damage, especially to water quality around disposal areas. Although it has a high potential for pollution, tofu liquid waste actually has potential that can be utilized, especially because of the high protein content with one of the waste treatment technologies, namely Microbial Fuel Cell (MFC). MFCs use bacterial metabolism to generate electricity from a variety of organic substrates, potentially reducing organic levels and providing sustainable energy production [5]

MFCs represent a promising technology for converting organic pollutants into bioenergy while simultaneously treating wastewater in a sustainable manner [5]. Despite this potential, most previous research has relied on naturally occurring or commercial microbial inoculants, with limited exploration of locally adapted microbial consortia capable of thriving under extreme environmental conditions. In addition, many of these studies employed batch-type MFC systems, which often suffer from inconsistent substrate availability, unstable power output, and limited treatment efficiency over time. For instance, the previous study reported that batch-mode MFCs treating cassava wastewater exhibited poor COD removal and erratic energy performance, highlighting the need for more robust reactor designs and microbial strategies [6].

To address these limitations, this study applied an MFC within a continuous series reactor system—a configuration designed to enhance microbial stability and ensure long-term operational performance. The reactor facilitates a steady flow of tofu liquid waste using an auto-dosing mechanism and a substrate recycling loop, enabling better control over residence time and microbial activity. This system represents a novel departure from conventional batch MFC designs, prioritizing consistent treatment efficiency while also enabling energy recovery as a secondary benefit.

In this study, two types of inoculants were used: Sidoarjo mud and *Bacillus subtilis*. The physical properties of Sidoarjo mud—being thick, water-rich, and nutrient-laden—provide a favorable environment for microbial growth. Bacterial isolates from this mud have demonstrated the ability to survive in extreme conditions, and various species with biotechnological potential have been identified [7].

Sequencing analysis revealed a rich microbial diversity consisting of 626 genera and over 1,200 species, including *Pseudomonas*, *Brucella*, *Klebsiella*, *Achromobacter*, *Stenotrophomonas*, and *Shewanella*. While *Shewanella* is known for its electrogenic capabilities, many of these genera are also recognized for their enzymatic capacity to degrade complex organic compounds, making Sidoarjo mud suitable not only for bioelectricity generation but more importantly for enhancing the breakdown of organic pollutants. Meanwhile, *Bacillus subtilis* was selected due to its strong proteolytic activity. It produces enzymes such as subtilisin, which can efficiently break down proteins into amino acids and peptides [8], thereby facilitating further microbial degradation.

This study primarily focused on the degradation of organic matter in tofu liquid waste, with electricity generation considered a secondary benefit. To support this objective, a continuous series reactor was employed, enabling stable substrate flow and precise control of microbial processes throughout the treatment. The integration of *Bacillus subtilis*, a proteolytic bacterium, with the diverse microbial community from Sidoarjo mud significantly improved biodegradation performance. The combination of these two inoculants, each contributing complementary metabolic capabilities, within a continuous reactor system represents the main novelty of this study—demonstrating a synergistic and more effective strategy for sustainable wastewater treatment, with electricity production as an added value.

II. METHOD

A. Reactor Preparation

This study focuses on optimizing bioelectricity production in a microbial fuel cell (MFC) system using a modified continuous-series Stacked MFC. The reactor features five horizontal stacks separated by double baffles, enhancing microbial colonization, electron transfer, and substrate utilization. The reactor, made of 8 mm thick glass, has dimensions of 100 cm in length, 20 cm in width, and 30 cm in height, providing a 40-liter operational volume to support stable microbial activity.

Key components include an auto-dosing pump for controlled substrate flow. Additionally, a closed-loop recycling system reintroduces 50% of processed tofu wastewater effluent back into the reactor, promoting sustainability and improving bioconversion efficiency over three 15-day cycles. This approach optimizes bioelectricity production while ensuring long-term system stability.

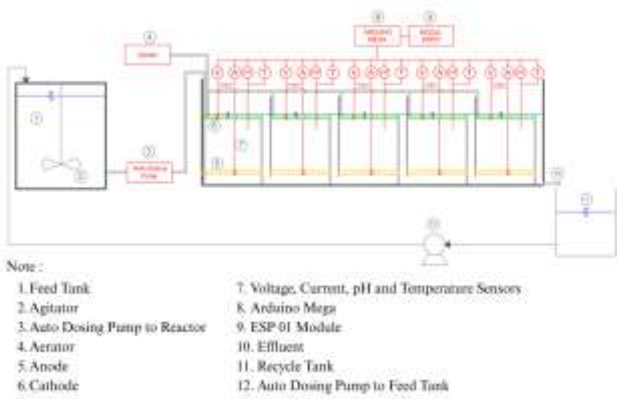


Figure 1. Design of Reactor

The tofu liquid waste substrate is first in the feed tank, then drained to the reactor using an auto dosing pump. The inflow starts from the base of the reactor and then rises and overflows through the double baffle to the last double baffle (right side of Figure 1). After that, the substrate goes to the recycle tank and will go through the recycle process three times which is drained using the second auto dosing pump.

B. Electrodes And Sensors Implantation

In this study, electrodes are modified with carbon felt 5F to improve performance and durability. The rectangular electrodes (17.5 cm x 10 cm) provide a surface area of 175 cm^2. The system includes pH, temperature, current, and voltage sensors for real-time data monitoring, with the data stored digitally for analysis. Arduino Mega is used for its capability in managing sensors, while ESP01 ensures internet connectivity. Sensors are calibrated to ensure accurate data measurement. The system is designed to log data via Wi-Fi to a spreadsheet, with coding for sensor data reading, internet management, and automated logging

C. Media Preparation

In this study, two types of media were utilized: Nutrient Broth and Minimum Medium. The Nutrient Broth was employed to support the general growth of bacterial cultures, providing a rich nutrient environment conducive to the proliferation of a wide range of microorganisms. On the other hand, the Minimum Medium was used to create a more defined and controlled environment, allowing for the study of bacterial growth under specific nutrient limitations.

The preparation of Nutrient Broth begins by weighing 0.8 grams of Nutrient Broth, 0.2 grams of yeast extract, and 0.2 grams of D-glucose. These components are then dissolved in 100 mL of distilled water in a beaker glass.

The preparation of Minimum Medium involves weighing 10 grams of NH_4NO_3, 10 grams of KH_2PO_4, 13.1 grams of K_2HPO_4, 1 gram of $MgSO_4.7H_2O$, 1 gram of $FeCl_3.6H_2O$, and 0.1 grams of $CaCl_2.2H_2O$. These components are then dissolved in 1000 mL of distilled water in a beaker glass.

The dissolution process is carried out using a hot plate set at a temperature of 80°C, accompanied by a magnetic stirrer rotating at 500 rpm until the solution becomes homogeneous. The final step is to sterilize the media using an autoclave for 15 minutes at 121°C to ensure that all potential microorganisms are eliminated, making the media sterile.

D. Inoculation

Bacillus subtilis bacteria are inoculated with both sterile media that have been made beforehand. Incubation for 4.5 hours according to the log phase of *Bacillus subtilis*, the inoculant is ready for use.

E. Start Up

The tofu liquid waste substrate is mixed with *Bacillus subtilis* inoculant according to the specified variables and left for 24 hours to react with the tofu wastewater. Meanwhile, the reactor assembly, with electrodes and sensors installed, is filled with Sidoarjo mud according to the predetermined variables. The auto-dosing pump is then activated to transfer the mixture of tofu wastewater substrate and *Bacillus subtilis* into the reactor. The process lasts for 15 days with three cycles of substrate recycling.

F. Materials

The tofu liquid waste utilized in this study was sourced from a tofu factory located at Jalan Tambang Boyo 132, Surabaya, Indonesia. Meanwhile, the Sidoarjo mud material employed in the research was collected from the gas drilling site operated by PT Lapindo Brantas, located in Siring Village, Porong District, Sidoarjo Regency. The precise coordinates of the sampling location are 112.704737° E longitude and -7.525114° S latitude.

G. Research Variable

This study will be conducted by varying the ratio of the mixed medium of tofu wastewater substrate and bacterial inoculants (V/V) as shown in Table 1.

TABLE 1.
RESEARCH VARIABLE

Code	Contain
V1	Tofu Liquid Waste 40 L (Control Variable)
V2	Tofu Liquid Waste 35 L + Sidoarjo Mud 5 L
V3	Tofu Liquid Waste 35 L + Sidoarjo Mud 2.5 L + *Bacillus subtilis* 2.5 L

Each variation was tested in a single operational run, as the aim of this study was to explore performance trends across inoculant types within a controlled system configuration. Although replication was not performed, the experiment was carefully monitored, and all key parameters were measured using consistent procedures to ensure data reliability.

H. Test Procedures

Bacterial cell enumeration was performed using a hemocytometer (Neubauer counting chamber). A diluted sample of the microbial suspension was stained and loaded onto the counting chamber. Under a light microscope at 400x magnification, the number of cells in five large squares (each 1 mm^2) was counted.

To analyze microbial diversity within the Sidoarjo mud inoculant, RNA-based full-length 16S rRNA sequencing was conducted using Oxford Nanopore Technology (ONT). This long-read sequencing platform enables coverage of all nine variable regions (V1–V9) of the 16S gene, allowing for high-resolution taxonomic identification at the genus and species levels. The process involved RNA extraction, reverse transcription to cDNA, library preparationing barcoded adapters, and sequencing using a portable MinION device. Basecalling and bioinformatics analysis were performed using ONT's

Guppy and EPI2ME pipelines, with taxonomic classification based on the NCBI 16S rRNA database.

COD was measured using the closed reflux method, which determines the oxygen equivalent of organic matter in the sample. In this process, the sample was mixed with potassium dichromate ($K_2Cr_2O_7$) as an oxidizing agent in the presence of concentrated sulfuric acid and silver sulfate catalyst. The reaction mixture was refluxed in a sealed digestion tube at 150°C for 2 hours. After cooling, the residual dichromate was titrated with standardized ferrous ammonium sulfate (FAS) using ferroin indicator. COD values were calculated based on the volume of FAS consumed.

BOD was determined using the dilution method with a dissolved oxygen (DO) meter. Initial DO concentration (DO_0) of the sample was measured using a calibrated DO probe. The sample was then incubated in the dark at 20°C for 5 days in a BOD bottle to prevent photosynthesis. After incubation, final DO (DO_5) was measured. BOD_5 was calculated as the difference between DO_0 and DO_5, representing the amount of oxygen consumed by microbial respiration in degrading organic matter.

Total protein content was determined using the Kjeldahl method, which quantifies total nitrogen as a proxy for protein. The procedure involved three stages. First is digestion, 1 g of sample was digested with concentrated sulfuric acid (H_2SO_4) and a catalyst mixture (copper sulfate + potassium sulfate) at 400°C until a clear solution was obtained, converting organic nitrogen into ammonium sulfate. Second is distillation, After cooling, the digested sample was neutralized with excess NaOH, releasing ammonia, which was distilled and absorbed in a boric acid solution. Third is titration, The absorbed ammonia was titrated with standardized H_2SO_4.

To determine the significance of different inoculants (Sidoarjo mud and *Bacillus subtilis*) on treatment outcomes (BOD, COD, and protein reduction), Analysis of Variance (ANOVA) was conducted using linear regression modeling. The statistical analysis tested both individual and interaction effects of variables, with significance determined by F-values and p-values (threshold: $p < 0.05$). The regression output quantified the relative influence of each inoculant type on treatment efficiency, enabling objective comparison across experimental conditions.

III. RESULTS AND DISCUSSION

A. Number of Bacteria

In the control variable, no pH adjustment was made using 1 M NaOH, resulting in extreme pH conditions from day 0 to day 5. This caused a 26% decrease in bacterial count, as shown in Figure 2, due to the stressful pH environment, leading to bacterial cell death. From day 5 to day 10, the pH conditions became more neutral, stabilizing the bacterial population. However, from day 10 to day 15, there was an additional 25% decrease in bacterial count, likely caused by the accumulation of acidic metabolic byproducts, which disrupted the pH balance in the microbial environment.

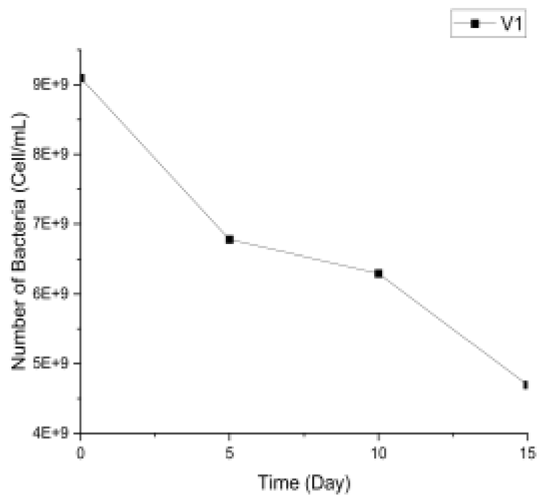


Figure 2. Number of Bacteria vs Time V1

In variables 2 and 3, pH adjustments were made to maintain an optimal microbial environment, as illustrated in Figure 3. In the second variable, an inoculant from Sidoarjo mud was utilized. From day 0 to day 5, bacterial counts increased by 45% and 51%, respectively, indicating a lag phase where microorganisms adapted to the new environment and began producing enzymes necessary for substrate degradation. The duration of this phase is influenced by environmental conditions and wastewater characteristics [9]. From day 5 to day 10, bacterial counts increased by 52% and 53%, respectively, marking the log or exponential phase, characterized by rapid microbial growth due to an abundance of nutrients [10]. From day 10 to day 15, bacterial counts stabilized, entering the stationary phase, where growth rates declined due to nutrient limitations.

Sequencing results of bacterial isolates from Sidoarjo mud revealed 626 genera, 1,277 species, and 133 microbial strains, with the top 10 genera being *Brucella, Castellaniella, Klebsiella, Pseudomonas, Achromobacter, Shewanella, Ochrobactrum, Stenotrophomonas, Rhizobium, and Agrobacterium*, as shown in Figure 4. *Shewanella* can utilize organic compounds as electron donors and transfer electrons to the anode, enhancing the efficiency of bioenergy conversion into electricity. In the Sidoarjo mud inoculant, *Shewanella* uses organic compounds in the substrate as an energy source and transfers electrons to the anode through anaerobic respiration, thereby increasing power and energy density. Other bacteria present in the mud may also contribute to more efficient substrate degradation, providing additional electron donors for the electrogenic bacteria. Several environmental strains of *Brucella* have demonstrated roles in the degradation of organic pollutants. A study found that *Brucella* sp., when combined with *Pseudomonas*, formed a synthetic consortium capable of degrading 2,4-dichlorophenol, a common industrial pollutant, more effectively than individual strains [7]. *Brucella* contributes to complex microbial networks that support co-metabolic degradation in wastewater environments. *Castellaniella* has been shown to play a key role in degrading polycyclic aromatic hydrocarbons (PAHs) and denitrifying pollutants in industrial waste. One study reported the biodegradation of pyrene, a toxic component of coking wastewater, by a novel *Castellaniella* strain under anoxic conditions with 97.2% removal efficiency, using nitrate as an electron acceptor [11]. This genus thus aids both in organic pollutant removal and nitrogen cycle regulation in wastewater. *Klebsiella* species are facultative anaerobes known for degrading a variety of pollutants such as chlorophenols and antibiotics in both aerobic and anaerobic systems. For instance, *Klebsiella* sp. demonstrated high tolerance to toxic compounds in industrial wastewater and was able to survive and grow in media containing over 40% toxic effluent when supplemented with a co-substrate [12]. *Pseudomonas* is one of the most well-studied genera for biodegradation. Strains such as *P. putida* and *P. stutzeri* have been reported to degrade a wide range of compounds, including PAHs, dyes, antibiotics, and synthetic detergents [13].

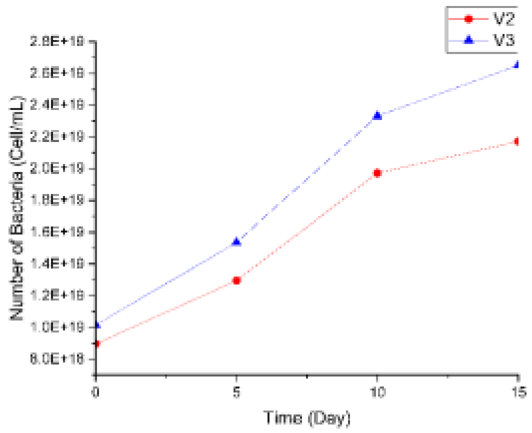


Figure 3. Number of Bacteria vs Time V2 and V3

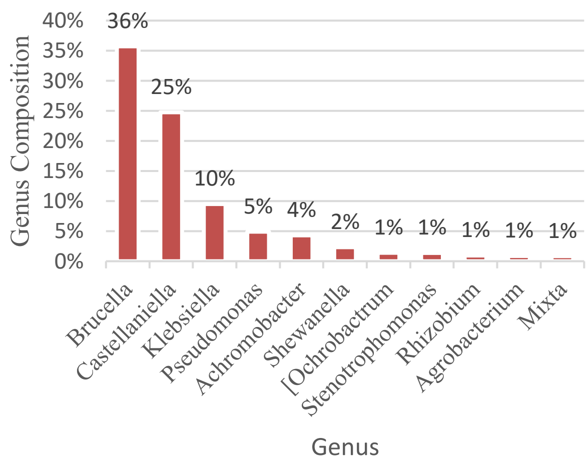


Figure 4. Sequencing Test

Their metabolic diversity and possession of genes like catechol 2,3-dioxygenase allow them to perform ring cleavage of aromatic compounds, crucial for breaking down complex wastewater pollutants. *Achromobacter* is known for its broad enzymatic capacity, participating in the degradation of sulfur compounds, aniline, and pharmaceuticals. Studies show that *Achromobacter* sp. strains can use industrial pollutants such as thioglycolic acid esters as sole carbon and energy sources and significantly reduce toxic loads in culture media [12] Their presence in microbial fuel cells and anaerobic reactors has also been linked to enhanced antibiotic and dye degradation [14].

B. %BOD And %COD Removal

The decrease in BOD and COD levels can be seen in Figure 5 and Figure 6. In the control variable, the natural %BOD and %COD removals were relatively low, at 70.43% and 47.92%, respectively. This is due to the absence of any added bacteria in the microbial fuel cell (MFC) system, meaning the degradation of organic matter relied solely on the naturally occurring microorganisms in the substrate. Without the presence of specialized bacteria capable of efficiently degrading organic matter, the reduction rate of BOD and COD was not significant.

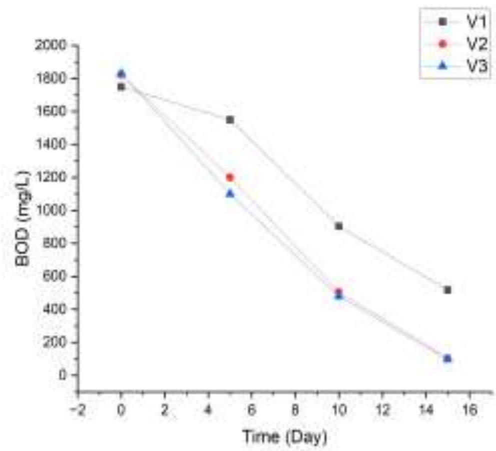


Figure 5. BOD vs Time

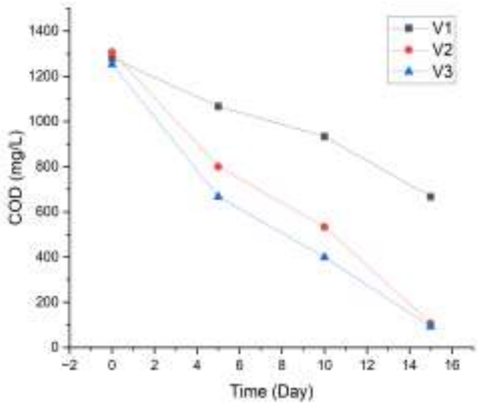


Figure 6. COD vs Time

In the second variable, the addition of Sidoarjo mud inoculant showed a significant increase in %BOD and %COD removal, reaching 94.30% and 91.84%. Sidoarjo mud contains various bacteria, including those that efficiently degrade organic matter. The mud is rich in microorganisms that contribute to the degradation process, including electricigenic bacteria like Shewanella, which help break down complex organic compounds into simpler substances. These simpler compounds can then be used by electricigenic bacteria to generate electrons and energy, leading to a substantial increase in BOD and COD removal.

In the third variable, the combination of Sidoarjo mud inoculant with the addition of *Bacillus subtilis* resulted in even higher %BOD and %COD removal, at 94.70% and 92.55%. *Bacillus subtilis* is known for its ability to degrade proteins and other complex organic compounds. It breaks down these complex substances, including proteins, into simpler forms such as amino acids. These simpler compounds are easily utilized by electricigenic bacteria in their metabolic processes, enhancing the efficiency of organic matter degradation. The synergy between *Bacillus subtilis* and the bacteria in Sidoarjo mud boosts overall MFC efficiency. *Bacillus subtilis* accelerates the degradation of complex organic substrates, while electricigenic bacteria use these degradation products to generate energy and electrons, leading to higher %BOD and %COD removal.

This study demonstrates that the presence and combination of microorganisms in an MFC significantly affect %BOD and %COD removal. The low %BOD and %COD removal in the control variable highlights the importance of bacteria capable of degrading organic matter within the system. The addition of Sidoarjo mud introduced bacteria that significantly enhanced %BOD and %COD removal through efficient degradation processes. Furthermore, the addition of *Bacillus subtilis* alongside Sidoarjo mud created a more favorable environment for electricigenic bacteria by providing more easily degradable substrates. This combination not only improved the efficiency of organic matter degradation but also increased the overall %BOD and %COD removal. This research opens up opportunities for further exploration of other microorganism combinations and optimization of operating conditions to enhance MFC performance in wastewater treatment.

C. Protein Test

The reduction of protein levels in liquid tofu waste can be done naturally through the decomposition process by microorganisms by 59% for 15 days. The microorganisms that are effective in this process are usually proteolytic bacteria, which have the ability to break down protein molecules into peptides and amino acids, which can then be further broken down into simpler compounds.

Sidoarjo mud contains a variety of microorganisms that have adapted to live in extreme environmental conditions, such as high organic content and pollutants. These mud microorganisms have a good protein degradation ability of 63.38% for 15 days. Isolates from Sidoarjo mud can increase the rate of decrease in protein levels because these microorganisms have efficient proteolytic enzymes.

Bacillus subtilis is a gram-positive bacterium known to have strong proteolytic abilities. *Bacillus subtilis* can produce a variety of protease enzymes that are able to break down proteins quickly and efficiently by 79.79% over 15 days. The addition of *Bacillus subtilis* to tofu liquid waste can accelerate the protein decomposition process, reducing protein levels more effectively.

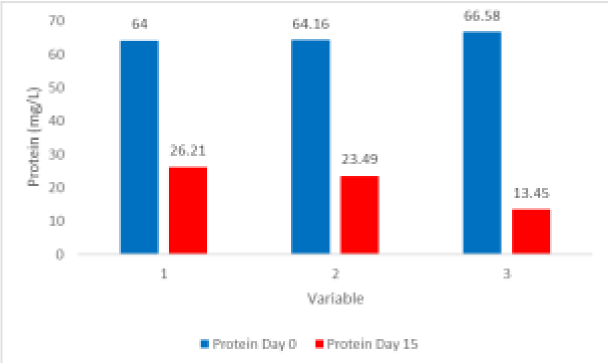


Figure 7. Protein Test Result

D. Electricity Generation

Although electricity generation was not the primary focus of this study, a consistent increase in voltage and power density was observed throughout the operation as shown in Figure 8. This trend reflects the progressive development of electroactive biofilms on the anode surface and the gradual adaptation of electrogenic microorganisms to the reactor environment. Notably, the highest total power density recorded over the 15 days period was 214,179.571 mW/m²—was achieved by Variable 3, as shown in Figure 9, which combined Sidoarjo mud with *Bacillus subtilis* as inoculants. This microbial synergy likely enhanced electron transfer processes, as the diverse consortium from Sidoarjo mud contributed a range of functional bacteria while *Bacillus subtilis* supported efficient protein degradation. The improved biofilm-electrode interaction in this configuration confirms the electrogenic potential of the system. These results demonstrate that, even when electricity production is a secondary goal, MFC-based treatment systems can contribute meaningful energy recovery alongside effective organic waste degradation.

E. ANOVA Test

The data from the ANOVA test results in Table 2 and Table 3, both Sidoarjo Mud (LS) and *Bacillus subtilis* (BS) sludge inoculants have a significant influence on BOD and COD removal. The p-value for both is 0.000, which indicates that the two inoculants contribute significantly to the waste treatment process by lowering BOD and COD. However, when viewed from the F-Value value, Sidoarjo mud inoculant (LS) has a greater value, which shows that Sidoarjo mud has a stronger influence on BOD and COD removal compared to *Bacillus subtilis*. This means that Sidoarjo sludge inoculants are more effective in improving waste treatment efficiency and reducing BOD and COD, although *Bacillus subtilis* also makes a significant positive contribution.

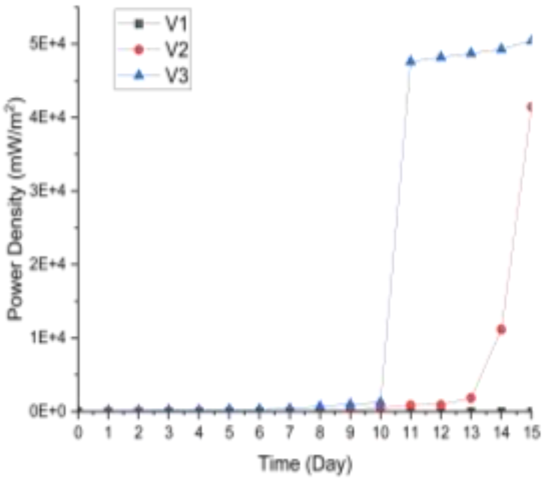


Figure 8. Power Density

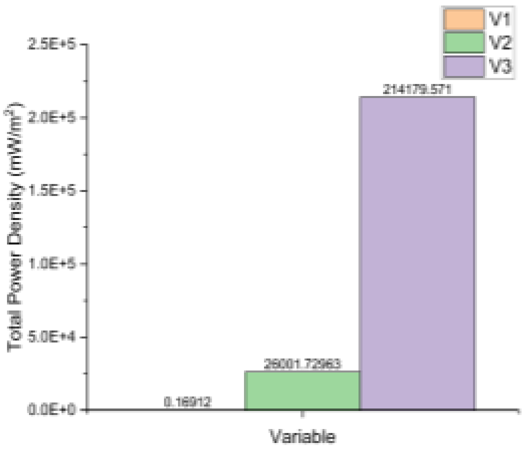


Figure 9. Total Power Density

Based on the results of the ANOVA test in Table 4, there is a very significant influence of two factors, namely LS (Sidoarjo Mud) and BS (*Bacillus subtilis*) on the level of protein degradation in tofu liquid waste. This can be seen from the very small P-Value (0.000) of both factors, which shows that both factors have a significant effect on the reduction of protein levels in tofu liquid waste. When compared to the two factors, the BS factor (*Bacillus subtilis*) showed a much higher F-Value (7226.77) compared to the LS factor (604.09). This indicates that *Bacillus subtilis* has a greater influence on reducing protein levels in tofu liquid waste compared to Sidoarjo Mud. Statistically, this shows that *Bacillus subtilis* is more effective in accelerating the process of protein degradation through the proteolytic enzymatic activity it produces.

The results of the ANOVA test for BOD, COD, and protein reduction showed p-values of 0.000, indicating a statistically significant difference between treatment groups. These values should be interpreted as $p < 0.001$, which means there is a less than 0.1% probability that the observed differences occurred by chance. The ANOVA analysis was conducted using one-way linear regression, comparing the effects of different inoculant treatments—control (V1), Sidoarjo mud (V2), and the combination of Sidoarjo mud with *Bacillus subtilis* (V3)—on wastewater degradation performance. The high F-values obtained for each parameter reflect substantial differences between groups, where the between-group variance far exceeded the within-group variance. These findings confirm that both Sidoarjo mud and *Bacillus subtilis* significantly influenced the degradation of organic content in tofu wastewater, supporting the effectiveness of the applied microbial treatments.

TABLE 2.
ANOVA BOD REMOVAL VS INOCULANT

Source	F-Value	P-value
Regression	15,539.19	0.000
LS	17,343.21	0.000
BS	13,735.17	0.000

TABLE 3.
ANOVA COD REMOVAL VS INOCULANT

Source	F-Value	P-value
Regression	3,652.42	0.000
LS	3,904.40	0.000
BS	3,400.44	0.000

TABLE 4.
ANOVA %PROTEIN REDUCE VS INOKULAN

Source	F-Value	P-value
Regression	3,915.43	0.000
LS	604.09	0.000
BS	7,226.77	0.000

Strong removal performance confirmed by ANOVA highlights the system's practical potential. MFCs are suitable for decentralized wastewater treatment in small agro-industries. Prior studies noted their cost-effectiveness and scalability, supporting tofu wastewater applications [15]. The system developed in this study aligns with these goals through use of local inoculants and continuous-flow design.

IV. CONCLUSION

In conclusion, the addition of Sidoarjo mud and *Bacillus subtilis* significantly improved the efficiency of organic waste treatment in microbial fuel cells. The control variable, without pH adjustment, showed a 26% decrease in bacterial count and low BOD (70.43%) and COD (47.92%) removal. In contrast, the Sidoarjo mud inoculant led to BOD and COD removal rates of 94.30% and 91.84%, respectively. The combination of Sidoarjo mud with *Bacillus subtilis* further enhanced these rates to 94.70% and 92.55%, respectively. Protein degradation was also significantly higher in the presence of *Bacillus subtilis*, reaching a reduction of 79.79% over 15 days, compared to 59% in the control and 63.38% with Sidoarjo mud alone. In addition to its primary treatment function, the system also exhibited electrogenic activity, with Variable 3 producing the highest total power density of 214,179.571 mW/m² over a 15-day period. These findings highlight the dual functionality of the reactor in treating high-strength organic wastewater while also enabling energy recovery as a valuable secondary benefit. ANOVA results confirmed that both inoculants significantly influenced waste treatment, with Sidoarjo mud being more effective for BOD and COD removal, while *Bacillus subtilis* played a dominant role in protein degradation. These findings highlight the potential of these microorganisms for optimizing MFC performance in wastewater treatment. Future studies should focus on increasing energy yield and system stability by optimizing electrode materials and reactor design, while maintaining microbial activity over longer operational periods. Exploring modular or stacked reactor configurations may further enhance scalability. With proper engineering, this MFC system shows promise for decentralized wastewater treatment in small-to-medium tofu industries, enabling both pollutant removal and energy recovery in line with circular economy principles.

V. ACKNOWLEDGMENTS

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