

Design and Testing of Stream as a Sterilization Tool for *Trichoderma* sp. Propagation Media Using a Palm Oil Mill Steam Boiler

Ihsanul Fajri¹, Edy Hartulistiyoso^{1,✉}, Rahayu Widyastuti²

¹ Department of Mechanical and Biosystem Engineering, Faculty of Agricultural Technology, IPB University, Bogor, INDONESIA.

² Department of Soil Science and Land Resources, Faculty of Agriculture, IPB University, Bogor, 16680, INDONESIA.

Article History:

Received : 19 July 2024
Revised : 31 October 2024
Accepted : 17 November 2024

Keywords:

Autoclave,
Basal stem rot disease,
Sterilization,
TPC,
Trichoderma sp.

Corresponding Author:

✉ edyhartulistiyoso@apps.ipb.ac.id
(Edy Hartulistiyoso)

ABSTRACT

Trichoderma sp. is a fungus used in oil palm plantations to control basal stem rot disease, which can reduce palm oil production by up to 80%. At PT. Bumitama Gunajaya Agro, the production of this fungus uses an autoclave, which can only produce 51 kg of *Trichoderma* sp. per day per unit. To meet the high demand for *Trichoderma* sp., a large-capacity sterilization tool called "stream" is needed for mass production. The optimal performance of the Stream shows that effective sterilization can be achieved in 20 min when the tool is operated for 45 min. The effectiveness of this sterilization time is proven by the Total Plate Count (TPC) test, which shows a bacterial count of 109×10^3 CFU/g and a fungal count of 35 CFU/g. The *Trichoderma* sp. product produced through this process has a conidium density of 8×10^8 , 100% conidium viability, and an inhibition power of 54%, all of which exceed the standard values of SNI 8027.3:2014. Based on its production capacity, Stream can achieve production of up to 1 ton per day with quality not significantly different from *Trichoderma* sp. production using an autoclave.

1. INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) is an economically valuable oil-producing plant that is widely cultivated in Africa, Latin America, and Southeast Asia, including Thailand and Indonesia (Barcelos *et al.*, 2015; Anothai & Chairin, 2020). Palm oil is used in various products, ranging from food to cosmetics, and its demand continues to increase annually. Alongside this rising demand, there is a serious problem that threatens palm oil production: basal stem rot disease caused by *Ganoderma boninense* (Musa *et al.*, 2018). This disease can cause extensive damage to the plant, reduce yield by up to 80%, and lead to significant economic losses. Basal stem rot disease is a major concern for the oil palm industry, given its highly detrimental impact (Rees *et al.*, 2009).

Basal stem rot disease not only reduces oil palm production but also increases plantation management costs. Infection by *G. boninense* can lead to the death of young oil palm plants within 6 months to 2 years after initial infection. Additionally, infected mature plants also experience significant productivity decline, ultimately affecting the profitability of plantation companies. The costs associated with managing this disease include replacing dead plants, applying fungicides, and implementing various other preventive measures, all of which add financial burdens to the companies (Tan *et al.*, 2021).

The development of biocontrol agents like *Trichoderma* sp. is essential to protect oil palms from fungal attacks from the early growth stages (Akter *et al.*, 2019; Anothai & Chairin, 2020; Samlikamnoed *et al.*, 2023). *Trichoderma* sp. is a biocontrol agent that exhibits antagonistic properties against pathogens, especially soil-borne and some airborne

pathogens (Kurniasari, 2021). This antagonism involves competition, parasitism, or predation, as well as the production of toxins, including antibiotics (Contreras-Cornejo *et al.*, 2016). *Trichoderma* sp. enhances parasitism activity and the formation of gliotoxin and viridin under acidic conditions (Akter *et al.*, 2019). In soil, the effect of *Trichoderma* on the root system is not visible; however, the direct effect of this fungus can be observed in in vitro studies (Narasswati *et al.*, 2017). Additionally, *Trichoderma* sp. also acts as a biofertilizer, improving the nutrient status of its host plants (Ho *et al.*, 2018).

Trichoderma sp. grows easily on various media, especially those rich in organic matter. For optimal growth of *Trichoderma* sp., sterilization using an autoclave or steam is necessary. Steam, under specific pressure and temperature, has high energy values, which are then used to transfer heat in the form of thermal energy for the process (Hikmawan *et al.*, 2020; Parinduri & Arfah, 2019). Sterilization is essential for killing microbes in culture media (Wulandari *et al.*, 2022). The production of *Trichoderma* sp. using an autoclave has limited capacity. To overcome this limitation, a new sterilization device with larger capacity, called "Stream," was developed. Stream is designed to utilize residual steam from palm oil mill boilers as an energy source, allowing the sterilization of larger quantities of *Trichoderma* sp. growth media. Therefore, this study aims to create a Stream prototype and test it according to SNI 8027.3:2014 standards (BSN, 2014). Tests were conducted on *Trichoderma* sp. production outcomes on media sterilized using Stream, and the results were also compared with products sterilized using an autoclave.

2. RESEARCH MATERIALS AND METHODS

This research was conducted from July 2023 to June 2024, involving a series of activities across several locations. The fabrication process took place at the Bumitama Central Pundu Workshop (BCPW), part of the facilities of PT. Bumitama Gunajaya Agro, located in Pantai Harapan Village, Cempaga Hulu District, Kotawaringin Timur Regency, Central Kalimantan. Equipment installation, on the other hand, was carried out at Pundu Nabatindo Mill (PNBM), a palm oil mill also located in Pantai Harapan Village, Cempaga Hulu District, Kotawaringin Timur Regency, Central Kalimantan.

Sample analysis was conducted at two laboratory locations: the Microbiology Laboratory of PT. Bumitama Gunajaya Agro in Pantai Harapan Village, Cempaga Hulu District, Kotawaringin Timur Regency, Central Kalimantan, and the Microbiology Laboratory of the Faculty of Agriculture at IPB University, Dramaga, Bogor.

The equipment used in this research included workshop tools for fabrication, such as electric welding for joining, acetylene grinders for cutting, drills and saws for drilling and cutting, as well as other manual tools like hammers, wrenches, and pliers. For testing purposes, laboratory equipment was utilized, including fungal and bacterial growth media (Potato Dextrose Agar/PDA, Dichloran Rose Bengal Chloramphenicol Agar/DRBC, Nutrient Agar/NA), and other supporting equipment such as microscopes and laminar air flow cabinets. The research methodology and testing workflow are illustrated in Figure 1.

2.1. Production Method Using an Autoclave

The production method for *Trichoderma* sp. begins with the preparation of raw materials in the field, which involves mixing fiber with dolomite (0.5%). Each plastic bag is filled with 300 grams of fiber, then wrapped in plastic, and sterilized using an autoclave at 121°C for 20 min. This sterilization process ensures the aseptic conditions necessary for inoculation with *Trichoderma* sp. starter culture. After sterilization, the fiber is inoculated with the *Trichoderma* sp. starter and incubated for at least 7 days. The propagation process of *Trichoderma* sp. using an autoclave can be seen in Figure 2. This method has proven effective in preparing an optimal growth medium for *Trichoderma* sp., illustrating the precise steps required to ensure successful microorganism production in a controlled environment.

2.2. Stream Fiber Prototype

Referring to sterilization using an autoclave, sterilization can also be achieved using steam, as the working principle is similar. The Stream is designed in such a way that it can sterilize the raw materials to be used. The Stream unit prototype utilizes steam flowing from the boiler at a pressure of 3 bar and a temperature of 130°C, and it has been successfully developed. This prototype was developed with components as shown in Figure 3.

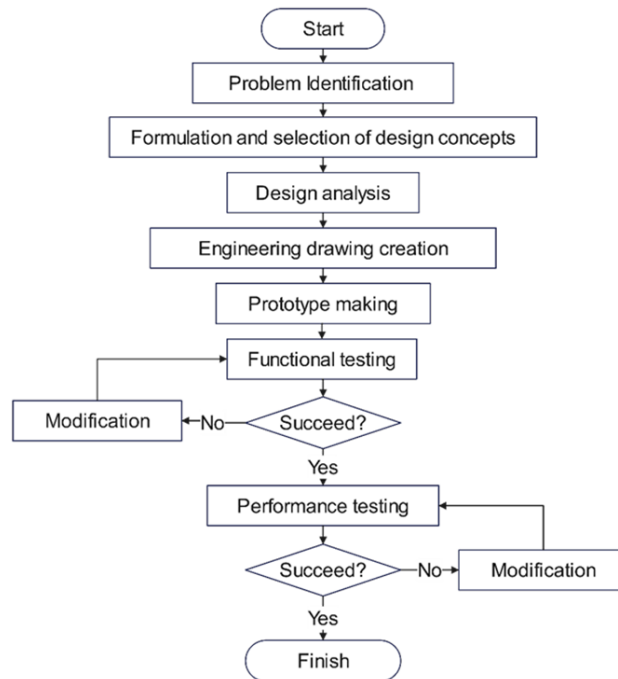


Figure 1. Production Method Using Autoclave



Figure 2. Mixing (left), wrapping (middle), and autoclave sterilization process (right)

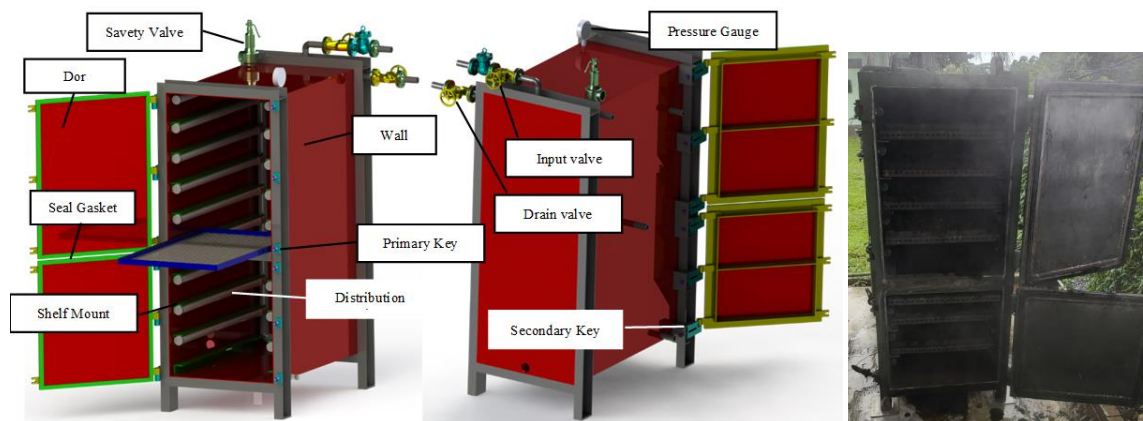


Figure 3. Stream fiber prototype: front view (left), rear view (center), and actual unit (right)

This prototype is designed to sterilize fiber with a capacity of up to 180 kg/hour and is capable of withstanding pressure up to 3 bar. The system is also equipped with a rack system for loading and unloading raw materials, which significantly simplifies the operational process. This design enhances efficiency, making it easier to manage the sterilization process on a larger scale while maintaining safety and effectiveness.

2.3. Raw Materials for *Trichoderma* sp. Growth Medium

In addition to producing CPO (Crude Palm Oil), the palm oil processing industry also generates various types of waste, one of which is fiber (Ruswanto, 2017). Fiber is a byproduct from the pressing of palm fruit. The palm kernel processing includes fiber separation (depericarping), shell cracking and separation, kernel drying, storage, and shipping (Nugroho, 2019). The chemical composition of the fiber is dominated by glucan (219 kg/ton dry weight), xylan (153 kg/ton dry weight), lignin (234 kg/ton dry weight), SiO₂ (632 kg/ton dry weight), K₂O (90 kg/ton dry weight), and CaO (72 kg/ton dry weight) (Kurniawan & Yulianto, 2020). Based on research conducted by the R&D Department of PT. Bumitama Gunajaya Agro, the growth medium used for *Trichoderma* sp. is fiber, which is a waste or byproduct from Crude Palm Oil (CPO) production. The following are the test results of the fiber used as the raw material for the *Trichoderma* sp. growth medium, conducted at the Analytical Laboratory, R&D Department, PT. Bumitama Gunajaya Agro with the analysis number: 0012OF/AL-BGA/INT/I/2024: (1) moisture content: 84.3%, (2) organic carbon: 55.42%, (3) nitrogen: 2.18%, (4) C/N ratio: 25.42, (5) phosphorus: 50 ppm, (6) potassium: 0.53%, and (7) magnesium: 0.32%.

These results provide an overview of the nutritional composition of the fiber used, which is an important parameter in determining the potential of the medium for *Trichoderma* sp. growth. The selection of the appropriate raw material can significantly influence the quality and outcome of this research.

2.4. Sterilization Temperature Testing

Temperature measurements were conducted using a thermocouple, which was installed at the top, middle, bottom, and the product section inside the Stream. Temperature measurements were taken using a type K thermocouple, which was installed on the walls of the sterilization unit. The measurements began when steam started flowing into the Stream and ended when the steam flow was stopped. The temperature was recorded every 10 seconds, allowing the data to be presented in a line graph with time on the X-axis.

2.5. Total Plate Count (TPC) Testing of Raw Materials and Products

This testing was conducted with six repetitions for each sample. The raw material (FB-00) was prepared and then sterilized according to different treatments, including autoclave sterilization for 20 min (FL-20) and sterilization using the stream for total times of 15 min (FP-15), 30 min (FP-30), 45 min (FP-45), and 60 min (FP-60).

2.6. Product Quality Testing

The results of *Trichoderma* sp. propagation must be tested to determine their quality and suitability for field application. This testing refers to the Indonesian National Standard SNI 8027.3:2014 (BSN, 2014) for Biological Control Agents (BCA) *Trichoderma* spp., which is designed to ensure the quality assurance (QA) of BCA. The quality requirements for *Trichoderma* spp. are outlined in Table 1.

Table 1. Quality requirements for *Trichoderma* spp. based on SNI 8027.3:2014 (BSN, 2014)

Parameter	Unit	Mark
Conidium density	per ml	> 10 ⁶
Conidium viability	%	> 60
Pathogenicity to tobacco plants	-	Negative
Antagonism *		
Antibiosis	-	Positive
Mycoparasitism	-	Positive
- Inhibition	%	> 50 %

*If one of the antagonism parameters is met, it means that the requirements have been met.

3. RESULTS AND DISCUSSION

3.1. Steam Operating Temperature Testing

Steam, with specific pressure and temperature, possesses high energy value (Siswanto, 2020; Yani & Ristyohadi, 2017). Steam can be used to transfer heat in the form of thermal energy to the process (Parinduri & Arfah, 2019). Before data collection, the type K thermocouple sensor needs to be calibrated to ensure the standardization and quality of the data generated. A graph of the calibration results for the sensor can be seen in Figure . Based on the graph above, the calibration results of the device show excellent performance, with an R^2 value of 0.9992, allowing the data collection phase to proceed. At this stage, temperature data were collected from the raw material and inside the steam sterilization unit (top, middle, and bottom), which was filled with 170 kg of fiber. Below are the recorded data results obtained:

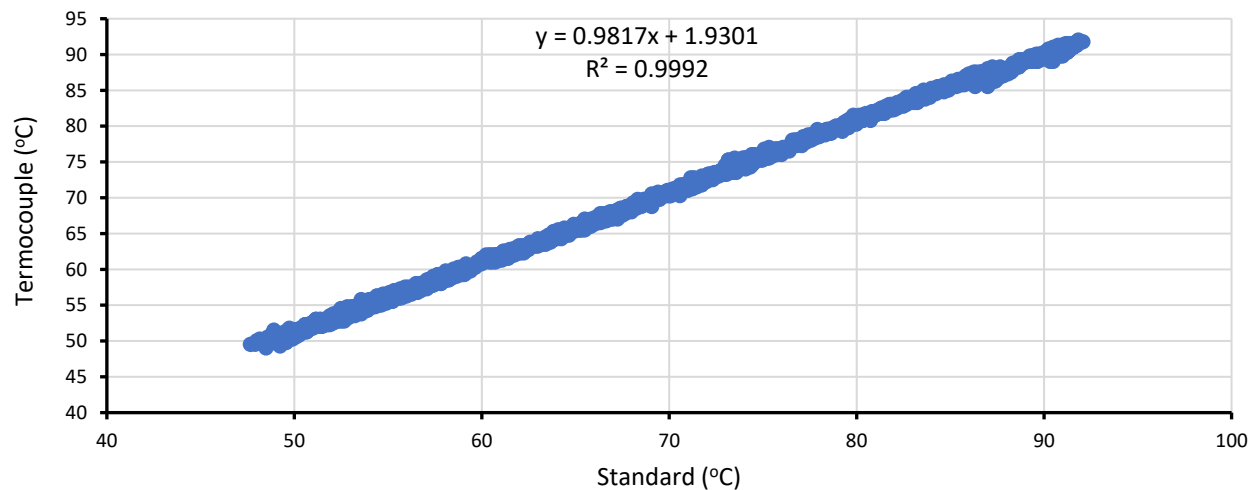


Figure 4. Graph of sensor calibration

3.1.1. Sterilization for 15 Min

Figure 5 shows temperature progress during sterilization process with different durations, namely 15 min, 30 min, 45 min, and 60 min. Based on the data above, steam was supplied from the first minute until the 15th minute, and then stopped at the 16th minute. The highest temperature was reached at the top of the sterilization unit, with a 6°C difference compared to the bottom. With a total duration of 15 min, the sterilization process was not optimal, as the maximum temperature was not reached, and the material temperature only reached 76°C.

Based on the data above, steam was supplied from the first minute until the 30th minute. However, the sterilization process only began at the 30th minute, meaning it was not optimal and additional sterilization time is required for better results.

Based on the data above, steam was supplied from the first minute until the 45th minute. The sterilization process at the peak temperature of 115°C lasted from the 30th minute to the 45th minute, with a total sterilization time of 15 min.

Based on the data above, steam was supplied from the first minute until the 60th minute. The sterilization process occurred from the 45th minute to the 60th minute, as the sterilization temperature reached its peak (115°C), with a total sterilization time of 15 min. This was due to the initially low temperature, which required additional time for heating the sterilization unit. This sterilization method is considered effective for the first sterilization of the day.

Based on the four sterilization time treatments above, in accordance with sterilization principles, the process requires a minimum of 15 min, with a total time of at least 45 min. This can be confirmed by calculating the number of bacteria and fungi before and after sterilization.

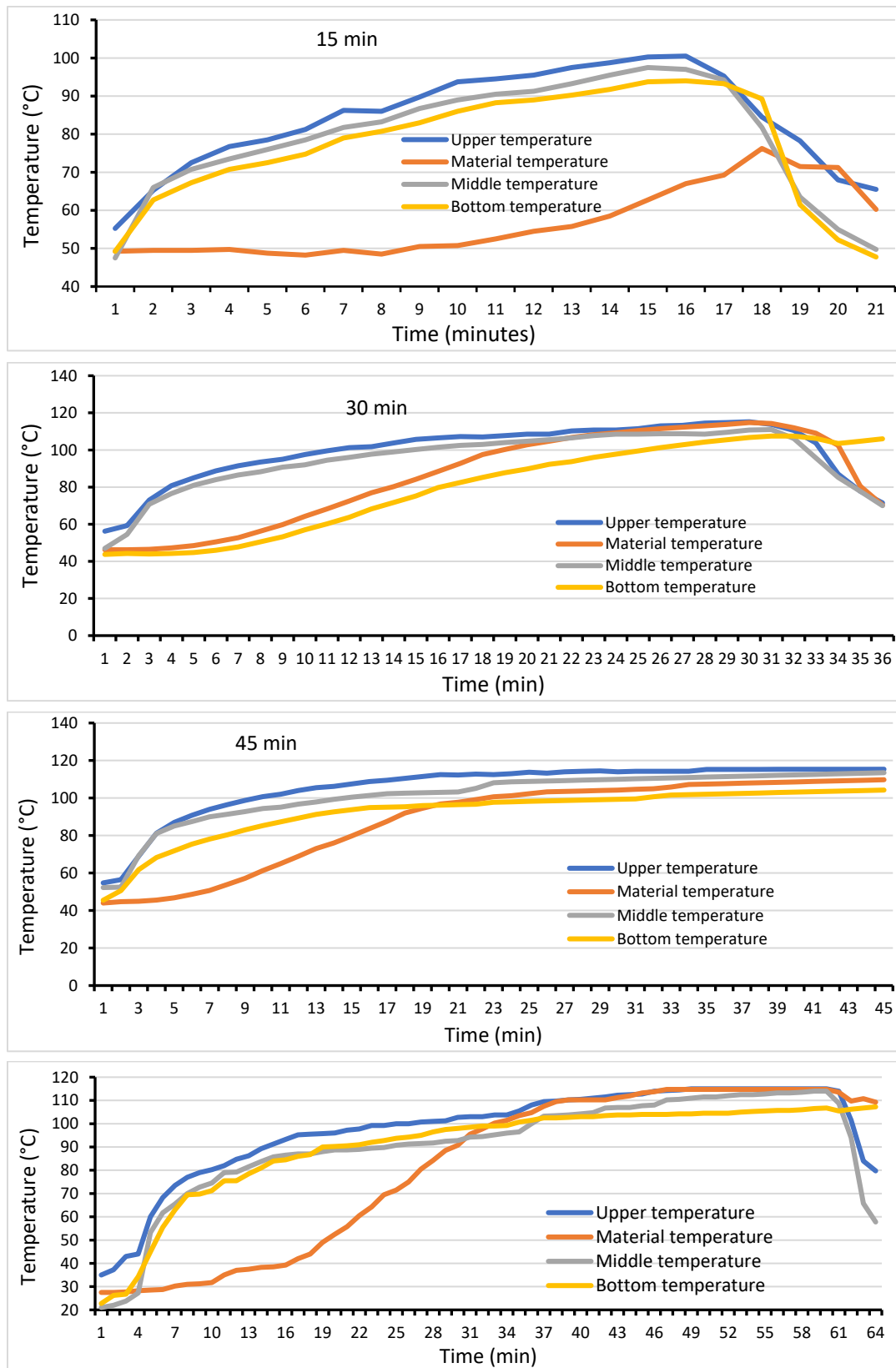


Figure 5. Fiber sterilization data for 15 min, 30 min, 45 min, and 60 min

Table 2. Properties of water vapor based on temperature and pressure (Moran & Shapiro, 2006)

Temperature (°C)	Pressure (bar)	Density (kg/m ³)	Enthalpy (kJ/kg)	Entropy (kJ/kg·K)
130	3.0	934.85	546.4	1.6346
115	1.8	947.09	482.6	1.4737

3.2. Analysis of Temperature and Pressure Changes in the Stream System

This analysis aims to understand the phenomena occurring within the Stream system. At atmospheric pressure (1 atm), saturated steam has a temperature of 100°C, and as the pressure increases, the temperature of saturated steam also rises (Winanti & Prayudi, 2006). The steam supplied to the Stream system has a temperature of 130°C and a pressure of 3 bar, while inside the Stream unit, the recorded temperature is 115°C and the pressure is 1.8 bar. The changes in steam pressure and temperature are presented in Table 2.

The decrease in enthalpy from 546.4 kJ/kg to 482.6 kJ/kg and the decrease in entropy from 1.63 kJ/kg·K to 1.47 kJ/kg·K during the use of the Stream tool for fiber sterilization indicate the release of thermal energy from the steam to the fiber during the process. The reduction in enthalpy suggests that the system experienced a decrease in temperature or the release of energy from the steam during the sterilization process. Meanwhile, the decrease in entropy may indicate that the process occurred in an adiabatic manner, with no heat lost from the system (no steam leakage). This could happen during the sterilization process where Stream uses pressurized steam to kill microorganisms on palm fiber.

3.3. Analysis of Heat Used

Heat is a form of energy that can cause a change in temperature, consisting of sensible heat and latent heat. Latent heat is the energy required for a phase change, represented as QL (latent heat), which is calculated by multiplying the mass (M) of the substance by the specific latent heat (L) (Haryanto, 2015). This analysis is conducted to calculate the amount of condensate produced in the sterilization system. The calculation uses the latent heat of water (2.26 MJ/kg) to determine the energy used in the system. Table 3 shows the heat calculation used in the system based on sterilization duration. All experimental repetitions were carried out with the same machine but at different times. This may result in variations in the machine's initial conditions, which can affect the amount of heat and condensate produced during the sterilization process.

Based on the data in **Error! Not a valid bookmark self-reference.**, it can be observed that the amount of heat used increases with the duration of sterilization. The data on the amount of condensate produced shows significant variation, as seen in the 15-minute sterilization time where the condensate produced in the first repetition was 57.3 kg, while in the third repetition it was only 31.8 kg. From this analysis, it can be concluded that the thermal energy used increases as the sterilization duration increases. The amount of condensate produced is also not constant for each time period, indicating variability in the system that may be caused by external or internal factors affecting the sterilization process. Additionally, the amount of condensate is likely influenced by the execution of the study, where the machine is not always at the same initial temperature when used.

3.4. Total Plate Count (TPC) Testing of Raw Materials and Products

The TPC observation was conducted without the use of a microscope, which makes it easier to obtain information about the number of microorganisms in the samples (Husen *et al.*, 2022). The data from the TPC tests for both raw materials and products are presented in Table 4.

Table 3. Calculation of heat used in the system

Time (min)	Condensate weight (kg)				Latent Heat of Water (MJ/kg)	Heat used (MJ)
	Test 1	Test 2	Test 3	Average		
15	57.3	33.12	31.8	40.7	2.26	92.1
30	96.8	55.6	67.3	73.2	2.26	165.5
45	109.1	90.6	106.3	102.0	2.26	230.5
60	145.4	131.8	143.0	140.0	2.26	316.5

Table 4. TPC testing data for raw materials and products

Sample	Bacteria (CFU/g)	Fungus (CFU/g)	Information
FB-00	18.3×10^{10} a	18.8×10^5 a	Raw material (without sterilization)
FL-20	6.9×10^4 c	3.3×10^1 cd	Autoclave sterilization for 20 min
FP-15	757.8×10^4 b	385.0×10^1 b	Stream sterilization for 15 min
FP-30	20.3×10^4 c	34.3×10^1 bc	Stream sterilization for 30 min
FP-45	10.9×10^4 c	3.5×10^1 d	Stream sterilization for 45 min
FP-60	10.6×10^4 c	2.0×10^1 d	Stream sterilization for 60 min

Note: significant difference is denoted using different lowercases following number at the same column.

The reduction in the number of microorganisms, both bacteria and fungi, indicates the effectiveness of sterilization in reducing microbial contamination. However, the variation in the number of bacteria and fungi across different sterilization durations suggests the presence of other factors that may affect sterilization effectiveness, such as the distribution of temperature and pressure within the sterilization unit. Table 4 also presents the results of the ANOVA test with Tukey's post-hoc test (logarithmic values), which shows that sterilization for 45 min using Stream is the optimal duration to achieve effective sterilization. This is important to ensure the quantity and quality of the produced product.

3.5. Conidium Density Testing

Samples sterilized using steam with a total sterilization time of 45 min and samples sterilized for 20 min using an autoclave were inoculated with *Trichoderma* sp. propagules that had been propagated using rice-based media. Table 5 summarizes the results of the conidium density testing for both sterilization methods. Based on SNI 8027.3:2014, the standard average conidium density is $>10^6$. The results of the testing show that the conidium density for both sterilization methods, whether using Stream for 45 min or autoclaving for 20 min, is higher than the standard set by the SNI. However, the conidium count for the autoclave sterilized sample is almost twice as high as the stream sterilized sample.

Table 5. Results of conidium density testing

Repetition	Stream (10^8)	Autoclave (10^8)
1	6.4	8.8
2	10.4	8.0
3	6.4	24.0
4	8.8	20.8
Average	8.0 a	15.4 a

This result indicates that sterilization using the autoclave is more effective in achieving a higher conidium density compared to the Stream sterilization for 45 min. This may be due to a more consistent heat distribution and more effective sterilization conditions in the autoclave. For *Trichoderma* sp. production with optimal conidium density, autoclave sterilization for 20 min is recommended. However, for capacity reasons, sterilization using Stream is more reliable, and this method still meets the SNI standard, even with a lower conidium density.

3.6. Conidium Viability Testing

Conidium viability testing was conducted using the same samples as the conidium density test. The testing method followed the procedure described in SNI 8027.3:2014 (BSN, 2014), with observations made at 8 h, 16 h, and 24 h. The results of the testing are presented in Table 6. The observations showed that the conidium viability in the steam method reached 98% at 16 h, while the autoclave method reached 93%. At 24 h, both methods achieved 100% conidium viability, exceeding the minimum standard of 60% set by the SNI. Although there is a difference in the recovery speed of viability between the two methods, both effectively meet the required standard. The implication of these findings suggests that the use of the steam sterilization method offers an advantage in maintaining the initial viability of conidium at the 16-hour mark. However, in the long term, both steam and autoclave methods are capable of achieving optimal conidium viability at 24 h.

Table 6. Conidium viability testing results

Observation Time (h)	Conidium Viability (%)	
	Stream	Autoclave
8	0	0
16	98	93
24	100	100

3.7. Inhibition Test

Trichoderma sp. can inhibit the growth of fungal pathogens that cause plant diseases (Verma *et al.*, 2007). Therefore, an inhibition test needs to be conducted according to SNI 8027.3:2014. The purpose of this test is to measure the inhibition of pathogen growth by *Trichoderma* sp. APH, with the pathogen used being *Ganoderma* sp. Figure 3 shows the comparison between the control (left) and the treatment (right). The test was conducted with 3 replications, resulting in an average colony diameter of *Ganoderma* sp. in the control group of 8.3 cm after an incubation period of 7 days. Meanwhile, the colony diameter of *Ganoderma* sp. inhibited by *Trichoderma* sp. was 3.8 cm, resulting in an inhibition percentage of 54%. This meets the minimum inhibition standard of 50% set by SNI 8027.3:2014 (BSN, 2014). Therefore, based on this observation, the *Trichoderma* sp. production has exceeded the values set by the SNI.

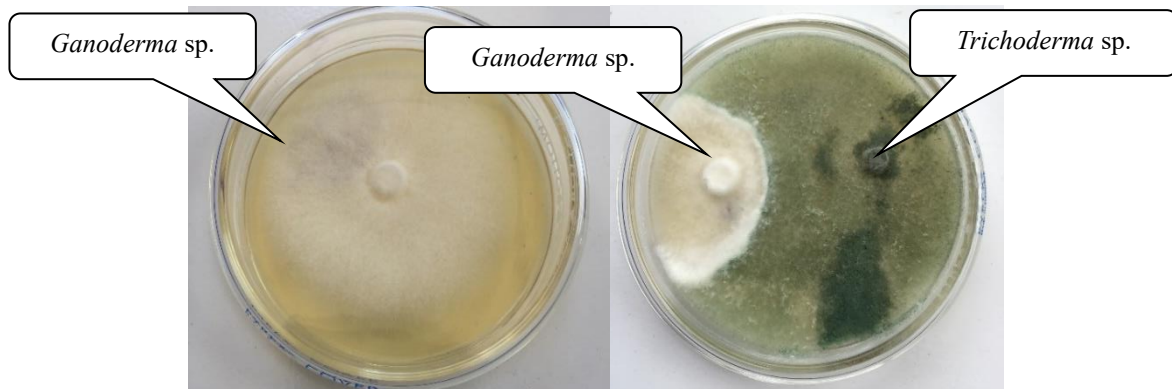


Figure 3. Control (left) and treatment (right)



Figure 4. Pathogenicity Test Control (left) and Treatment (right)

3.8. Pathogenicity Test

Pathogenicity is the relative ability of an organism to cause disease (infect) in its host. This is used to observe the response of oil palm plants applied with *Trichoderma* sp. The observation was carried out by looking for necrotic lesions on tobacco leaves after injecting a suspension of *Trichoderma* sp. onto the midrib of the tobacco leaf (Figure 11). If no

necrotic lesions were observed during the study (observations were made daily for 5 days), the result was negative or non-pathogenic to tobacco (Jumadi *et al.*, 2021). The pathogenicity test was conducted using tobacco plant media in accordance with the SNI 8027.3:2014 guidelines, to observe the possible occurrence of necrotic lesions on leaves inoculated with *Trichoderma* sp. APH produced by the Stream method with a single dilution and conidium density meeting the standard ($>10^6$). The results from 18 repetitions showed that no necrotic lesions were formed, indicating that the pathogenicity test was negative, in accordance with the SNI 8027.3:2014 standard, both for *Trichoderma* incubated using Stream and autoclave.

3.9. Production Capacity

Production conducted in the laboratory using an autoclave (capacity 18 kg) and production using Stream (capacity 170 kg) is briefly summarized in Table 7. Sterilization using an autoclave with a capacity of 18 kg takes 2.5 h per cycle. The long production time is due to the autoclave's setup process, where it takes time to reach a temperature of 121°C before sterilization and to cool down to 60°C after sterilization. Although the sterilization process lasts for 20 min, the actual time for one sterilization cycle is 2.5 h. The daily production capacity using the autoclave is around 51 kg (3 cycles) during a 7.5-h operational day.

Table 7. Comparison of autoclave and stream usage

Parameter	Autoclave	Stream
Production per cycle	18 kg	170 kg
Time per cycle	2.5 h	45 min
Effective sterilization time	20 min	20 min
Maximum sterilization temperature	121°C	115°C
Loading and unloading time	15 min	15 min
Daily production (7.5 h)	51 kg (3 times production)	1 ton (6 times production)

Meanwhile, production using Steam has a much larger capacity, which is 170 kg per hour. One hour of operation on the Steam system consists of 5 min of loading time, 45 min of sterilization, and 10 min of unloading time. This short unloading time is made possible by the Steam system's drain system, which facilitates the release of pressure and temperature through a special valve. The daily production capacity using Steam can reach up to 1 ton (i.e., 6 cycles) during a 7.5-h operational a day.

The significant difference in production capacity between the autoclave and Steam shows that the use of Steam is not only more efficient in terms of time, but it also produces a much higher output in the same period. This provides a competitive advantage in large-scale production and enables savings in operational costs and energy resources. Additionally, since Steam does not require electricity, it also has the potential to reduce overall operational costs. Therefore, switching from autoclave to Steam can enhance operational efficiency and support sustainable production growth in laboratory or industrial facilities.

4. CONCLUSION

Stream has been successfully designed to overcome the production constraints of *Trichoderma* fungus. Temperature analysis shows that Stream achieves maximum efficiency with an optimal sterilization time of 20 min within a total operation of 45 min. This success is proven by the TPC test results, which show a bacterial count of 109×10^3 CFU/g and a fungal count of 35 CFU/g. On the other hand, the autoclave control shows a fungal count of 68.8×10^3 CFU/g and a bacterial count of 33 CFU/g.

Additionally, the conidium density analysis shows that the product produced using Stream achieves a density of 8×10^8 , with conidium viability reaching 100%, pathogen inhibition at 54%, and a Negative result for pathogenicity. These results consistently exceed the standards set in SNI 8027.3:2014, with tests conducted independently and showing no significant difference from products sterilized using an autoclave.

REFERENCES

- Akter, F., Ahmed, M.G.U., & Alam, M.F. (2019). *Trichoderma*: A Complete tool box for climate smart agriculture. *Madridge Journal of Agriculture and Environmental Sciences*, **2**(1), 40–43. <https://doi.org/10.18689/mjaes-1000107>
- Anothai, J., & Chairin, T. (2020). Soil physicochemical properties closely associated with fungal enzymes and plant defense enzymes in *Ganoderma*-infected oil palm orchards. *Plant and Soil*, **456**(1–2), 99–112. <https://doi.org/10.1007/s11104-020-04705-y>
- Barcelos, E., De Almeida Rios, S., Cunha, R.N.V., Lopes, R., Motoike, S.Y., Babiychuk, E., Skirycz, A., & Kushnir, S. (2015). Oil palm natural diversity and the potential for yield improvement. *Frontiers in Plant Science*, **6**(MAR), 1–16. <https://doi.org/10.3389/fpls.2015.00190>
- BSN (Badan Standardisasi Nasional). (2014). *SNI 8027.3.2014 – Agens Pengendali Hayati (APH) - Bagian 3 : Trichoderma spp.* Badan Standardisasi Nasional, Jakarta.
- Contreras-Cornejo, H.A., Macías-Rodríguez, L., del-Val, E., & Larsen, J. (2016). Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: Interactions with plants. *FEMS Microbiology Ecology*, **92**(4), fiw036. <https://doi.org/10.1093/femsec/fiw036>
- Haryanto, A. (2015). *Perpindahan panas*. Innosain, Yogyakarta: 516 p.
- Hikmawan, O., Naufa, M., & Simarmata, L.H. (2020). Pemanfaatan cangkang dan serat kelapa sawit sebagai bahan bakar boiler utilization of palm kernel shell and fiber as boiler fuel. *Jurnal Riset Industri*, **15**(29), 18–26.
- Ho, C.L., Tan, Y.C., Yeoh, K.A., Lee, W.K., Ghazali, A.K., Yee, W.Y., & Hoh, C.C. (2018). Transcriptional response of oil palm (*Elaeis guineensis* Jacq.) inoculated simultaneously with both *Ganoderma boninense* and *Trichoderma harzianum*. *Plant Gene*, **13**(January), 56–63. <https://doi.org/10.1016/j.plgene.2018.01.003>
- Husen, E., Pratiwi, E., Suroño, & Widowati, L.R. (2022). *Metode Analisis Biologi Tanah*. Balai Penelitian Tanah, Bogor: 394 p.
- Jumadi, O., Junda, M., Caronge, M.W., & Syafruddin. (Eds.). (2021). *Trichoderma dan Pemanfaatan*. Penerbit Jurusan Biologi FMIPA UNM, Makassar: 89 p. <http://eprints.unm.ac.id/id/eprint/21426>
- Kurniasari, S. (2021). *Mengenal Agen Hayati Trichoderma sp.* Unit Pelaksana Teknis Perlindungan Tanaman Pangan dan Hortikultura Provinsi Kalimantan Barat.
- Kurniawan, A.D., & Yulianto, D. (2020). Pemanfaatan limbah serat (fiber) buah kelapa sawit dan plastik daur ulang (polypropylene) sebagai material komposit papan partikel (particle board). *Journal of Renewable Energy and Mechanics*, **3**(02), 60–70. <https://doi.org/10.25299/rem.2020.vol3.no02.4884>
- Moran, M.J., & Shapiro, H.N. (2006). Engineering Thermodynamics. In *Mechatronic Systems, Sensors, and Actuators: Fundamentals and Modeling*. John Wiley & Sons, Inc.
- Musa, H., Nusaibah, S.A., & Khairulmazmi, A. (2018). Assessment on *Trichoderma* spp. mixture as a potential biocontrol agent of *ganoderma boninense* infected oil palm seedlings. *Journal of Oil Palm Research*, **30**(3), 403–415. <https://doi.org/10.21894/jopr.2018.0035>
- Narasswati, N., Oktavia, R., Nenci, N., Eryanti, Y., Nugroho, T.T., & Nurulita, Y. (2017). Potensi metabolit sekunder dari *Trichoderma* sp. LBKURCC22 tanah gambut hutan sekunder sebagai antibiotik. *Chimica et Natura Acta*, **5**(2), 85. <https://doi.org/10.24198/cna.v5.n2.14692>
- Nugroho, A. (2019). *Teknologi Agroindustri Kelapa Sawit*. Lambung Mangkurat University Press, Banjarmasin: 183 p.
- Parinduri, L., & Arfah, M. (2019). Pendekatan energi dalam pengelolaan limbah pabrik kelapa sawit studi kasus PT. Perkebunan Nusantara IV Kebun Adolina. *Journal of Electrical Technology*, **4**(2), 85–92.
- Rees, R.W., Flood, J., Hasan, Y., Potter, U., & Cooper, R.M. (2009). Basal stem rot of oil palm (*Elaeis guineensis*); Mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathology*, **58**(5), 982–989. <https://doi.org/10.1111/j.1365-3059.2009.02100.x>
- Ruswanto, A. (2017). *Mengenal Teknologi Pengolahan Tandan Buah Sawit (TBS) Menjadi Minyak Kelapa Sawit*. Instipier, Yogyakarta: 58 p.
- Samlikamnoed, P., Anothai, J., & Chairin, T. (2023). Defense-related enzyme production in oil palm seedlings against basal stem rot pathogen *Ganoderma boninense* and its biological control by *Trichoderma asperellum*. *Physiological and Molecular Plant Pathology*, **128**, 102154. <https://doi.org/10.1016/j.pmpp.2023.102154>

- Siswanto, J.E. (2020). Analisis limbah kelapa sawit sebagai bahan bakar boiler dengan menggunakan variasi campuran antara fiber dan cangkang buah sawit. *Journal of Electrical Power Control and Automation (JEPCA)*, **3**(1), 22. <https://doi.org/10.33087/jepca.v3i1.35>
- Tan, M.I.S.M.H., Jamlos, M.F., Omar, A.F., Dzaharudin, F., Chalermwisutkul, S., & Akkaraekthalin, P. (2021). *Ganoderma boninense* disease detection by near-infrared spectroscopy classification: A review. *Sensors*, **21**(9), 3052. <https://doi.org/10.3390/s21093052>
- Verma, M., Brar, S.K., Tyagi, R.D., Surampalli, R.Y., & Valéro, J.R. (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochemical Engineering Journal*, **37**(1), 1–20. <https://doi.org/10.1016/j.bej.2007.05.012>
- Winanti, W.S., & Prayudi, T. (2006). Perhitungan efisiensi boiler pada industri-industri tepung terigu. *Jurnal Teknik Lingkungan, Edisi Khusus* (Juni), 58-65.
- Wulandari, S., Nisa, Y.S., Taryono, T., Indarti, S., & Sayekti, R.S. (2022). Sterilisasi peralatan dan media kultur jaringan. *Agrotechnology Innovation (Agrinova)*, **4**(2), 16-19. <https://doi.org/10.22146/a.77010>
- Yani, A., & Ristyohadi, R. (2017). Analisis kehilangan steam dan penurunan temperatur pada jaringan distribusi steam dari PT. KDM ke PT. KNI. *Turbo : Jurnal Program Studi Teknik Mesin*, **6**(2), 123–135. <https://doi.org/10.24127/trb.v6i2.558>