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The Potential of Tofu Liquid Waste and Rice Washing Wastewater as Cheap Growth Media for *Trichoderma* sp.

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Abstract

The tofu processing industry in Indonesia is massively increasing. The increasing activity of tofu production causes the disposal of tofu industrial waste to the environment to be higher as well. This condition is exacerbated by the fact that generally, the tofu industry producers do not have their appropriate tofu waste management system. The liquid waste generated from the tofu processing industry will be discharged directly into the waterways. Additionally, people in Indonesia generally use rice as their staple food. The rice washing process produces washing wastewater which is usually discharged into the environment directly. The disposal of these wastes raises the environmental pollution rate. Also, the tofu liquid waste and rice washing water contain some nutrients such as proteins, fats, carbohydrates, and vitamins. The presence of these nutrients will increase the population of pathogenic microbes in the environment. Thus, in this study, the authors discussed the potential of tofu wastewater and rice washing wastewater as alternative media for the growth of *Trichoderma* sp. *Trichoderma* sp. is an antagonistic fungus that can be used as a biocontrol agent for farmers. The use of waste as an alternative medium reduces the mass production and propagation cost of biological agents. In this study, this isolate was cultivated in tofu liquid waste and rice washing wastewater by adding a carbon source. Based on the research conducted, it was known that the average conidial density of *Trichoderma* sp. cultivated in tofu liquid waste was 2.9 x 10^6, while the conidial density of *Trichoderma* sp. cultivated in alternative rice washing wastewater was only 1.6 x 10^6. The growth of this isolate in tofu liquid waste was higher than rice washing water. It indicated that tofu liquid waste potentially to be used as a cheap alternative growth medium for *Trichoderma* sp.

Keywords: Cheap Media, Tofu liquid waste, *Trichoderma* sp., Rice Washing Wastewater

1 Introduction

Trichoderma sp. is one of the biological control agents that have been used widely to control pathogenic microbes in many plants [1]. *Trichoderma* sp. produces cellulase enzymes to damage the cell walls of pathogenic fungi from the Pythiaceae family such as *Phytophthora infestans*. Also, the fungus can wrap and penetrate pathogenic hyphae and produce antibiotics that are toxic to the pathogens. The antagonistic mechanism is also done by producing antibiotics killing *P.infestans*. Meanwhile, the mechanism of antibiosis depends on the type and nature of the soil as a growth substrate [2]. Additionally, this fungus can produce gliotoxin and viridin which will inhibit plant pathogenic microorganisms. Also, it produces chitinolytic enzymes. Those enzymes will destroy the mycelium cell wall of pathogenic fungi. Those components are essential in biological mechanisms toward plant pathogenic microorganisms [3].

Some *Trichoderma* spp. can produce siderophores that chelate iron. *T.harzianum* is one of the species of *Trichoderma* spp. that is known to successfully inhibit the growth of other pathogenic fungi such as *Fusarium* sp. The chelation of iron by *Trichoderma* sp. reduces the germination of pathogenic fungi spores. *Trichoderma* sp. inhibits the growth of fungal pathogen of *Fusarium oxysporum* by colonizing the rhizosphere and taking more nutrients than that pathogen [4]. *Trichoderma* spp. can also be used to control the pathogenic fungus of *Rigidoporus lignosus* which causes white root disease on rubber. *Trichoderma* spp. effectively inhibited the growth of the pathogenic fungal mycelium [5].

Trichoderma sp. grows on some media. The common medium for fungal growth is Potato Dextrose Agar (PDA). This medium is made from potato extract supplemented with glucose and agar. Meanwhile, the alternative growth medium can be rice and corn. Rice and corn media are often used in the propagation of *Trichoderma* sp., However, the media require high cost. Besides, the media also compete with food considering that rice and corn are considered as staple foods in some area [6].

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Trichoderma sp. is a promising and potential biological agent for controlling pathogenic microorganisms in the farm. However, this fungal availability is still a problem for the farmers due to limited knowledge and high costs for application in their field directly. Needs for *Trichoderma* sp. on the large scale requires large propagation media. Therefore, alternative and cheap media are needed [7]. Propagation of *Trichoderma* sp. on a massive scale needs to conduct in tackling plant pathogens. The propagation on large scale is necessary to use cheap alternative media but still contain sufficient nutrients for the growth of this fungus [8].

Organic wastes that are potentially used as growth media for growing this fungus are tofu liquid waste and rice washing wastewater. The tofu industry is one of the fastest-growing industries in Indonesia. In the process of processing materials into products, the tofu industry produces waste, both solid and liquid waste. Tofu industrial liquid waste is produced from the washing, boiling, pressing, and printing of tofu. Although tofu liquid waste contains various organic materials, its use and utilization are still rare so that it is still a source of environmental pollutants [9].

Tofu liquid waste contains several nutrients essential for the growth of fungi. It contains N 1.24%, P_2O_5 5.54%, K_2O 1.34%, and C-Organic 5.803%. N, P, and K contained in tofu wastewater are important in the physiological and metabolic processes of fungi [10]. Generally, tofu liquid waste contains protein, carbohydrates, and fat. The protein contained in tofu wastewater ranges from 40% to 60%, carbohydrates 25% to 50%, and fats about 10% [11].

The utilization of tofu liquid waste is the right way to increase its use-value and reduce the impact of environmental pollution it causes. Organic liquid waste is potential as a medium for the propagation of biological agents because it contains some essential nutritional compositions for microbial growth such as carbohydrates, protein, water, amino acids, fats, mineral salts, and other nutrients. These nutrients can be used by microorganisms, such as fungi, for their development and growth processes [12].

Meanwhile, rice washing wastewater is an organic waste from household activities. It contains carbohydrates, some nutrients, vitamins, and other mineral substances. These contents can be useful as growth enhancement for microorganisms [13]. Rice undergoes a washing process before being cooked into the rice. In the washing process, rice is usually washed or rinsed 3 times to clean rice from dirt. This milky white wastewater contains protein, phosphorus, and vitamin B1 stimulating growth and metabolism [14].

Thus, in this article, the authors discussed the characteristics and density of the fungal conidia of *Trichoderma* sp. which is cultivated using alternative media in the form of tofu liquid waste and rice washing wastewater.

2 Materials and Methods

2.1 Time and Place

This research was conducted from 14 May 2018 to 14 June 2018, at the Laboratory of the Technical Service Unit Protection Estate Crops Centre, South Sumatera Estate Crops Service, Indonesia (UPTD BPTP Dinas Perkebunan Sumatera Selatan).

2.2 Materials

The tools used in this work were aluminum foil, glass bottle, bunsen, Petri dishes, steamer, Erlenmeyer, measuring cup, hemocytometer, hot plate, ose needle, rubber band, label paper, stove, microscope, tray, ruler, pencil, dropper, knife, filter paper, spatula, test tube, and analytical balance. The materials used were agar, distilled water, 70% alcohol, amoxicillin, detergents, sugar, cotton, potatoes, and the fungal culture of *Trichoderma* sp. obtained from the Laboratory of the Technical Service Unit Protection Estate Crops Centre, South Sumatera Estate Crops Service, Indonesia (UPTD BPTP Dinas Perkebunan Sumatera Selatan).

2.3 Sterilization

Glass bottles (cultivation bottles) and Petri dishes wrapped in paper. Then sterilized conventionally using a steamer on the stove at 70 ºC for 5-8 hours [15]. Meanwhile, the ose needle was sprayed with 70% alcohol, then heated until it turned red using Bunsen.

2.4 Media Preparation

2.4.1 Potato Dextrose Agar (PDA) Media Preparation

The potatoes are cleaned and peeled, then cut into cubes. Then, they were weighed 200 grams and washed using water. The potato slices were put into the Erlenmeyer. Added 1 liter of distilled water. Covered with cotton and aluminum foil and secured with a rubber band. Then it is heated to a boil. Then filtered the potato extract. Aquadest Added to the extract until the volume reached exactly 1 liter. Also added 20 grams of agar, 20 grams of sugar (modified carbon sources), and 500 mg of amoxicillin [16]. Reheated until boiling. Then it was steamed or heated on the stove for 5 - 8 hours [15].

2.4.2 Tofu-Liquid-Waste Agar Alternative Media Preparation

One liter of tofu liquid waste was added to the Erlenmeyer flask. Then, added 20 grams of sugar, 20 grams of agar, and 500 mg amoxicillin. The Erlenmeyer flask was covered with cotton and aluminum foil and tied using a rubber band [17]. Then, it is steamed or heated on the stove for 5 - 8 hours [15].

2.4.3 Rice-Washed-Wastewater Agar Media Preparation

One liter of rice washing water was poured into the Erlenmeyer flask. Then, added 20 grams of sugar, 20 grams of agar, and amoxicillin 500 mg. The was flask covered with cotton and aluminum foil and tied using a rubber band [17]. Then was steamed or heated on the stove for 5 - 8 hours [15].

2.5 Rejuvenation of Trichoderma sp.

Trichoderma sp. isolate was taken aseptically by cutting the agar medium using an ose needle. Then, it was put into a petri dish that already contained a modified PDA medium [18]. Then incubated for 5 - 7 days at room temperature [19].

2.6 Innoculation of Trichoderma sp. into Tofu-Liquid-Waste Agar

Fungi Trichoderma sp. which had been rejuvenated aseptically inoculated by cutting the agar medium overgrown with *Trichoderma* sp. using ose needle. The slices were put into a petri dish that already contained tofu-liquid-waste agar media. Then it was incubated for 5 - 7 days at room temperature [19].

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2.7 Innoculation of Trichoderma sp. Into Rice-Washed-Wastewater Agar Media

Fungi Trichoderma sp. which had been rejuvenated aseptically inoculated by cutting the agar medium overgrown with *Trichoderma* sp. using ose needle. The slices were put into a petri dish that already contained Rice-Washed-Wastewater Agar Media. Then it was incubated for 5 - 7 days at room temperature [19].

2.8 Data Observation

2.8.1 Macroscopic Morphology Observation of Fungus Trichoderma sp. Cultivated in Alternative Media

Trichoderma sp. which was grown in alternative waste media from tofu liquid waste and rice washing water observed its macroscopic morphology. The characters included colony diameter, colony color, colony texture, concentric lines, and reversed-colony color [18]. The observation was carried out until the 7th day.

2.8.2 Microscopic Characters Observation of Fungus Trichoderma sp. Cultivated in Alternative Media

The slide and cover glass were sterilized with alcohol 70%, then heated with bunsen for a while. One ose of the mycelium of *Trichoderma* sp. was taken and scratched on the slide. Then dripped with lactophenol and covered with a cover glass. The microscopic characteristics were observed using a light microscope [20]. The observed microscopic characters of *Trichoderma* sp. included the structure of the mycelium, hyphae, conidia, and the conidia-producing bodies [21]. The observation was done on the 7th day of the incubation.

2.9 The Conidial Density Calculation of Trichoderma sp.

Conidia of *Trichoderma* sp. were taken from both alternative media by using an ose needle. The conidia were put in a test tube containing 10 mL of distilled water, then homogenized. The dilution is carried out to obtain a suspension with a conidial density of 10^6/mL. 0.5 mL of the suspension was poured into a test tube containing 4.5 mL of sterile distilled water and homogenized. one mL of the suspension was dropped into the syringe of the counting chamber. The number of conidia was counted using a microscope [22]. The formula for calculating conidia density was:

$$C = \frac{t}{(n \times 0.25)} \times 10^6$$

where C is conidial density/mL solvent, t is the total conidial number, n is a total small unit (80 small units), and 0.25 is the correction factor for the use of the small unit.

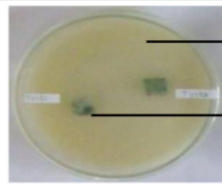
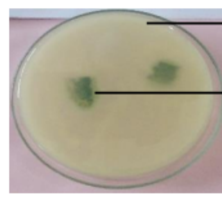
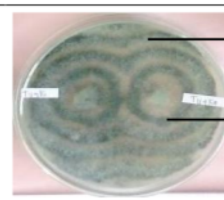
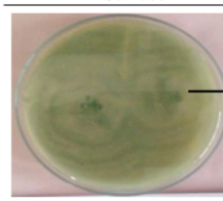
3 Result and Discussion

3.1 Macroscopic Morphology Observation of Trichoderma sp. Cultivated in Tofu-liquid-waste Agar Media

The initial colony of *Trichoderma* sp. cultivated on tofu-liquid-waste agar was white with a fibrous surface and fine texture. Incubation on the first day did not show concentric lines. The reverse colony had a creamy white in color. The average diameter of the isolate on the first day of incubation was 1.54 cm from the initial diameter of 1.42 cm or equivalent to 0.12 cm growth. According to [23], the initial phase of microbial growth, including fungi, is the adaptation phase. In the adaptation phase, fungi have a low growth rate. In this stage, fungi will adjust and adapt to the new environmental conditions and nutrients.

The colony of *Trichoderma* sp. was turned green after three days of incubation. On the third day, it already had concentric lines with a light green reverse media. The colony diameter on the third day of incubation was 5.07 cm. Meanwhile, on the 7th day of incubation, the fungal colony diameter was 7.07 cm with green reverse media. The mycelium had filled the entire surface of the media. The macroscopic morphology of *Trichoderma* sp. cultivated in tofu-liquid-waste agar media is shown in table 1.

Table 1: Macroscopic Morphology of *Trichoderma* sp. Cultivated on Tofu-liquid-waste Agar Media

Day	Macroscopic Morphology of *Trichoderma* sp.
1	 Surface
	 Reverse
7	 Surface
	 Reverse

The growth of *Trichoderma* sp. on the media is triggered by the adaptation phase of the isolate to the media condition. The adaptation allowed the isolate to utilize the nutrients contained in the media. The adaptations also enhanced the physiological processes of the fungus with the environmental conditions surrounding the media. According to [24], an increase in the spread of fungal mycelium on the surface of the medium showed that the fungus had been able to adapt to the conditions therefore it can grow on the medium. This adaptability enables the fungus to multiply by using the available nutrients.

Fungi will grow extensively after entering the exponential phase. In the exponential phase, fungal cells divide rapidly and then grow rapidly as well. The exponential phase is a state of rapid growth with a specific growth rate and fixed cellular composition. Meanwhile, the chemical composition of the medium changes due to product synthesis in the use of the substrate by fungal cells [25]. The growth of *Trichoderma* sp. on the alternative media mainly composed of tofu liquid waste was due to the nutritional contents contained in that organic waste. The nutrients contained in tofu liquid waste such as protein about 40% to 60%, carbohydrates 25% to 50%, and fats about 10% [11] are useful in supporting the growth process of this potential fungus. The macroscopic characters of *Trichoderma* sp. cultivated on tofu-liquid-waste agar are shown in table 2.

Table 2: Macroscopic Characters of *Trichoderma* sp. Cultivated on Tofu-liquid-waste Agar Media

Day	Diameter (cm)	Colony Color	Colony Texture	Reversed Media Color	Concentric Ring
0	1.42	Green	Smooth	White	-
1	1.54	White	Smooth	White	-
2	3.31	White	Smooth	Light Green	-
3	5.07	Green	Smooth	Light Green	√
4	5.34	Green	Rough	Light Green	√
7	7.07	Green	Rough	Dark Green	√

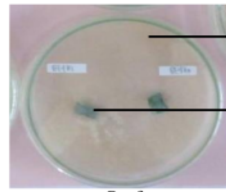
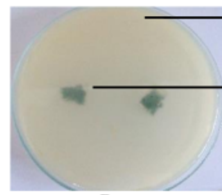
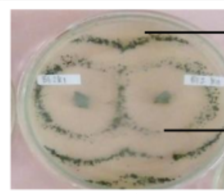
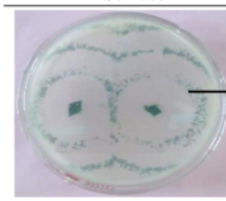
Tofu liquid waste contains many organic nutrients. Disposing of this waste into the environment directly without being processed will result in pollution of the environment. In Indonesia, most tofu processing industries do not process liquid waste properly due to the additional cost needed. Therefore, it is still a problem for the environment.

Meanwhile, the ability of *Trichoderma* sp. to grow in the tofu liquid waste medium is the potential to increase the usefulness of organic waste. Nowadays, tofu liquid waste in Indonesia is disposed of directly into the environment which causes environmental pollution. Thus, the use of tofu liquid waste as an alternative medium for growing *Trichoderma* sp. is one of the right solutions to overcome the problem.

3.2 Macroscopic Morphology Observation of Trichoderma sp. Cultivated in Rice-Washing-Wastewater Agar Media

The growth of the fungus *Trichoderma* sp. cultivated on an alternative medium of rice-washing-wastewater agar was less optimal compared to the tofu-liquid-waste agar media. Fungi *Trichoderma* sp. cultivated in alternative media of rice washing water had thinner colonies compared to tofu liquid waste media. The growth of the fungal mycelium *Trichoderma* sp. on the medium was uneven and thin. The mycelium did not cover the entire surface of the medium. This can be seen in table 3.

Table 3: Macroscopic Morphology of *Trichoderma* sp. Cultivated on Rice-Washing-Wastewater Agar Media

Day	Macroscopic Morphology of *Trichoderma* sp.
1	 Surface
	 Reverse
7	 Surface
	 Reverse

On the first day of incubation, *Trichoderma* sp. on the rice-washing-wastewater media did not increase its colony diameter. During the first day of incubation, it did not have a clear colony form. Colony color appeared white after incubation for two days. Two days old isolate only had a colony diameter growth of 0.78 cm. Growth of Fungi *Trichoderma* sp. Meanwhile, on the 7th day of incubation, the colony diameter was 6.42 cm. However, these fungal colonies appeared thin and patchy. The growth of the colony only forms concentric lines which appeared to be tenuous from each other.

The slow growth of the fungus *Trichoderma* sp. in the alternative rice-washing-wastewater media indicated that the fungus had a low growth rate in the media. This phenomenon is caused by the limited availability of nutrients in the media. Nutritional limitations could be caused by the water used for washing rice which is runny. According to [24], the spread of mycelium on the surface of the media occurs when the fungus has been able to adapt to the conditions of the media. One of the factors that cause the success of the adaptation process is nutrition. Lack of nutrients interferes with the process of spreading mycelium on the surface of the media.

The nutrition available in the medium affected the increase in the diameter of the fungus *Trichoderma* sp. cultivated in the alternative media as shown in Figure 4. The isolate cultivated in tofu liquid waste media had increased in diameter since the first day of incubation. The diameter of the fungus increased with the incubation time of the fungus. Meanwhile, the fungi that were cultivated in an alternative medium of rice washing wastewater had a lower and slower growth rate, particularly in diameter. This phenomenon might be caused by the low nutrient content of the alternative media. According to [17], the availability of nutrients greatly affects the diameter of a fungus. The macroscopic characters of *Trichoderma* sp. cultivated on Rice-washing-wastewater agar are shown in table 4.

Table 4: Macroscopic Characters of *Trichoderma* sp. Cultivated on Rice-Washing-Wastewater Agar Media

Day	Diameter (cm)	Colony Color	Colony Texture	Reverse Media Color	Concentric Ring
0	1.04	Green	Smooth	White	-
1	1.04	White	Smooth	White	-
2	1.82	White	Smooth	Light Green	-
3	4.44	White	Smooth	Light Green	√
6	5.48	Green	Rough	Light Green	√
7	6.42	Green	Rough	Light Green	√

5.3 Microscopic Characters of Trichoderma sp. Cultivated in Tofu-Liquid-Waste Agar and Rice-Washing-Wastewater Agar Media

Observation of the microscopic characters of *Trichoderma* sp. performed by using modified Potato Dextrose Agar and alternative media. After incubated for three days, some characters of *Trichoderma* sp. could be seen including conidia, phialides, and conidiophores as shown in figure 1. According to [26], conidia are asexual reproductive cells that have various forms depending on the species. Conidial morphology is essential in the conventional identification of fungi to the species level.

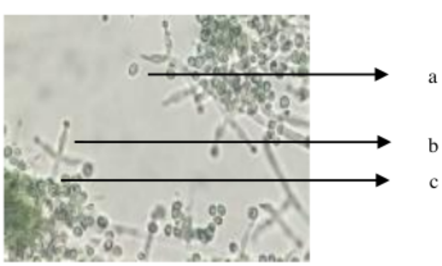
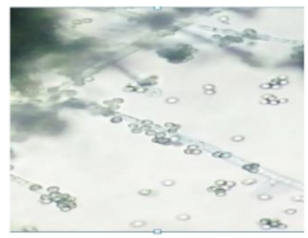
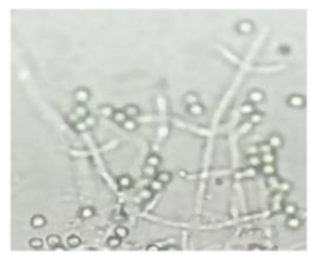


Figure 1: Microscopic characters of *Trichoderma* sp. cultivated in modified Potatto Dextrose Agar (a) conidia, (b) phialide, and (c) conidiophore

Furthermore, the microscopic morphology of *Trichoderma* sp. cultivated in tofu-liquid-waste agar and rice-washing-wastewater agar media are presented in table 5.

Table 5: Macroscopic Morphology of *Trichoderma* sp. Cultivated in Cheap Alternative Media

Media	Day	Microscopic Morphology
Tofu Liquid Waste Agar	7	
Rice Washing Wastewater Agar	7	
Modified Pottato Dextrose Agar	3	

Conidiophores are part of the fungi that function as stalks for the formation of conidia. Conidiophores consist of some branches, some are paired and some are not. Each conidiophore branch forms a new branch. At the end of the conidiophore branch, found a phialide [26]. Phialides are the ends of the conidiophores where conidia grow. phialides can be shaped like slender bottles and grow singly or irregularly. Conidia will grow at the end of the phialides [27].

Based on Figure 1, it can be seen that *Trichoderma* sp. used in this study had oval conidia located at the tip of the phialides. Its phialides were pointed and short. Meanwhile, the conidiophores of the *Trichoderma* sp. used in this study were erect and branched. These characters have some similarities to the fungal species of *Trichoderma koningii*. According to the colonies of *Trichoderma koningii* on the initial growth will be white with a smooth surface. The presence of aerial hyphae on the edge of the colony makes the edges filamentous. The colony will then grow dark green. The phialides are shaped like a bottle and are slender, narrow at the base, the middle is wide and coned at the top.

The results of microscopic morphological observations of *Trichoderma* sp. cultivated in the alternative media did not show the complete and perfect morphological form as shown in table 5. It was due to the microscopic observation of the isolate in the alternative media was carried out on the 7th day of incubation, it should have been observed on the second or third day of the incubation [6].

3.4 The Conidial Density of *Trichoderma* sp. Cultivated in Cheap Alternative Media

The conidial density of *Trichoderma* sp. was estimated after seven days of incubation. Based on the estimation, the average conidial density of the isolate is presented in table 6.

Table 6: Conidial Density of *Trichoderma* sp. Cultivated in Tofu-Liquid-Waste Agar and Rice-Washing-Wastewater Agar Alternative Media

No.	Alternative Media	Conidial Density
1.	Tofu-Liquid-Waste Agar	2,9 x 10^6
2.	Rice-Washing-Wastewater Agar	1,6 x 10^6

Based on table 6, it can be seen that the conidial density of *Trichoderma* sp. is higher in the alternative medium of tofu liquid waste compared to the rice washing water. The average conidial density of *Trichoderma* sp. cultivated in tofu liquid waste was 2.9 x 10^6. Meanwhile, the conidial density of *Trichoderma* sp. cultivated in rice washing water media was only 1.6 x 10^6. Based on these data, it can be concluded that the growth of *Trichoderma* sp. is more optimal in the alternative medium of tofu liquid waste than rice washing water. This different growth rate and conidial density are caused by the differences in the nutritional composition of the tofu liquid waste and the rice washing wastewater. Additionally, the tofu liquid waste used has a higher concentration than the rice washing water.

Tofu liquid waste contains protein, carbohydrates, and fat. The protein contained in tofu wastewater ranges from 40%-60%. Carbohydrates contained in tofu liquid waste range from 25% - 50%, and contain about 10% fat [11]. Meanwhile, the highest nutritional content in rice washing water is carbohydrates by 41.3%, while protein was only 26.6% and fat 18.3% [28]. The high protein content in tofu wastewater is essential for the growth of *Trichoderma* sp. Protein is a nutrient that is used to stimulate the growth of fungal mycelium. The protein contained in tofu wastewater increases the division rate of hyphae cells to form mycelium. Cell division requires nitrogen compounds found in these proteins. Nitrogen which is a building block of these proteins is useful in the synthesis of nucleic acids in fungal cells. A sufficient amount of protein enhances the optimal mycelium growth rate [17].

The carbohydrates contained in the medium can be used by *Trichoderma* sp. as the main energy source in the form of ATP to carry out the cellular respiration process. The use of carbohydrates as an energy source will be carried out through the catabolism of carbohydrates to form glucose. The breakdown of complex carbohydrates into glucose is carried out by excreting certain enzymes, such as amylase. According to [26], the substrate can be utilized by fungi after the fungi excrete extracellular enzymes that can break down complex compounds from the substrate into simpler compounds.

The higher percentage of nutrients in tofu wastewater supported the growth rate of *Trichoderma* sp. faster than rice washing water. The higher nutrient content was able to provide a sufficient source of energy for the growth of *Trichoderma* sp. Based on [17], the main function of nutrition is as a source of energy for cells, the building blocks of cells, and as an electron acceptor in the process of producing energy in the form of ATP. Although rice washing water contains several components such as B vitamins and phosphorus which can support the activity of fungal cells, the concentration of these components was insufficient. It causes the growth rate of the fungus *Trichoderma* sp. on the alternative medium, the rice washing was slower.

4 Conclusion

Fungi *Trichoderma* sp. cultivated in tofu liquid waste and rice washing wastewater media had green colonies with circular colony growth. However, in the tofu liquid waste medium, the mycelium filled the entire surface of the media on the 7th day of incubation, while in the alternative made of rice washing wastewater mycelium did not cover the entire surface of the media. The average conidial density of *Trichoderma* sp. cultivated in tofu liquid waste was 2.9 x 10^6, while the conidial density of *Trichoderma* sp. cultivated in an alternative rice washing wastewater was only 1.6 x 10^6.

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Ethical issue

Authors are aware of and comply with, best practices in publication ethics specifically about authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests, and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution to data collection, data analyses, and manuscript writing.

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