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The Effect of Storage Time of Water Hyacinth Liquid Organic Fertilizer on Microorganism Viability and Potency

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Abstract: The quality of biological organic fertilizer is determined by the viability of microorganisms during storage until the fertilizer is applied to the field. The purpose of this study was to determine the effect of length of storage of biological organic fertilizer on the viability of microorganisms, and identify the types of microorganisms that can survive in several storage periods and their potential. The research was conducted at the Payakumbuh State Agricultural Polytechnic Screenhouse from September to November 2024 using a non-factorial Completely Randomized Design (CRD) with five treatments and five replications. The treatment of storage duration of biological organic fertilizer includes: T1 = 0 days storage time, T2 = 7 days storage time, T3 = 14 days storage time, 21 days storage time, and 28 days storage time. The results showed that the best storage length for bacterial viability was from 0 to 14 days after fermentation with a bacterial population of 1.0,107-3.3,107 CFU/ml and a fungal population of <10-1.4,102 CFU/ml. Seven potential and multifunctional bacterial isolates were found, B. altitudinis, Bacillus cf. zanthoxylli, Bacillus cereus, Pseudomonas aeruginosa, Pseufomonas. flexibilis, Pseudomonas cf. pharmacofabricae, and Ectopseudomonas mendocina. All bacterial isolates were able to produce IAA hormone with concentrations between 0.0039-0.0057 µg/ml. Bacterial species that have high viability in water hyacinth POH are bacteria from the genera Bacillus spp. and Pseudomonas spp.

Keywords: Length of storage; Potency; Viability.

Introduction

Biological organic fertilizers are fertilizers derived from organic matter, both from plants and animals, which have gone through a decomposition process so that they become nutrients that are beneficial for plant growth. Bio-organic fertilizers can be in solid or liquid form. A distinctive feature of biological organic fertilizer is the presence of live microorganisms (viability) that function as decomposers and phosphate solvents (Nursanti, 2017; Allouzi et al., 2022). The viability of microorganisms in biological organic fertilizer greatly affects the quality of the fertilizer (Sarikhani et al., 2019). Bacterial viability is defined as the ability of bacteria to survive in certain media. Some factors that affect bacterial viability are media, temperature, pH, moisture content, number of bacteria, and packaging (Cabello-Olmo et al., 2020), Bacterial viability is measured by detecting the growth of single cells into colonies in terms of colony forming units (CFU) (Wang & Wu, 2024).

Microorganisms act as bioactivators in the decomposition process. Microorganisms are bacteria and fungi that are beneficial. Bacterial consortia have a more significant impact than single bacteria on the degradation process of organic matter in increasing nitrogen and potassium nutrients in the material and optimizing pH (Liu et al., 2023), but the nitrogen content in fertilizers is influenced by the absorption of exogenous nitrogen by decomposing microbes that meet their metabolic needs (Bani et al., 2018). Fungi have the

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ability to produce certain enzymes and the ability to access new substrates through hyphae (Bani et al., 2018). The type of substrate or carrier medium is an important factor affecting the microorganism community along with various environmental factors such as temperature and humidity, and the microbial community is also able to influence the characteristics of the substrate itself (Bani et al., 2018). The carrier medium of biofertilizer will determine the overall quality of the fertilizer (Sondang et al., 2020a). The carrier material should allow the bacteria to remain alive and thrive during the fermentation process, storage, and when ready for application.

Bio-organic fertilizers are often not available in time for application, so a storage method is needed so that they can be used at any time. Nowadays, more and more bio-organic fertilizers are available in the market, but there is no certainty whether the microorganisms contained in them are still active and functioning. The benefit of biofertilizers lies in the active role of the available microorganism population (Ngampimol & Kunathigan, 2008). Therefore, it is important to consider appropriate storage methods, so that the microorganisms contained in biological organic fertilizers remain viable and can develop. According to Berninger et al (2018) the survival of bacterial cells during the storage process is the key to success in the commercialization of biofertilizer products. One of the advantages of bio-organic fertilizers is the long storage mass, which is 1.5 to 2 years, without the risk of contamination Mahanty et al (2017) reported that a long shelf life can be achieved by adding ingredients that support the growth of microorganisms.

The results of Adeleke & Oluwaseun (2022) stated that water hyacinth is a good carrier medium, judging from the many types of microorganisms that can be harvested. There are two important bacterial genera that are often found in biological organic fertilizers, namely the genera Bacillus spp. and Pseudomonas spp. Kumawat et al (2017) stated that Bacillus spp is able to dissolve phosphate and micronutrients such as zinc (Zn) and silicate (Si). Etesami et al (2017) reported that K dissolution can be done by bacteria such as B. mucilaginosus, B. edaphicus, B. cereus, Pseudomonas sp, and Paenibacillus spp. Added by Setiaji et al (2023) that Bacillus spp. can act as biological control agents and stimulants in plants. The objectives of the study were to determine the effect of the length of storage of water hyacinth biological organic fertilizer on the viability of microorganisms, identify the types of microorganisms that can survive during storage, and the potential of these microorganisms.

Method

Place and Time

The research was conducted at the Payakumbuh State Agricultural Polytechnic Screenhouse and the Biology Laboratory of FMIPA IPB Bogor. The research was conducted for 3 months from September to November 2024.

Materials and Tools

Materials consisted of cow feces, water hyacinth, cow bone meal, shallots, molasses, bacteria Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis, Nutrient Agar (NA), Tryptone Soya Agar (TSA), Potato Dextrose Agar (PDA), Plate Count Agar (PCA), Nutrient Broth (NB). 70% alcohol, and sterile aguades. The tools used were plastic jars, glass storage jars, microscopes, scales, petri dishes, measuring cups, erlenmeyers, hand sprayers, spatulas, stirring spoons, and ose needles.

Research ImplementationPreparation of Biological Organic Fertilizer

The biological organic fertilizer (POH) was made by fermenting cow feces for one week, then adding finely chopped water hyacinth, cow bone meal, shallots, and molasses. Then inoculated with a bacterial consortium of Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus, Bacillus thuringiensis) from previous research (Sondang et al., 2023). Biological organic fertilizers can be produced using one or a mixture of several microorganisms based on the role of the biofertilizer produced (Allouzi et al., 2022). The fermentation process of water hyacinth POH was carried out for 4 weeks with POH stirring done once a week. After 4 weeks, the liquid POH was removed from large jars with no carrier media and continued the storage process in glass jars with a time of 0, 7, 14, 21, and 28 days. POH that has been made is stored at room temperature 27-28oC.

POH storage experiments were carried out using a non-factorial completely randomized design (CRD) with 5 treatments and 5 replicates. Treatment T1 = 0 days storage, T2 = 7 days storage, T3 = 14 days storage, T4 = 21 days storage, and T5 = 28 days storage. The experiment used analysis of variance with a 5% F test and to determine the effect of each treatment followed by a 5% Duncan's Multiple Range Test (DMRT) to determine differences between treatments.

Viability Test of Microorganisms in POH

The technique for growing microorganisms in agar media is carried out using the pour plate method. Pour 1 ml of serially diluted POH 104, 105, 106, 107 into a petri dish and add 10 ml of TSA media to grow bacteria and

10 ml of PDA media to grow fungi, then shake the petri dish until the POH solution is evenly distributed on the surface of the agar or in the agar. Incubate for 2-3 days to see the growth of microorganisms. Observations were made 2 x 24 hours after growth on TSA and PDA media.

Counting the number of microorganisms was done using the Total Plate Count (TPC) method (Wang & Wu, 2024). TPC was performed by taking 10 µ of liquid POH in a certain dilution using peptone water. Petri dishes were incubated for 24 hours at 28-30 ^{O}C.

Purification of Microorganisms

Each bacterium was scratched on NA media using an ose needle. The results of purification in the form of one colony of each bacterium were taken using an ose needle. Then scratched again on new NA media and incubated for 24 hours, and continued until pure isolates were obtained. Furthermore, bacterial identification uses molecular analysis based on the 16S rRNA gene sequence using PCR.

Qualitative test of P and K solubilizing bacteria

Quantitative testing is done by growing bacteria on liquid Alexandrov media with Feldspar as a potassium source. The observation variables for qualitative tests are the diameter of bacterial growth and the diameter of the clear zone formed by bacteria. In the qualitative test, the quality of bacteria can be seen from the solubility index (IK) value. The higher the IK value, the more capable the bacteria are in dissolving potassium. Testing of bacterial isolates in dissolving phosphate is characterized by the presence of colonies and clear zones formed. Testing of bacterial isolates in dissolving potassium is characterized by the presence of colonies and yellow zones formed on Alexandrov media.

Bacterial IAA production test

Pure isolates of bacteria were tested for their ability to produce the hormone IAA. Bacterial isolates were cultured on nutrient agar media. After 24 hours, they were inoculated into 50 ml of Luria Bertani (LB) containing 0.1% DL-Trytophan and incubated in a shaking incubator for 24 hours at 125 rpm. The bacterial culture was centrifuged at 10,000 rpm for 10 minutes. Separate 1 ml supernatant was mixed with 4 ml Salkowski reagent and incubated in a dark room for 30 minutes. The mechanism is that IAA containing Indole groups have the ability to interact with Fe3+ ions, when FeCl3 (Salkowski reagent) is added to a solution containing IAA, Fe3+ ions will bind to phenol or indole groups on IAA. This process forms a complex that is pink in color.

Result and Discussion

Viability of microorganisms in POH

Viability of microorganisms at various lengths of POH storage was observed based on the types of bacteria and fungi that survived and the population counts (TPC) of bacteria and fungi treated with POH storage for 0, 7, 14, 21, and 28 days are presented in Table 1 and Table 2.

Table 1. Bacterial viability at various storage lengths of water hyacinth POH

Storage Duration POH	Total bacterial population (CFU/ml)
T1 (0 day)	$3.3.10^{7}$ a
T2 (7 days)	$2,.4.10^{7}$ ab
T3 (14 days)	$1.0.10^{7}$ c
T4 (21 days)	$3.6.10^{6}$ d
T5 (28 days)	$1.5.10^{6}$ d

Numbers followed by the same lowercase letter in the same column are not significantly different in the DMRT further test at the 5% level.

One of the factors that determine the quality of biological organic fertilizer is the number of microorganisms living in it. Table 1 shows the number of bacterial populations (TPC) and the most abundant types of bacteria at 0, 7, and 14 days of POH storage, because the nutrients needed by bacteria are still sufficient. When POH is stored for 21 and 28 days, the amount of nutrients decreases along with the start of bacterial proliferation, resulting in competition between bacteria, where the stronger bacteria survive and other bacteria die, at this time an increase in temperature occurs. The carrier medium for water hyacinth POH acts as a medium in which the microorganisms live. The carrier material should allow the bacteria to survive and thrive during the fermentation process, storage, and when applied. The presence of phosphate-solubilizing bacteria in the carrier medium allows the decomposition process of organic matter to continue. According to Nursanti (2017) storage at low temperatures can maintain the survival of microorganisms and reduce the death of microorganisms.

Research by Hidayati et al (2017) stated that freeze-dried bacteria storage has stable bacterial viability up to two months of storage, after two months bacterial viability will decrease. Added by Berninger et al (2018) that the ability to live bacterial cells in increasingly long storage is a prerequisite for the successful commercialization of biofertilizer products. According to Mahanty et al (2017) the advantage of biological fertilizer is that it has a long shelf life of 1.5-2 years without being contaminated by adding materials that increase the growth of microorganisms.

Water hyacinth is an abundant source of bacterial inoculum that can be harvested and processed as a bacterial suspension. In accordance with the opinion of Adeleke & Oluwaseun (2022) that microbiological analysis of water hyacinth plant parts contains total heterotrophic, nitrogen-fixing, phosphate-solubilizing, and heterotrophic fungi.

Table 2. Viability of fungi at various lengths of POH storage

Storage Duration POH	Total bacterial population (CFU/ml)	
T1 (0 day)	$1{,}4.10^2$	a
T2 (7 days)	$5{,}5.10^1$	b
T3 (days)	$1{,}5.10^1$	b
T4 (days i)	< 10	c
T5 (days i)	< 10	c

Numbers followed by the same lowercase letter in the same column are not significantly different in the DMRT further test at the 5% level.

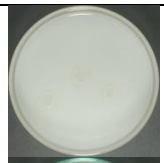
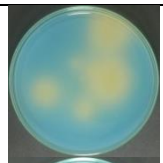
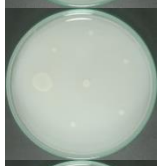
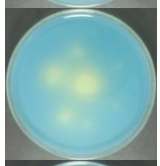
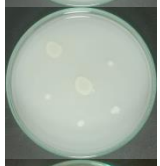
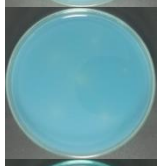
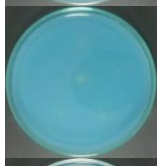
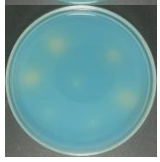
Table 2 shows that the total population of fungi is very low. From the results of dilution 101, only a few fungi were found, and even then only at 0, 7, 14 days of POH storage, but at the 21th and 27th storage, no fungal growth was detected. It should be noted that water hyacinth POH contains live microorganisms that must be properly managed to survive until the time of application to soil or plants, and microorganisms have their own very important roles in the process.

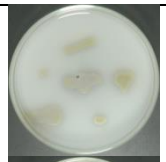
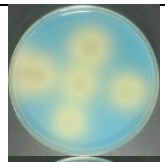
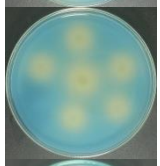
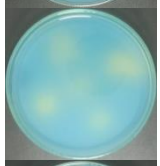
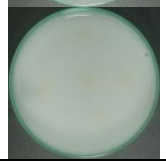
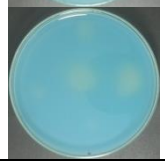
The main factors that play a role in the growth of microorganisms are nutrient supply, time, temperature, water presence, pH, and oxygen availability. Each type of microorganism has a certain pH range that supports its growth. According to Arfarita et al (2019) most microorganisms grow optimally in the pH range of 6.0-8.0.

Qualitative Test of P and K

After the purification process of bacteria from their colonies, 10 species of bacteria were obtained from the POH storage treatment on days 0, 7, 14, 21, and 27. Furthermore, the ten bacterial species were tested qualitatively to assess the ability of bacterial isolates to dissolve P and K. The results of the qualitative testing can be seen in Table 3.

Table 3. Bacterial species and P and K solubilizing ability

Bacterial species	Solvent P		Solvent K	
Bacillus altitudinis		+		+++
Pseudomonas flexibilis		-		-
Bacillus cereus		-		++
Bacillus cf. zanthoxylli		-		-
Pseudomonas cf. pharmacofabricae		-		-
Bacillus altitudinis		++		++

Bacterial species	Solvent P		Solvent K	
Pseudomonas aeruginosa		++		+++
Pseudomonas aeruginosa		++		+++
Bacillus altitudinis		+		++
Ectopseudomonas mendocina		-		+

Notes: + = Weak
++ = Medium
+++ = strong

Based on the observation of bacterial isolates that are able to dissolve phosphate, some isolates show a clear zone as presented in Table 3. The table shows that in biological organic fertilizer derived from water hyacinth, there are 3 species of phosphate solubilizing bacteria that also function as potassium solubilizers, meaning that these bacteria have a multifunctional role. All species are from the genera Bacillus spp. and Pseudomonas spp. Phosphate solubilizing microorganisms play an important role in converting insoluble phosphate into a soluble form, so that it can be absorbed by plants. The utilization of POH containing phosphate solubilizing microorganisms is expected to overcome the problem of phosphorus nutrient deficiency in soil and in plants.

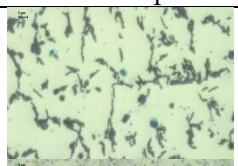
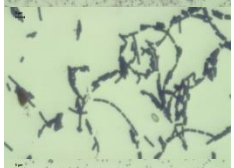
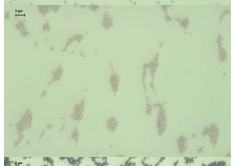
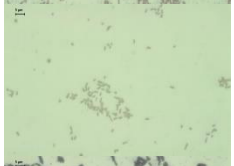
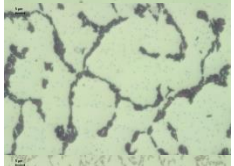
The addition of several species of bacteria with different functions to the POH is expected to maintain the viability of the bacteria until its application in the field. In accordance with the opinion of Jacoby et al (2017) microbial inoculation into organic fertilizers aims to take advantage of the ability of microbial roles as decomposers. Microorganism inoculants have a significant ability to increase plant growth, enrich nutrient content, and support the absorption process and plant health. Castiglione et al (2021) reported that inoculation using microbial consortia with selected beneficial microbes, such as plant growth-promoting bacteria, has been shown to improve nutrient use efficiency, especially phosphorus, nitrogen, and carbon.

There are four species of K-solubilizing bacteria in water hyacinth POH, two from the genera Bacillus spp. and two from the genera Pseudomonas spp. Potassium-solubilizing microorganisms are microorganisms that can dissolve bound potassium into solubility. Potassium bacteria are usually fixed by aluminum (Al) and iron (Fe) on soil colloids. The mechanism of potassium dissolution by microorganisms is by producing organic acids. Handayani & Amin (2024) stated that microbes play a role in the K cycle through the mechanism of organic acid production. This organic acid mineralizes organic K so that it is available to plants. Potassium-solubilizing bacteria can produce organic acids such as citric acid, oxalic acid, malic acid, succinic acid, and tartric acid (Prajapati & Modi, 2012) and reduce pH (Bagyalakshmi et al., 2017).

In Table 4, isolates of Pseudomonas flexibilis, Bacillus cf. zanthoxylli, and Pseudomonas cf. pharmacofabricae did not show the ability to dissolve nutrients P and K. This means that bacteria cannot increase phosphate and potassium nutrients. Microorganisms have a life cycle that at a certain period the microorganisms will die, and do not function at all, so the fertilizer needs to be packaged in a dormant state and needs to reactivate the microorganisms before being applied to the soil or plants.

Microorganisms in biological organic fertilizers play a role in increasing the availability of nutrients for plants, as bioactivators of organic matter breakers, as pest and plant disease control agents, and produce active substantives to spur plant growth (Nursanti, 2017; Saraswati et al., 2004).

Table 4. Molecular characteristics of bacterial cells

Isolate Code	Cell shape	Color	shape	Gram
Bacillus altitudinis		Lilac	Basil	Positive
Pseudomonas flexibilis		Red	Basil	Negative
Bacillus cereus		Lilac	Basil	Positive
acillus cf. zanthoxylli		Red	Coccus	Negative
Pseudomonas cf. pharmacofabricae		Red	Basil	Negative
Bacillus altitudinis		Lilac	Basil	Positive
Pseudomonas aeruginosa		Red	Basil	Negative
Pseudomonas aeruginosa		Red	Bacil	Negative
Bacillus altitudinis		Lilac	Basil	Positive
Ectopseudomonas mendocina		Red	Basil	Negative

Based on microscopic observations, the bacterial cell shapes of all isolates show many similarities. Among these forms, bacillus cells are the most dominant form with dark and pink colors. In addition, liquid organic fertilizers have better advantages than inorganic fertilizers and solid organic fertilizers in terms of lasting longer, improving crop quality, and requiring less quantity for application (Allouzi et al., 2022).

Bacillus spp. are gram-positive bacteria that are strong and able to form endospores (Setiaji et al., 2023), so they can compete with other organisms, for example B. altitudinis, B. cereus. Pseudomonas spp. is a

hemoorganotroph bacterium, straight rod-shaped, cell size 0.5-0.1-1 µm x 1.5-4 µm, does not form spores and reacts negatively to gram staining. Pseudomonas spp is classified as a non-symbiotic bacterium that is able to fix nitrogen and phosphate, so that these bacteria are able to survive and compete with other microorganisms (Sondang et al., 2020b). There is a synergistic interaction between bacteria genera Bacillus spp. and Pseudomonas spp. related to functional water hyacinth POH. The incorporation of several microbes from the rhizosphere and phylloplane into a microbial consortium plays an important role in helping plants obtain minerals, organic matter, and many other small metabolites including amino acids and phytohormones (Bhattacharyya et al., 2023). This contributes to increasing plant productivity, as well as providing biological control against biotic and abiotic stress.

Indole-3-Acetic Acid (IAA) Producing Bacteria

IAA hormone-producing bacteria is a phytohormone that can spur plant growth. The ability of bacteria to produce IAA hormone is presented in Table 5.

Table 5. IAA concentration in bacterial isolates

Bacterial species	Concentration IAA (µg/ml)	
Bacillus altitudinis	0.0057	a
Bacillus altitudinis	0.0047	b
Bacillus cf. zanthoxylli	0.0042	c
Pseudomonas cf. pharmacofabricae	0.0041	d
Pseufomonas. flexibilis	0.0041	d
Pseudomonas aeruginosa	0.0041	d
Pseudomonas aeruginosa	0.0041	d
Bacillus altitudinis	0.0040	e
Bacillus cereus	0.0040	e
Ectopseudomonas mendocina	0.0039	f

Numbers followed by the same lowercase letter in the same column are not significantly different in the DMRT further test at the 5% level.

Table 5 shows that all pure bacterial isolates are able to produce the hormone IAA with concentrations between 0.0039-00057 µg/ml. IAA is included in the class of auxin growth regulators that play a role in stimulating plant growth. Dash et al (2017) stated that growth stimulating substances produced by microorganisms play a role in stimulating faster plant growth. Microorganisms that can dissolve P also play a role in promoting plant growth. This is supported by the findings of Sondang et al (2019) that water hyacinth POH can act as a biofertilizer and biostimulant for corn plants.

Based on the observations, it can be seen that the bacterial consortium inoculated into the manufacture of POH, the POH product will also contain the bacteria. This is supported by the results of research (Sondang et al., 2020b) that organic fertilizer inoculated with a consortium of bacteria will produce POH products with the same bacteria.

The role of POH for plants is as a source of macro and micro nutrients (Sondang et al., 2023). The multifunctional role of bacteria as growth promoters, biological fertilizers and plant disease suppressants (Yasmin et al. 2016); Vejan et al. 2016), in addition to improving land productivity and quality (Kumawat et al., 2017), all of which represent great potential in the development of sustainable agriculture.

Conclusion

The quality of water hyacinth biological organic fertilizer is influenced by the viability of microorganisms contained in it. The best storage length for bacterial viability is from 0 to 14 days after fermentation with a bacterial population of 1.0,107-3.3,107 CFU/ml and a fungal population of <10-1.4,102 CFU/ml. Seven potential and multifunctional bacterial isolates were found. The surviving bacterial species were bacteria from the genera Bacillus spp and Pseudomonas spp.

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Author Contributions

Y.S.: Developing ideas, analyzing observational data, writing, and responding to reviewers' comments; T.W. and R.S.: collecting research data, analyzing data, creating test documents, reviewing manuscripts, and writing.

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Conflicts of Interest

The authors declare no conflict of interest.

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