



Optimization of the Degradation Power of *Aspergillus flavus* DFSP.J1 and *Aspergillus niger* DFSP. J4 in Degrading Sago Bioplastics

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bioplastics made from sago flour have the potential to be developed into plastic-based industrial materials that are environmentally friendly and easily degraded by microorganisms. *A. flavus* DSFP.J1 and *A. niger* DFSP.J4 are microorganisms from a type of fungus that are proven to be able to degrade sago bioplastics, but have not yet obtained optimal degradation power. Therefore, this study aimed to obtain optimal conditions of the two isolates of fungi in degrading bioplastics made from sago flour at variable inoculum concentrations of 10, 20, 30,40, 50% v/w. The manufacture of sago bioplastics is done by adding acetic acid as a catalyst and added glycerol to form plastic properties in 15% sago flour, after being formed and cut to a size of 1 cm². Observations to determine the degradation power were carried out after an incubation period of seven days to the next five weeks. The results obtained showed that the optimal conditions of *A. flavus* DFSP.J1 and in degrading sago bioplastics at 40% inoculum concentration with a degradation power of 56.52% in the fifth week of observation and tended to increase the degradation power on the following day, as well as *A. niger* DFSP.J4 optimally degrades at 30% inoculum concentration with a degradation power of 55.90% in the fifth week of observation. The results of the research, it can be concluded. Both the fungus *A. flavus* DFSP. J1 and *A. niger*

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DSFP.J4 showed that potentially to be developed for further research to increase its degradation power higher and faster.

Keywords: Bioplastics; degradation; fungi.

1. INTRODUCTION

The world community's need for plastic is increasing in line with the progress of the plastic-based industry with an increasing rate of demand approaching 400 metric tons per year [1]. (While the use of biodegradable plastics has a low growth rate of only 13% per year, there needs to be a campaign to increase its use [2]. Generally, bioplastics are made from renewable natural materials in the form of polysaccharides in the form of cellulose, starch, pectin from chitin and from proteins such as gluten, casein, and gelatin, while organic lipids come from animal fats and fats from plants [3]. The use of plastic in everyday life is an inseparable part of this because it is cheap, quality and elastic [4] the use of plastic is not only limited to wrapping food ingredients but also used in industry and buildings [5]. Plastics made from hydrocarbons are resistant to degradation by microorganisms so that they will accumulate in the environment [6]. In the plastic environment, large sizes will be degraded for a long time, need to be reduced in size before being discharged into the environment (Collignon, et al, 2014).

Various biodegradable plastics have been developed from various sources of biological raw materials to overcome environmental problems caused by hydrocarbon plastic waste [6]. Degradable bioplastic raw materials generally come from various types of polysaccharides, including starch [7]. Starch is a food reserve for plants and a source of energy for every living thing [8]. In addition, starch is a raw material in the manufacture of bioplastics, this is because it is easy to obtain, cheap and abundant. [7]. Starch consists of amylose and amylopectin parts (Shanks & Kong, 2012), both compounds are formed from glucose monomers and bonded by glycosidic bonds. Amylose is composed of chains of 100 to 10000 glucose units, while amylopectin is composed of glucose chains and the bonds are branched. In flour, starch and lipids were also found in small amounts derived from the rest of the plant cell parts that dissolved and precipitated with starch during the extraction process [9]. Starch is naturally granular and becomes dry when heated and is easily thermally degraded before its boiling point is reached (Shanks & Kong, 2012).

Starch has a thermoplastic character that is useful in the formation of bioplastics. The structure and composition of starch-forming starch and amylose [10] from each plant causes differences in the efficiency of starch as a bioplastic raw material [11]. The starch source from the sago plant has been proven to be used in the manufacture of bioplastics [12]. The nature of whether or not it is degraded by microorganisms of any bioplastic made needs to be studied to increase its efficiency [13]. Bioplastic degradation by microorganisms can occur in the environment, changes in the structure of bioplastics will make it easier to degrade [14]. The speed and duration of bioplastic reform depends on the variation in the composition of the polymer making up the bioplastic [15]. Biodegradability of bioplastics can be observed from changes in weight before and after the bioplastic is broken down at certain time intervals [16]. In addition to the complexity of making bioplastics, the availability of bioplastic-degrading microorganisms in the environment is also a determining factor for the rate of degradation of bioplastics [17], including from various types of bacteria such as *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp. , and *Moraxella* sp. Of the types of fungi such as *Aspergillus niger*, *A. versicolor*, *Cladosporium* sp, *Fusarium* sp, *Tricoderma* sp and *Verticillium* sp [18]. *A. flavus* DSFP.J1 and *A. niger* DSFP.J4 are two species of fungi that can degrade sago bioplastic [12]. The necessary conditions are biotic and abiotic factors. Biotic factors can be in the form of the presence of other species or the same species that can affect its degradation power. The existence of other species creates a symbiotic effect that can be beneficial or detrimental. Meanwhile, the presence of the same species in conditions of adequate amounts of nutrients can accelerate the degradation power. Variations in the concentration of inoculum inoculated on the substrate, in this case sago bioplastic, is one of the variables used in the optimization process.

2. MATERIALS AND METHODS

2.1 Production of Sago Bioplastics

The sago flour used is sago flour from Keder Kampong, District of East Coast, Sarmi Regency,

Province of Papua, which is traditionally processed and dried and then blended, filtered 95 mesh and packaged. The manufacture of bioplastics was done by adding 100 ml sterile distilled water to sago flour in a stainless steel pan with a thermostat at 70°C with a composition of 1%v/v acetic acid, 1%v/v glycerol and 15 gram sago flour. Heating is done in 20 minutes while stirring until the sago flour dissolves and the suspension looks clear, then 20 ml suspension bioplastic printed in a 15 cm x 2.5 cm petri dish. The formed bioplastic is put into an oven at 45°C for 48 hours [12].

2.2 Test of Degradation Power of *A. flavus* DFSP.J1 and *A. niger* DFSP.J4 against Sago Bioplastics

The cultures of *A. flavus* DSFP.J1 and *A. niger* DSFP.J4 isolates before use were rejuvenated first by transferring the spores to be grown on Potato Dextrose Agar (Oxoid) media to form spores from each fungal isolate. The spore suspension was prepared by incubating pure cultures of the fungi *A. flavus* DFSP.J1 and *A. niger* DFSP.J4 at 30°C for 10-14 days. The petri dish containing the spore culture was tilted and then rubbed with a solution containing 20% (v/v) glycerol and 0.1% (v/v) Tween 80 and the resulting spore suspension was then passed through a sterilized cotton swab. The collected spores were stored in a freezer at -20°C. The spore concentration in the suspension was determined by the spread plate method. This was done by pouring the spore suspension on potato dextrose agar medium and incubating for 48 hours at 30°C. Then the spore concentration could be determined using the cup count

method. To obtain an initial spore concentration of 10³-10⁷ spores/mL, the spores were inoculated into a 500 mL Erlenmeyer flask containing 200 mL of Potato Dextrose Broth (Oxoid) fermentation medium and incubated at 30°C with shaking at 200 rpm [19]. Furthermore, 10, 20, 30, 40, 50% v/w suspensions of fungal spores were tested for degradability by growing them in a petri dish containing 1 gram of sago bioplastic with a size of 0.5 cm², for 14 days, at room temperature. Mushroom Degradation Power is determined by Formula 1: $DP = (W_i - W_t) / W_i \times 100\%$ [12]. Description of DP : percentage of degradability, W_t = weight in time and W_i = initial weight.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Sago bioplastics

Bioplastic made from sago flour with acetic acid catalyst and glycerol as plasticizer. The bioplastics were molded in petridishes and after drying they were cut into 1 cm² pieces to be tested for their degradation power by *A. flavus* DFSP.J1 and *A. niger* DFSP.J4, as shown in Fig. 1 A and B.

3.1.2 Sago bioplastic degrading fungi

Aspergillus flavus DFSP.J1 has the ability to degrade sago bioplastic, this fungus has a pale yellow conidiospores and can degrade sago bioplastic in low water conditions, as shown in Fig. 2 A and B.

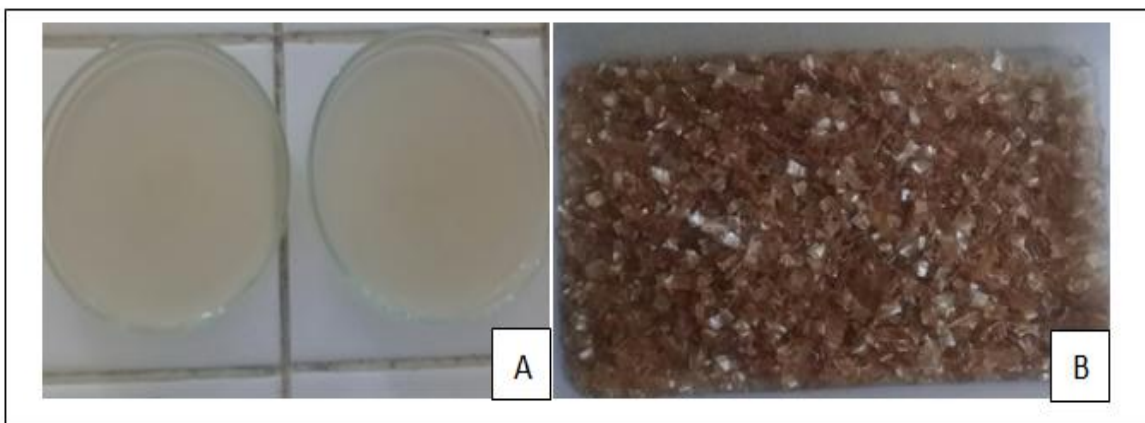


Fig. 1. Bioplastic sago in a petri dish mold (A) and pieces of sago bioplastic (B)

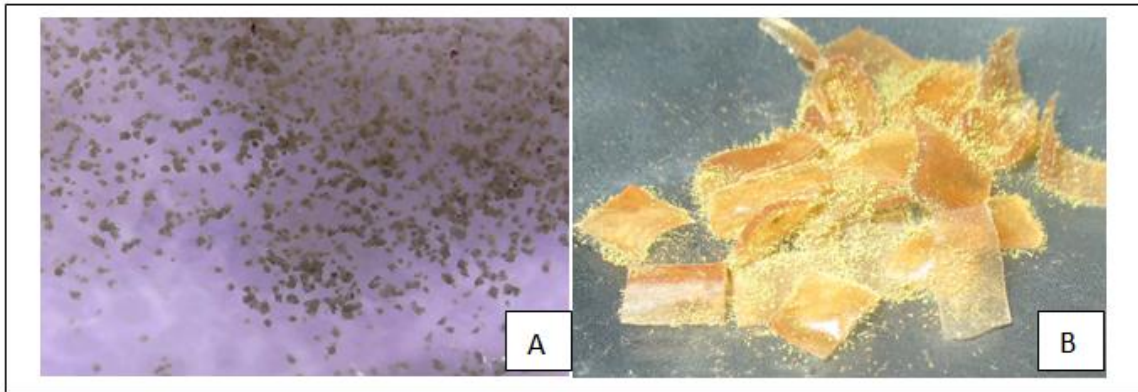


Fig. 2. *A. flavus* DFSP. J1 with a pale yellow conidospores (A), and medium degrade sago bioplastics (B)

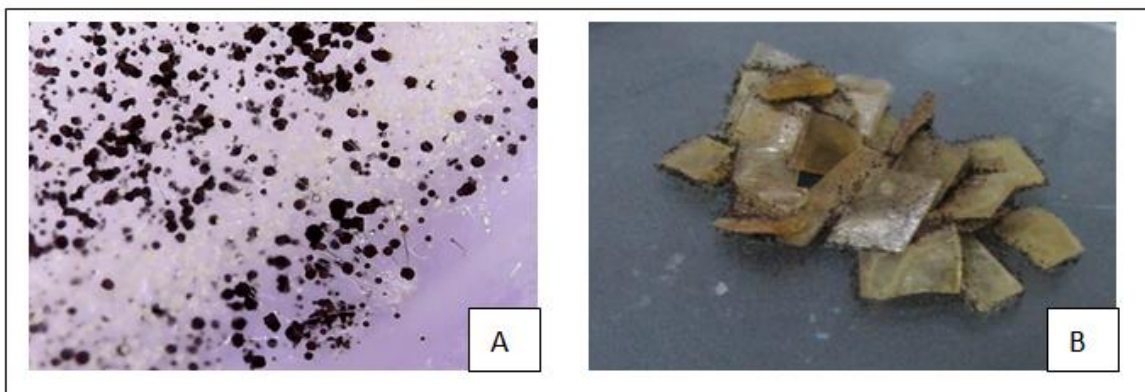


Fig. 3. *A. niger* fungus with brownish black conidiaspores (A) and fungus currently degrading sago bioplastic (B)

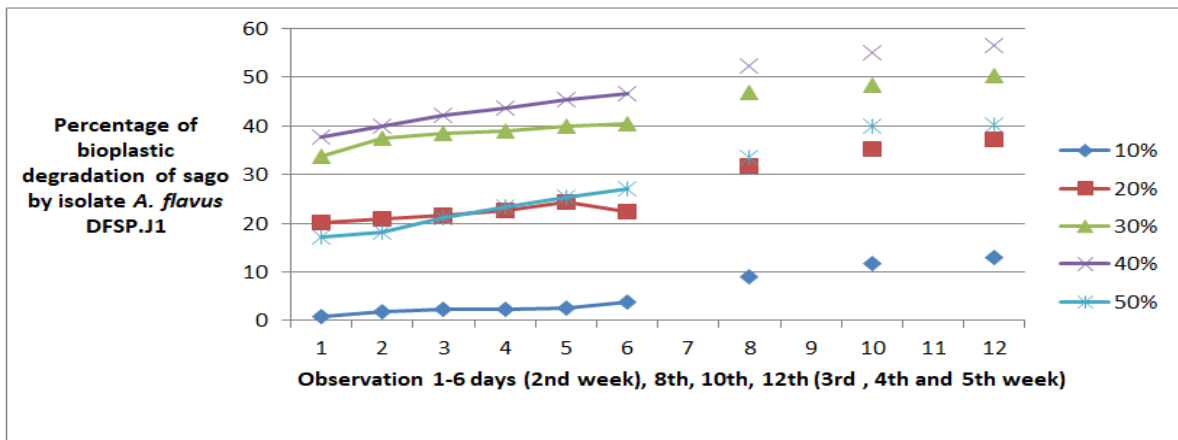


Fig. 4. Percentage of sago bioplastic degradation by isolate *A. flavus* DFSP. J1 based on variations in inoculum concentrations of 10, 20, 30, 40 and 50% five weeks observation

Aspergillus niger DFSP.J4 has a brownish black conidiaspores, this fungal isolate also has the ability to degrade sago bioplastic as shown in Fig. 3.

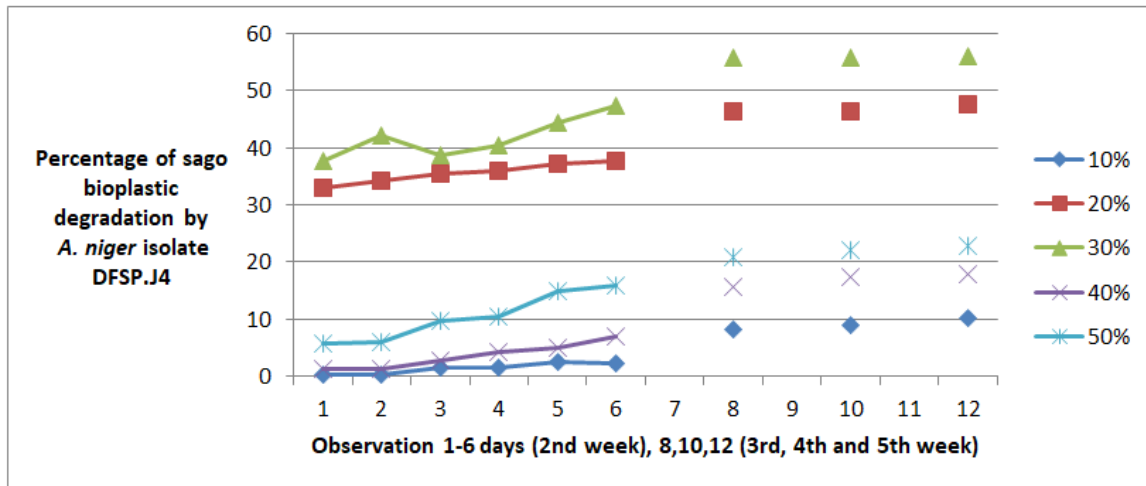


Fig. 5. Degradability of the fungus *A. niger* DFSP. J4 on sago bioplastics with variation inoculums concentrations of 10, 20, 30, 40 and 50%

3.1.3 Optimization of the degradation of fungi for sago bioplastics

Optimization of the degradation power of *A. flavus* DFSP.J1 was carried out by inoculating it into 1 gram pieces of sago bioplastic in a petri disk. The inoculum concentrations used were 10, 20, 30, 40 and 50% and observations were carried out for five weeks starting with observations with one day intervals to six days of observation and continued with one week intervals for 5 weeks. The results obtained using an inoculum concentration of 40% fungi can degrade bioplastic by 56.52%, as shown in Fig. 4 which shows the percentage of sago bioplastic degradation during the 5-week observation period and the concentration of inoculum used and observations started after an incubation period of 7 days in room temperature.

The fungus *A. niger* DFSP.J4 also has the ability to degrade sago bioplastics. This type of fungus in optimizing the inoculum concentration variable was showed the most optimal degradation power at 30% inoculum concentration with a percentage of 55.90% degradation power, in the fifth week of observation, as shown in Fig. 5.

3.2 Discussion

Sago bioplastic is a plastic made from sago flour added with acetic acid as a catalyst and glycerol as a plasticizer. Its nature is rather rigid and transparent, this is due to the content of amylose and amylopectin (21.7% - 62.51%), compared to arrowroot starch (19.4% - 59.35%) and cassava

starch (18.0%). - 60.15%). The results showed that the content of amylose and amylopectin greatly affected the tensile strength and elongation of biodegradable plastics. The higher the amylose content, the higher the value of the tensile strength and elongation of the biodegradable plastic. Bioplastics from sago has elongation and tensile strength (97.83 kgf/mm² – 4.83%) meanwhile arrowroot starch ((98.97 kgf/mm² – 3.38%) and cassava starch. (89.83 kgf/mm² – 2.26%) [20]. This plastic made from sago flour must be environmentally friendly, that is, it can be easily degraded by microorganisms, both fungi and bacteria. At the time of manufacture, glycerol is used as a plasticizer, besides that the addition of glycerol can accelerate the microbial degradation process and increase humidity. Bioplastics can be resistant to biodegradation if stored in a dry place [13]. Microorganisms that have the potential to degrade bioplastics are bacteria from the genus *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp., and *Moraxella* sp [21]. While the fungi group were *Aspergillus niger*, *A. versicolor*, *Cladosporium* sp, *Fusarium* sp, *Trichoderma* sp and *Verticillium* sp [18].

Aspergillus flavus DFSP.J1 and *Aspergillus niger* DFSP.J4 isolates were fungi derived from sago bioplastic which were accidentally overgrown by both fungi during drying and storage at room temperature. After going through re-testing, characterization and identification of the fungus was able to degrade sago bioplastic. Fungi of the *Aspergillus* genera have several species and have been tested and found in bioplastic

biodegradation experiments. *Aspergillus niger* has also been used by other researchers in degrading bioplastics by burial method with compost soil as a source of microbes and can reduce the weight of bioplastics 29.89% with the sample color becoming darker and brittle after 10 days of observation [22]. Furthermore, *Aspergillus terreus* (LM 1021) was able to degrade PHB plastic by 44.96%, plastic bags by 4.28% and pure LDPE by 4.9% [23]. The ability of the isolates of *A. flavus* DFSP.J1 and *A. niger* DFSP.J4 was higher than that of *A. terreus* LM 1021.

Biodegradable bioplastics are polymers that can be mineralized into carbon dioxide, methane, water, inorganic compounds, or biomass through enzymatic activity produced by microorganisms. Due to its biodegradability by microorganisms, bioplastics can be a substitute for conventional petrochemical plastics. The influencing factors can be overhauled by microorganisms in addition to the presence of microorganisms around the bioplastic, as well as the physico-chemical structure of the biopolymer and environmental conditions [17]. The bioplastic degradation process begins with reducing the physical size of the bioplastic so that it is easily degraded by microorganisms biochemically using extracellular enzymes released by microorganisms [24]. Biodegradation testing by burial in the ground, bioplastics were cut into 10 mm x 10 mm, for 12 days with 3 day observation intervals, The difference in weight before the bioplastic was buried and after showing the presence of a biodegradation process [25].

In this study, sago bioplastics were cut with a size of 1 cm² after being inoculated with fungal isolates *A. flavus* DFSP.J1 and *A. niger* DFSP.J4. After 5 weeks of observation, the fungus was able to degrade bioplastics by more than 50%. Degradation still shows a tendency to increase even though the bioplastic conditions have started to dry up. This is due to its ability to grow even though it is not on a specific substrate, even under acidic environmental conditions. Under stressful conditions, fungi are able to produce several enzymes that can be used to degrade organic compounds [23]. Most fungi have lignolytic enzymes, namely laccase, lignin peroxidase and manganese peroxidase which are extracellular enzymes and have been studied for their ability to degrade plastics [26].

Fungi that have the potential to degrade sago bioplastics need to be optimized to see their

degradation power. The isolates of *A. flavus* DFSP.J1 and *A. niger* DFSP.J4 after optimization of the inoculum concentration variable showed their degradation power above 50%. Optimization of inoculum concentration is important to increase the production of high cellular material [27]. In the optimization process, optimal conditions will be obtained in degrading sago bioplastics. Low concentrations below 30% the degradation power is not very good and above 40% the degradability tends to be constant. The addition of inoculum concentration in the fermentation medium resulted in competition among microorganisms in using nutrients, so that the growth of microorganisms and production were disrupted [28]. Optimal inoculum concentration causes rapid fungal growth characterized by the formation of a dense mass of mycelium accompanied by a rapidly ripening conidiospore [19].

4. CONCLUSIONS

Based on the results of research and discussion related to research on optimizing the degradation power of *Aspergillus flavus* DFSP.J1 and *Aspergillus niger* DFSP.J4 in degrading sago bioplastics, it can be concluded. First, the optimal conditions of *A. flavus* DFSP.J1 and in degrading sago bioplastics at 40% inoculum concentration with a degradation power of 56.52% in the fifth week of observation and tended to increase the degradation power, as well as *A. niger* DFSP.J4 optimally degrades at 30% inoculum concentration with a degradation power of 55.90% in the fifth week of observation. Second, based on the magnitude of the degradation power value and the tendency to increase its degradation power, Both the fungus *A. flavus* DFSP. J1 and *A. niger* DFSP.J4 showed that potentially to be developed for further research to increase its degradation power higher and faster.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Chamas A, Moon H, Zheng J, Qiu Y, Tabassum T, Jang JH, et al. Degradation rates of plastics in the environment. *ACS Sustainable Chem. Eng.* 2020;8:2494-3511.
- Meereboer KW, Misra M, Mohanty AK. Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites. *Green Chem.* 2020;22.
- Shafqat A, Alsalmeh A. Synthesis and characterization of starch based bioplastics using varying plant-based ingredients, plasticizers and natural fillers. *Saudi Journal of Biological Sciences.* 2021, March;28(3):1739-1749.
- Cho HS, Moon HS, Kim M, Nam K, Kim J. Cho H.S, Mo Biodegradability and biodegradation rate of poly(caprolactone)-starch blend and poly(butylene succinate) biodegradable polymer under aerobic and anaerobic environment. *Waste Management.* 2011;31:475-480.
- Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastic ever made. *Science Advances.* 2017; 3:e1700782.
- Mohee GR, Unmar. Determining biodegradability of plastic materials. *Waste Management.* 2007;27:1486-1493.
- Torres F, Troncoso O, Torres C, Diaz D, Amaya E. Torres F, Troncoso O, Torres C, Diaz D. & Amaya E. Biodegradability and mechanical properties of starch films from Andean crops. *Int J Biol Macromol* 48: Int J Biol Macromol. 2011;48:603-606.
- Carvalho AJ. Starch: Major sources, properties and application as thermoplastic materials. In I. E. S. (Ed.), *Handbook of Biopolymers and Biodegradable Plastics.* 2013;129-152. Elsevier.
- Khan B, Bilal KN, Samin G, Jahan Z. Thermoplastic starch: A possible biodegradable food packaging material—A review. *J Food Process Eng.*; 2017.
- Pérez S, Bertoft E. The molecular structures of starch components and their contribution to the architecture of starch granules: a comprehensive review. *Starch/Starke.* 2010;62:389-420.
- Carpenter MA. Association mapping of starch chain length distribution and amylose content in pea (*Pisum sativum* L.) using carbohydrate metabolism candidate genes. *BMC Plant Biol.* 2017;17(132).
- Gunaedi T, Mawardi A. Sequence analysis of 18S rDNA gene from sagoplast degrading fungi Berkala PENELITIAN HAYATI. Berkala PENELITIAN HAYATI. 2021, June;26(2):73-78.
- Mateus M, Branciforti M, Brienza M. Review biodegradation of hemicellulose-cellulose-starch-based bioplastics and microbial polyesters. *Recycling.* 2021;6:1-22.
- Lucas N, Bienaime C, Belloy C, Queneudec M, Françoise S, Edmundo JN. Polymer biodegradation: Mechanisms and estimation techniques - A review. *Chemosphere.* 2008;73(4): 429-442.
- Emadian SM, Onay TT, Demirel B. Biodegradation of bioplastics in natural environments. *Waste Management.* 2017;59:526-536.
- Adhikari DM, Kubota K, Kai T, Kaneko N, Araki K, Kubo M. Degradation of bioplastics in soil and their degradation effects on environmental microorganisms. *Journal of Agricultural Chemistry and Environment.* 2016;5:23-34.
- Folino A, Karageorgiou A, Calabro AP, Komilis D. Biodegradation of wasted bioplastics in natural and industrial environments: A review. *Sustainability.* 2020;12:1-37.
- Szumigaj Z, Zakowska ZLKJ, RK, AB. Assessment of polylactide foil degradation as a result of filamentous fungi activity. *J. Environ. Stud.* 2008;17(3):335-341.
- Ghojavand H, Bonakdarpour B, Heydarian SM, Hamedi J. The inter-relationship between Inoculum concentration, morphology, Rheology and erythromycin Productivity in submerged cultivation of *Saccharopolyspora erythraea*. *Brazilian Journal of Chemical Engineering.* 2011; 28(4):565-574.
- Khairun N. Study pengaruh kandungan amilosa dan amilopektin umbi-umbian Terhadap karakteristik fisik plastik biodegradable dengan Plastizicer gliserol. *Jurnal Biotik.* September; 2017;5(2):106-113.
- Prakash MJ, Sivakumar V, Thirugnanasambandham K, Sridhar R.

- Degradation behavior of biocomposites based on cassava starch buried under indoor soil conditions. *Carbohydr. Polym.* 2014;101 (1):20–28.
22. Nissa R, Fikriyah AK, Abdullah AH, Pudjaraharti S. Preliminary study of biodegradability of starch-based bioplastics using ASTM G21-70, dip-hanging, and Soil Burial Test methods. 1,23rd International Symposium on Green Technology for Value Chains 2018. IOP Conf. Series: Earth and Environmental Science. 2019;277.
 23. Rohmah UM, Shovitri M, Kuswyasari ND. Degradasi Plastik Oleh Jamur *Aspergillus terreus* (LM 1021) Pada pH 5 dan 6; Serta Suhu 25°C dan 35°C. *JURNAL SAINS DAN SENI ITS.* 2018; 7(2):2337-3520.
 24. Di Franco CR, Cyras VP, Busalmen JP, Ruseckaite RA, Vázquez A. C. R. Di Franco Degradation of polycaprolactone/ starch blends and composites with sisal fibre. *Polym. Degrad. Stab.* 2004;86(1):95-103.
 25. Wahyuningtyas N, Suryanto H. Analysis of biodegradation of bioplastics made of cassava starch. *Journal of Mechanical Engineering Science and Technology.* 2017;1(1):24-31.
 26. Bhardwaj AT, Hilmani, Gupta R. Microbial population associated with plastic degradation. *Open Access Sci. Reports.* 2012;1(5).
 27. Hidayati NR, Pujiati RD, Rahayu DT. Pengaruh Konsentrasi Inokulum dan Lama Hidrolisis Bagasse Oleh *Aspergillus niger* pada Proses Produksi Bioetanol. *Proceeding Biology Education Conference (ISSN: 2528-5742).* 2016;13:827-832. I.
 28. Yanti A, Ahmad SW, Muhiddin NH. Evaluatiion of Inoculum size and fermentation period for bacterial cellulose production from sago liquid waste. *International Conference on Science and Technology.* 2018;116. IOP Publishing.

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