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Synthesis and Characterization of Biodegradable Plastic from Tropical Marine Microalgae *Navicula* sp. TAD01

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Abstract: The use of plastics made from petrochemicals has a bad environmental impact. One of the efforts to overcome this is using microalgae *Navicula* sp. as a raw material for making biodegradable plastics. *Navicula* sp. TAD01 is a type of marine microalgae that is spread Inner Bay of Ambon and can be used to manufacture biodegradable plastics. In this study, biodegradable plastic was synthesized from the biomass of *Navicula* sp. TAD01, characterization and degradation test. The method used in this research includes three stages. *Navicula* sp. TAD01 cells were grown in the first stage to obtain biomass. The second stage is making biodegradable plastic from the biomass of *Navicula* sp. TAD01. The last step is to characterize biodegradable plastics and perform a degradation test. Cultivation of *Navicula* sp. TAD01 obtained dry biomass of 6.4398 grams, with a productivity value of 0.0524 $gL^{-1}h^{-1}$. The biodegradable plastic made has a slippery texture and a greenish opaque color with a thickness of 0.05 mm. It has tensile strength and elongation values at break of 1.36 MPa and 30.7%, respectively. The results of the analysis of the biodegradation test on this biodegradable plastic film have a mass loss percentage value of 85.27% with an immersion time of 10 days. This shows that *Navicula* sp TAD01 has the potential to be used as an ingredient in the manufacture of biodegradable plastics that can degrade well.

Keywords: Biodegradation; Biodegradable Plastic; *Navicula* sp. TAD01; Tensile strength

Introduction

Plastics are synthetic polymers that are widely used because they are stable, waterproof, light, transparent, flexible, and strong but are not easily decomposed by microorganisms. The decomposition of plastic waste by burning will produce dioxin compounds that are harmful to health. One of the efforts to overcome this problem is to use biodegradable plastic. Biodegradable plastics are biopolymer plastics that are readily decomposed by microorganisms so that they are more environmentally friendly when compared to commercial plastics. The material that is often used in the synthesis of biodegradable plastics is starch. This raises the problem of food competition, so efforts to continue to make alternative sources of raw materials to overcome this problem.

Biodegradable plastics are polyhydroxyalkanoates (PHA) (Abdo & Ali, 2019; Akhlaq, et al., 2023). PHA polymers can be used to produce biodegradable plastics, one of which is polyhydroxyl butyrate (PHB). PHB polymers are polymers belonging to the polyester class with thermoplastic properties, which are naturally produced by certain bacteria as storage compounds and are 100% biodegradable. These products can then be extracted and further processed to produce biodegradable plastics (Lathwal, Nehra, & Rana , 2015; Mahitha & Madhuri , 2015; Onen , et al., 2020; (Mostafa, Alrumman, & Alfaify, 2022); Adnan, et al., 2022)

Polyhydroxyl butyrate is the simplest polyester PHAs, as an intracellular biopolyester produced by microorganisms. Microorganisms that synthesize PHB in their cells are microalgae. PHB from microalgae can improve the recyclability of plastics by reducing the amount of petroleum used to produce plastics (Hempel,

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et al., 2011; Largade, et al., 2016; Rocha, et al., 2020; Rahman, Anthony, Sathish, Sims, & Miller, 2014)). The biodegradability and biocompatibility of PHB, which is compatible with petroleum polymers make PHB applicable in industry, medicine, agriculture, and various fields (Abdo & Ali, 2019). Microalgae, a stock of raw materials suitable for use as biomass producing biodegradable plastics, has several advantages, including high yields and the ability to grow quickly in the environment (Hadiyanto & Azim, 2012; Apriliyani, Rahayu, & Thohari, 2019; Klein, et al., 2014) .

Indonesia is known as a country that has the greatest wealth of marine biodiversity in the world because it has distinctive coastal ecosystems such as mangrove forests, coral reefs, and seagrass beds. One sea area with abundant biodiversity is the Ambon Bay area, which is located in Maluku Province. Ambon Bay is an estuarine water divided into two by a narrow and shallow threshold. The eastern part of this threshold is known as inner Ambon bay. This area is protected from big waves throughout the year, so it has much biodiversity that grows marine microorganisms such as microalgae (Sumadiharga and Yulianto, 1987). The type of microalgae that grows a lot in the sea is the diatom class microalgae *Navicula* sp. obtained from the inner Ambon bay (TAD), in which the cells are elongated oval in shape with a brown-blond color with cell walls composed of silicates. (Sheehan et al., 1998; Telussa, Hattu, & Sahalessy, 2022)

Navicula sp. has several essential chemical components. Research conducted by Lee et al. (2009) showed that the microalgae *Navicula* sp. have a carbohydrate content of 13.5%, 16.9% protein, 2.1% lipid, and 63.9% ash content. All these chemical components are produced from photosynthesis and its derivatives. The content of *Navicula* sp. components can be used for various important purposes, such as a source of bioactive substances for animal feed and agricultural needs and a source of renewable alternative energy (Orona-Navar, Aguilar-Hernández, López-Luke, Pacheco, & Ornelas-SotO, 2020; Kumar , Anand, Shah , & Bharadvaja, 2022; Telussa I., Fransina, Singerin, & Taipabu, 2023;). Based on the chemical components contained in the *Navicula* sp., one application of microalgae as an alternative renewable energy source is using microalgae as a raw material for making biodegradable plastic.

Method

Cultivation of Navicula sp. TAD01

Navicula sp. TAD01 cells were grown in a modified medium (Telussa, Rusnadi, & Nurachman, 2019). Cultivation was carried out with an initial cell density of 5×105 cells mL^{-1} in a simple photobioreactor at room temperature under a light intensity of 5000 lux with a photoperiod of 12:12 hours (dark: light), 28 ppt salinity, pH 8.2–8.5 and aerated with free air bubbles. The simple photobioreactor used in this study was made of a transparent jar with a height of 26 cm, an external diameter of 29 cm, and a working volume of 12 L. Cell growth in culture was measured by counting the number of cells (in units of cells mL^{-1}) using a Neubauer Haemocytometer below. light microscope Research design and method should be clearly defined**.** *Navicula* sp. TAD01 cells that had been cultivated were harvested on day 11 using sedimentation and filtration techniques. A light and electron microscopes (SEM) were used to observe cell morphology.

Biodegradable Plastic Synthesis

Making biodegradable plastic is done by dissolving 1 g of chitosan with 150 mL of distilled water and 1% acetic acid. Then 2 g of biomass and 1 g of sorbitol were added and stirred using a magnetic stirrer until homogeneous. Next, 1 mL of glycerol was added to the mixture while heating at 310 °C and stirring constantly. Mixing is done until the mixture thickens and resembles glue. The mixture was then left at room temperature to remove bubbles. The bubbles formed will damage the structure of the plastic film to be made. After that, the mixture is ready to be printed on a mold made of plastic. The biodegradable plastic molds were then dried in an oven at 50°C for 24 hours. After drying, the plastic can be removed from the mold.

Characterization of Biodegradable Plastics

Characterization analysis of the mechanical properties of biodegradable plastics can be carried out with several stages of mechanical testing, including; tensile strength test and Elongation at break test. Biodegradable plastics were characterized using SEM (FEI, Type: Inspect S50) and FT-IR (Perkin-Elmer). Tensile strength testing was carried out using ASTM (American Society for Testing and Material) standards with a thickness of ± 0.05 mm biodegradable plastic. Furthermore, the sample tested for tensile strength is calculated for the percentage of elongation by looking at the percentage of the final length and initial length of the bioplastic.

Biodegradation Test of Biodegradable Plastic

The biodegradation test was carried out by being buried in a medium made from a mixture of soil and compost. The simplest quantitative method to characterize the biodegradation of a polymer is to determine the mass loss and degradability of the polymer material. Mass loss was determined by weighing the polymer mass before and after the biodegradation process for a specific time. This method is done by calculating the percent loss of mass of biodegradable plastic. The biodegradable plastic was weighed, then tested for degradation by being buried in a mixture of soil and compost for ten days. Furthermore, this biodegradable plastic is weighed for the weight of the degradation product (Sumartono et al., 2015)

Research Scheme

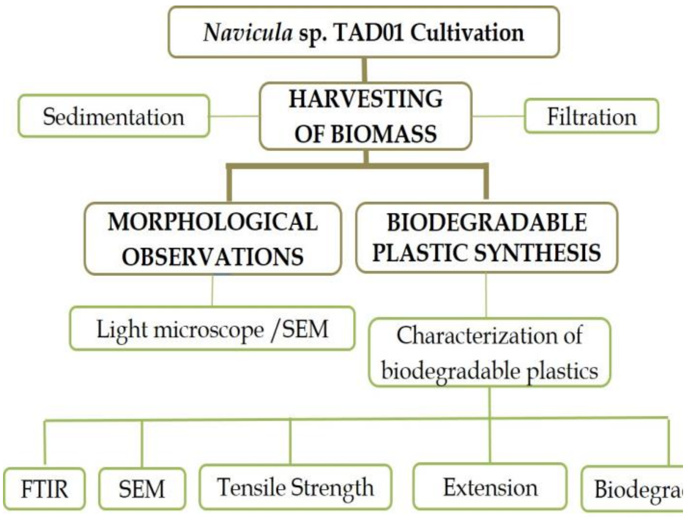
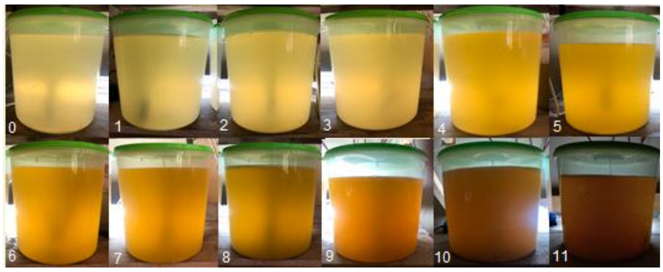


Figure 1. Research scheme

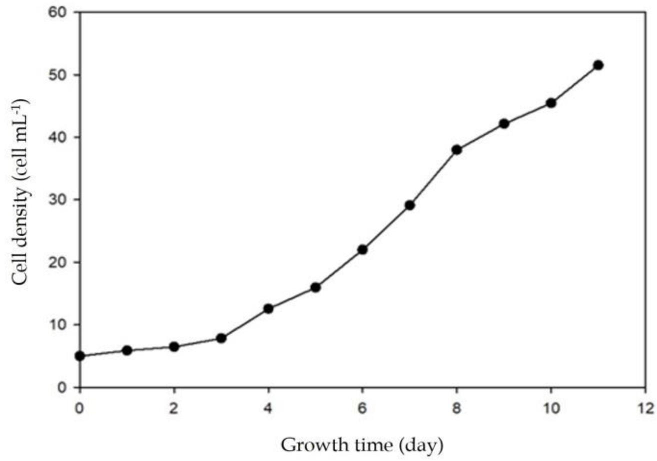
Result and Discussion

Cultivation of Navicula sp. TAD01

Navicula sp. TAD01 is a microalgae derived from the diatom class used as a raw material to synthesize plastic biodegradable. *Navicula* sp. TAD01 cells were grown indoors using 5×105 cells/mL initial cells at a light intensity of 5000 lux in a modified medium. For 11 days, *Navicula* sp. TAD01 cells were grown and showed changes in culture color and cell density during growth. Observations during cell growth showed increased cell density and a darker color change of the culture. The darker color change of the culture indicated that the number of cells was increasing, and the biomass productivity was higher (Figure 2a). This is evidenced by the increase in cell density during growth. Figure 2b shows the growth of *Navicula* sp. TAD01 cells with the number of cells increasing rapidly with the highest cell density obtained on day 11, which is 51.5000 x 105 cells/mL with a specific growth rate of 0.3490. This specific growth rate indicates the rate of cell division during the growth of *Navicula* sp. TAD01. Dry biomass obtained from cell cultivation for 11 days in 12 L culture was 6.4398 g with biomass productivity of 0.0524 $gL^{-1}h^{-1}$.



(a)



(b)

Figure 2. Growth of *Navicula* sp. TAD01 (a) Culture color (days 0-11 (from left to right)); (b). growth curve

Cell morphology observations were carried out using light and electron microscopy (SEM). The light microscopy image showed that the cell morphology of *Navicula* sp. TAD01 was oval, yellow (Figure 3a). The yellow color in *Navicula* sp. cells indicates the content of carotenoid pigments that dominate the pigment in *Navicula* sp cells (Telussa, Rusnadi, & Nurachman, 2019; Lilipaly, Telussa, Fransina, & Efruan, 2022; Telussa, Fransina, Lilipally, & Efruan, 2022; Pane, Risjani, Yunianta, & Maulana, 2023; El Amin & Prihantini, 2023). Observations of *Navicula* sp. TAD01 cells were also observed by SEM, which showed the unique characteristics of *Navicula* sp. TAD01 with oval-shaped frustule with large pores at the periphery of the cell wall and nanopores that lay on the surface for the exchange of nutrients or other materials (Figure 3b).

Figure 3. *Navicula* sp. TAD01 cells. (a) Observation under a

light microscope with a magnification of 400x; (b) SEM observations with 3000x magnification.

Synthesis and Characterization of Plastic Biodegradable

The result of biodegradable plastic printing has a thickness of 50 µm. biodegradable plastic has a smooth surface, yellowish green and opaque which indicates the presence of photosynthetic pigments from the biomass of *Navicula* sp. TAD01 (Figure 4a). The opacity of plastic is caused by the high protein, fat, and fiber content. Films with a high opacity are very good for protection against light exposure, especially for products sensitive to light-catalyzed degradation. (Kumoro & Purbasari, 2014).

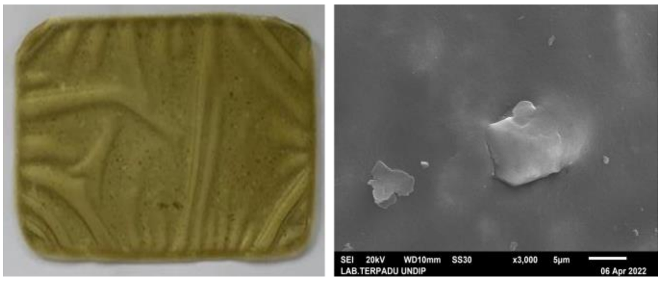


Figure 4. Biodegradable Plastic surface of *Navicula* sp TAD01(a) and SEM image (b)

Morphological observations on the surface of biodegradable plastic were carried out using an electron microscope (SEM) with a magnification of 3,000X (Figure 4b). In the figure, it can be seen that the average thickness of the biodegradable plastic produced is approximately 50 µm. The resulting biodegradable plastic is more like a laminated material, less dense and less homogeneous in structure with several small cracks. According to Otsuki et al. (2004), Cracks in biodegradable plastic have a stronger interaction between the biomass material and the polymer than within the biomass material itself. This is supported by Fabra et al. (2018) who observed cross-sections of the resulting biocomposite films and concluded that the microalgal cells remained intact even after mixing. The surface of the biodegradable plastic tends to be rough, reflecting the low gloss value and high opacity. The gloss value represents the surface texture and polishing degree, which are strongly influenced by the particle size distribution of the film raw material (Moraes, et al., 2008). Therefore, the more uniform the particle size of the film-making material, the smoother and brighter the film will be (Trezza and Krochta, 2001). The high opacity is due to the high protein, fat and fiber content. Tapia-Blácido et al. (2005) and Batista et al. (2005) also reported an increase in film blurring with increasing levels of protein and fat in films made from spinach flour. Fakhouri et al. (2007) argued that the film's opacity also depends on the content of amylose, which is a compound with a linear molecular structure with strong hydrogen bonds between opposite hydroxyl groups. This phenomenon weakens the interaction between the biopolymer and water and triggers the formation of an opaque polymer matrix. Films with a high opacity are very well used to protect against light exposure, especially for products sensitive to light-catalyzed degradation.

Characterization with FTIR spectrometer showed that the spectrum appearing on biodegradable and commercial plastics was very different (Figure 5). The difference in the spectrum that appears causes differences in the properties between conventional and biodegradable plastics in terms of their degradation properties. Table 1 presents the FTIR analysis of biodegradable plastics for the functional groups.

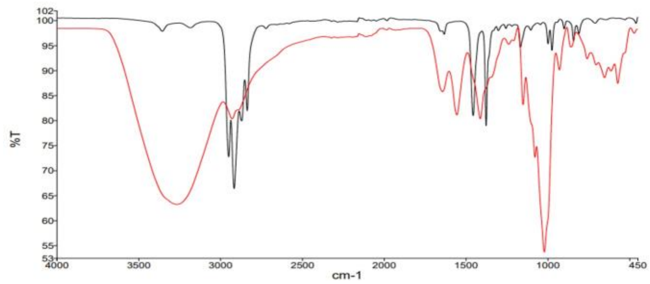


Figure 5. FTIR spectrum of biodegradable plastics from *Navicula* sp TAD01 (red line) and commercial plastics (Black line)

Table 1. FTIR Analysis Results

Functional groups	Wavenumber (cm^{-1})	
	Biodegradable plastic	commercial plastic
C-H	2930.31	3000-2850
CH_3	-	1376.26
CH_2	-	1455.91
H bond of O-H	3270.69	-
C-O of C-O-C	1150.70	-
C-O of C-H-O	1020.67	-
N-H (Amina)	1555.41	-
C=O (Amida)	1644.24	-

FTIR analysis of biodegradable plastic showed a stretching vibration of the C-H bond at 2930.31 cm^{-1}, a typical vibration with a wide absorption band with a weak intensity indicating the stretching H bond of O-H at 3270.69 cm^{-1}. The C-O bond of C-O-C at 1020.67 cm^{-1} with a sharp absorption band and strong intensity is in the fingerprint region, and the C-O bond of C-H-O at a wave number of 1150.70 cm^{-1} with a weak absorption band. and sharp. At 1555.41 cm^{-1} denotes an N-H bond. The C=O (amide) bond at 1644.24 cm^{-1} indicates a carbonyl compound functional group with a sharp absorption band and weak intensity indicating the presence of protein from biomass and chitosan. The resulting uptake corresponded to the functional group of the *Navicula* sp. TAD01 (protein, fat, and carbohydrate), sorbitol, chitosan and glycerol.

In contrast to the spectrum that appears on commercial plastics. Commercial plastic containing Polypropylene compounds (Ozzetti, De Oliveira Filho, Schuchardt, & Mandelli, 2002) at 3000-2850 cm^{-1} shows C-H bonds from CH_3 or CH_2 groups. This spectrum difference that appears is what distinguishes the properties of biodegradable plastics and commercial plastics. This causes biodegradable plastic to have still properties such as its constituent components, namely plastic, and easy to decompose.

Analysis of the mechanical properties of biodegradable plastics was carried out by testing the tensile strength value of biodegradable plastic products with a thickness of 0.05 mm. the tensile strength of biodegradable plastic from *Navicula* sp TAD01 biomass, which is 1.3 MPa, is smaller than that of biodegradable plastic from *Chlorella* sp. and *Spirulina* sp. which has tensile strengths of 5.7 and 3 MPa, respectively (Zeller, Hunt, Jones, & Sharma, 2013). The value of the tensile strength of biodegradable plastic is influenced by its constituent components. In contrast to the value of elongation at break obtained by 30.7%. According to Darni & Utami, 2010, the higher the percent elongation at the plastic break indicates that the plastic film is more flexible.

Biodegradation Test of Biodegradable Plastic

Determine the degradation of biodegradable plastic, it is done by calculating the mass loss of biodegradable plastic before it is degraded and after it is degraded (Sumartono, Handayani, Desiriana, & Novitasari, 2015). A biodegradation test on biodegradable plastic was conducted to determine the ability of biodegradable plastic to be degraded naturally. In this study, the biodegradation test of biodegradable plastic was carried out by immersing a sample of biodegradable plastic in a media made of a mixture of soil and compost. Mass loss was calculated after ten days of immersion in biodegradable plastic.

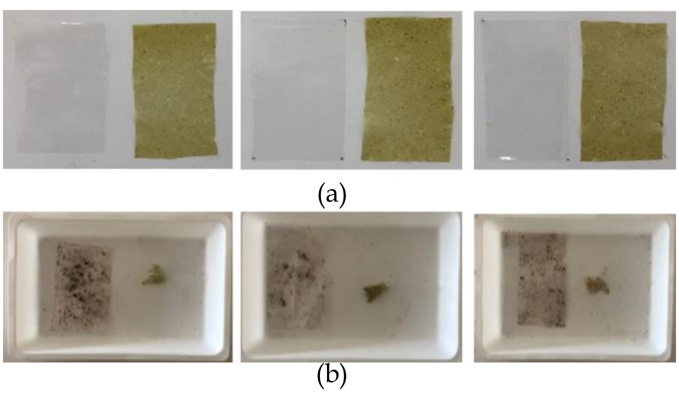


Figure 6. Comparison of biodegradable plastics and commercial plastics before degradation (a); after degradation (b).

Figure 6 compares the structure of biodegradable plastic before and after it is degraded. In contrast, for commercial plastics it does not degrade while for biodegradable plastics it degrades well which can be seen from the form of biodegradable plastic that breaks down into small pieces. The percentage of mass loss of biodegradable plastic can be seen in Table 2.

Table 2. Percent Mass Loss of Biodegradable Plastic

Biodegradable plastic	Mass before Degradation (g)	Mass after Degradation (g)	Percent Loss of Mass Degradation
1	0.0719	0.0106	85.25
2	0.0786	0.0114	85.49
3	0.0723	0.0108	85.06

Polymers are degraded due to damage or loss of quality due to breaking of the polymer chain bonds. The results obtained from the degradation test on biodegradable plastic after being buried for ten days with three repetitions had an average percent mass loss value of 85.27±0.0021%. This indicates that the biomass of *Navicula* sp. TAD01 contains biomolecules with an OH group that initiates the hydrolysis reaction to absorb water from the soil (Ihsan & Ratnawulan, 2023; An, Hwang, & Cho SH, 1998; Hong, Park, & Kim, 2000) The hydroxyl group decomposes into small pieces until it disappears in the soil.

Conclusion

Navicula sp. TAD01 cells were cultivated to obtain biomass as an additive in the manufacture of biodegradable plastics, having wet and dry biomass productivity of 0.5564 $gL^{-1}h^{-1}$ and 0.0524 $gL^{-1}h^{-1}$. Mechanical properties of biodegradable plastic from *Navicula* sp. TAD01, with a thickness of 50 µm, has a tensile strength of 1.3 MPa and an elongation at a break value of 30.7%. Degradation test on biodegradable plastic of 85.27 ± 0.0021% within ten days.

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Conflicts of interest
The authors declare that there is no conflict of interest regarding the publication of this article.

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