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ANALYSIS OF THE DEGRADABILITY OF AZO DYES AND MICROBES BIOPLASTICS FROM CELLULOSE *Kappaphycus alvarezii*

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ABSTRACT

The increasing accumulation of non-biodegradable plastic waste has encouraged the development of environmentally friendly alternatives derived from renewable natural resources. One promising material is cellulose extracted from the red seaweed *Kappaphycus alvarezii*, which possesses favorable characteristics for bioplastic production. This study aimed to evaluate the degradability of azo dyes and microbial activity in bioplastics produced from *K. alvarezii* cellulose with different cellulose-to-cassava starch ratios. The research was conducted from July to October 2025 using five formulations: F1 (5:5), F2 (8:2), F3 (2:8), F4 (0:10), and F5 (10:0). Cellulose extraction was performed through alkaline and acid hydrolysis methods, while bioplastic films were produced through solution casting with glycerol as a plasticizer. Azo dye degradation was analyzed using UV–Visible spectrophotometry, whereas microbial activity was evaluated through Total Plate Count (TPC) analysis. The UV–Vis results revealed no characteristic absorption peaks of azo dyes in the 300–500 nm wavelength range, indicating the absence or successful degradation of azo chromophores in all formulations. Cellulose-rich formulations exhibited higher optical transparency and greater film homogeneity than starch-dominated formulations. TPC values ranged from 0 to 420 CFU g^{-1} and remained substantially below the maximum limit established by SNI 7388:2009 (4,400 CFU g^{-1}), confirming excellent microbiological quality and product safety. Statistical analysis using the Kruskal–Wallis test showed no significant differences among formulations ($p > 0.05$). The combined findings demonstrate that cellulose-based bioplastics derived from *Kappaphycus alvarezii* possess good biodegradation potential, are free from detectable azo dye contamination, and maintain low microbial contamination levels. Therefore, these bioplastics have strong potential as sustainable alternatives to conventional petroleum-based plastics for food packaging, cosmetic packaging, and other environmentally sensitive applications.

INTRODUCTION

Plastic pollution has become one of the most pressing environmental challenges worldwide. The increasing production and consumption of conventional petroleum-based plastics have resulted in the accumulation of persistent waste in terrestrial and aquatic ecosystems. According to the United Nations Environment Programme (UNEP, 2023), global plastic production exceeds 400 million tons annually, with a substantial proportion ending up in landfills, rivers, and oceans. In Indonesia, plastic waste contributes significantly to environmental degradation, accounting for approximately 18% of the total national waste generation, equivalent to millions of tons each year (Ministry of Environment and Forestry, 2023). The resistance of synthetic plastics to natural degradation processes causes long-term environmental impacts, including soil contamination, marine pollution, and the formation of microplastics that threaten ecological and human health (Rosadi et al., 2024).

Growing environmental concerns have stimulated intensive research into biodegradable materials derived from renewable biological resources. Bioplastics have emerged as promising alternatives to conventional plastics because they can be decomposed naturally by microorganisms into environmentally benign compounds such as carbon dioxide, water, and biomass (Folino et al., 2020). Unlike synthetic polymers, bioplastics are produced from renewable feedstocks including starch, cellulose, proteins, and marine biomass. Their biodegradability and lower environmental footprint make them attractive materials for sustainable packaging and other industrial applications (Peng et al., 2019).

Among various renewable resources, seaweed has gained considerable attention as a raw material for bioplastic production due to its abundance, rapid growth rate, and high polysaccharide content. Marine macroalgae do not compete with agricultural land resources and require neither freshwater nor chemical fertilizers for cultivation, making them environmentally sustainable biomass sources (FAO, 2022). In particular, red seaweeds are rich in structural polysaccharides that can be converted into biodegradable films and packaging materials. According to Norhayati et al. (2021), seaweed-based bioplastics possess desirable biodegradability characteristics and have the potential to reduce dependency on petroleum-derived plastics.

Indonesia is one of the world's leading seaweed-producing countries and possesses extensive coastal areas suitable for seaweed cultivation. Among commercially important species, *Kappaphycus alvarezii* represents one of the most extensively cultivated red seaweeds due to its high economic value and broad industrial applications. The species is primarily utilized for carrageenan extraction, a hydrocolloid widely employed as a thickening, stabilizing, and emulsifying agent in food, pharmaceutical, cosmetic, and textile industries (Widigdo et al., 2022). FAO (2020) reported that global production of *K. alvarezii* reached approximately 1.5 million tons, highlighting its significance as an industrial commodity. Furthermore, Indonesia ranks among the world's largest producers of carrageenan-producing seaweeds, contributing substantially to national export revenues and coastal community livelihoods (Parenrengi et al., 2021).

In addition to carrageenan, *Kappaphycus alvarezii* contains substantial amounts of cellulose, a natural polymer that has attracted considerable interest in the development of biodegradable materials. Cellulose is the most abundant biopolymer on Earth and possesses several advantageous properties, including biodegradability, renewability, mechanical strength, and compatibility with other biopolymers (Peng et al., 2019). Recent studies have demonstrated that cellulose extracted from marine biomass can be incorporated into bioplastic formulations to improve structural integrity while maintaining environmental friendliness (Folino et al., 2020). The utilization of cellulose from *K. alvarezii* therefore offers a sustainable approach to producing bioplastics with enhanced functional properties.

Despite these advantages, seaweed-based bioplastics often exhibit limitations such as brittleness, low flexibility, and relatively poor moisture resistance. To overcome these challenges, plasticizers such as glycerol are commonly incorporated into bioplastic formulations to improve flexibility and elongation properties (Marsa et al., 2023). Additionally, the incorporation of natural preservatives and antimicrobial compounds has been explored to extend product shelf life and enhance functional performance. Therefore, understanding the degradation characteristics and microbial interactions of seaweed-based bioplastics is essential for evaluating their suitability as substitutes for conventional plastics.

Besides plastic pollution, industrial wastewater contamination represents another major environmental concern. Synthetic dyes released from textile, food, paper, cosmetic, and pharmaceutical industries are among the most problematic pollutants due to their chemical stability and resistance to natural degradation processes. Azo dyes constitute the largest group of synthetic dyes used globally, accounting for more than 60% of total dye production (Saratale et al., 2011). These dyes are characterized by one or more azo bonds (-N=N-) connecting aromatic structures, which contribute to their high stability and persistence in aquatic environments.

The discharge of azo dyes into water bodies can significantly reduce light penetration, inhibit photosynthetic activity, and disrupt aquatic ecosystems. Furthermore, the breakdown products of certain azo dyes may generate toxic, mutagenic, and carcinogenic aromatic amines that pose risks to both environmental and human health (Katheresan et al., 2018). Conventional wastewater treatment methods such as coagulation, adsorption, and chemical oxidation often involve high operational costs and may generate secondary pollutants. Consequently, environmentally friendly and sustainable approaches for azo dye removal have become increasingly important.

Biodegradation using microorganisms has emerged as an effective and eco-friendly strategy for treating azo dye contamination. Various bacteria, fungi, and mixed microbial consortia possess enzymatic systems capable of reducing azo bonds and transforming complex dye molecules into simpler compounds (Solís et al., 2019). The efficiency of microbial degradation depends on several factors, including dye structure, environmental conditions, nutrient availability, and the characteristics of the supporting matrix. Recent studies have shown that biodegradable polymer matrices can provide favorable environments for microbial colonization and activity, thereby enhancing pollutant degradation processes (Sharma et al., 2021).

Seaweed-derived cellulose bioplastics offer a unique opportunity to integrate sustainable materials development with environmental remediation applications. Due to their biodegradable nature and compatibility with microbial growth, cellulose-based bioplastics may serve not only as environmentally friendly packaging materials but also as substrates that support microbial degradation processes. Understanding the interactions among cellulose bioplastics, azo dye pollutants, and microbial communities is therefore essential for developing multifunctional biomaterials with both environmental and industrial benefits.

Although numerous studies have investigated seaweed-based bioplastics and microbial degradation of azo dyes separately, research focusing on the degradability of azo dyes and microbial activity in cellulose bioplastics derived from *Kappaphycus alvarezii* remains limited, particularly in Indonesia. Comprehensive evaluation of these aspects is necessary to determine the environmental performance and biodegradation potential of the resulting bioplastic materials. Therefore, this study aims to analyze the degradability of azo dyes and microbial activity in cellulose-based bioplastics produced from *Kappaphycus alvarezii*. The findings are expected to contribute to the development of sustainable bioplastic technologies while providing valuable insights into their potential role in environmental pollution mitigation and circular bioeconomy initiatives.

METHOD

This study was conducted from July to October 2025. Samples of the red seaweed *Kappaphycus alvarezii* were collected from coastal cultivation areas in Jeneponto Regency, South Sulawesi, Indonesia, one of the major seaweed-producing regions in eastern Indonesia (FAO, 2022; Widigdo et al., 2022). Sensory quality evaluations were carried out at the Institute of Maritime Technology and Business Balik Diwa (ITBM Balik Diwa), Makassar, while chemical and microbiological analyses were performed at the Research and Development Laboratory, Faculty of Mathematics and Natural Sciences, Hasanuddin University. The raw materials consisted of cellulose extracted from *K. alvarezii* as the primary biopolymer matrix, cassava starch as a reinforcing agent, glycerol as a plasticizer, distilled water as a solvent, and azo dye solution as the target pollutant for degradation testing. Cellulose extraction followed the procedure reported by Putri et al. (2025), involving alkaline treatment using NaOH at 80°C to remove carrageenan, proteins, and other non-cellulosic components, followed by sulfuric acid hydrolysis to obtain purified cellulose fractions. The extraction of cassava starch was conducted according to Dewi (2023), including sorting, washing, peeling, grinding, filtration, sedimentation, and decantation processes to obtain starch with high purity. Bioplastic production was adapted from Rosadi et al. (2024), where cellulose was dispersed in distilled water and heated at 70°C under continuous stirring for approximately 25 min to obtain a homogeneous suspension. Subsequently, glycerol (1.5 mL) and cassava starch were incorporated to improve flexibility, film-forming ability, and mechanical stability, as commonly applied in cellulose-based bioplastic formulations (Folino et al., 2020; Peng et al., 2019).

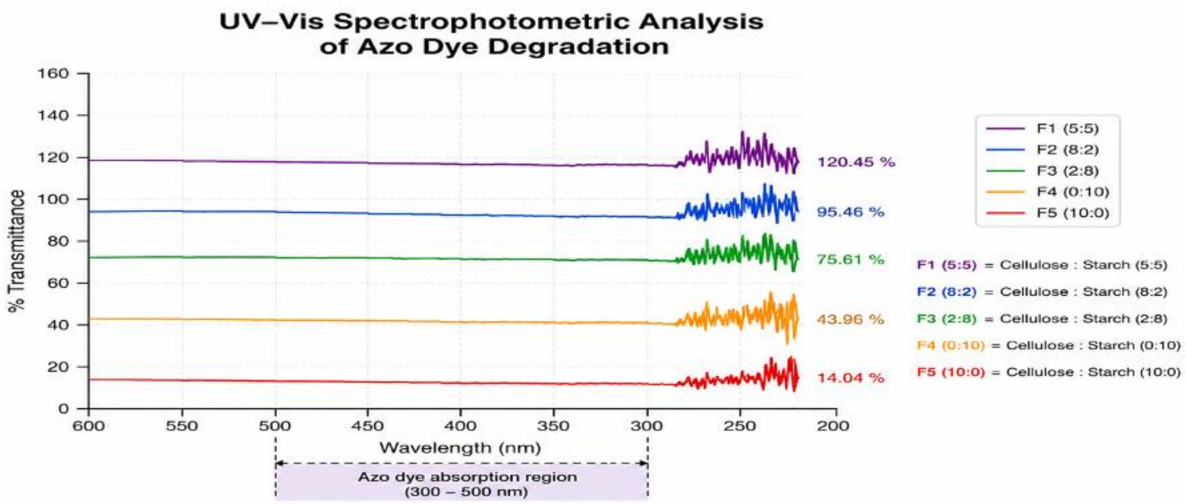
The degradability of azo dye compounds was assessed using UV–Visible spectrophotometry by measuring absorbance changes before and after exposure to the cellulose-based bioplastic matrix. The percentage of dye degradation was calculated from the reduction in absorbance at the maximum wavelength of the azo dye solution following established spectrophotometric procedures for dye-removal studies (Saratale et al., 2011; Solís et al., 2019). Microbial activity associated with biodegradation was evaluated using the Total Plate Count (TPC) method. Samples were aseptically collected, serially diluted, and cultured on standard growth media, and microbial abundance was expressed as colony-forming units per milliliter (CFU mL^{-1}), following standard microbiological protocols (APHA, 2017). The TPC analysis was conducted to determine the susceptibility of the bioplastic material to microbial colonization and to evaluate the role of microorganisms in the biodegradation process (Sharma et al., 2021). All data obtained from spectrophotometric and microbiological analyses were evaluated using descriptive and quantitative approaches. UV–Vis measurements were used to determine the degradation efficiency of azo dyes, whereas microbial counts were used to assess biodegradation performance and biological activity within the bioplastic matrix. The integration of dye degradation and microbial growth analyses provided a comprehensive assessment of the environmental degradability, biodegradation behavior, and ecological compatibility of cellulose-based bioplastics derived from *Kappaphycus alvarezii* (Katheresan et al., 2018; UNEP, 2023).

RESULT AND DISCUSSION

UV–Vis Spectrophotometric Analysis of Azo Dye Degradability

The UV–Vis spectrophotometric analysis was conducted to evaluate the ability of cellulose-based bioplastics derived from *Kappaphycus alvarezii* to degrade azo dye compounds and to assess the optical quality of the resulting films. The results presented in **Figure 1** indicate differences in absorbance intensity among the five formulations, namely F1 (cellulose = 5:5), F2 (8:2), F3 (2:8), F4 (0:10), and F5 (10:0).

Figure 1.
UV–Vis Spectrophotometric Analysis of Azo Dye Degradation



The UV–Vis spectra demonstrated that none of the bioplastic formulations exhibited characteristic absorption peaks within the azo dye absorption region (300–500 nm), indicating the absence or substantial degradation of azo chromophores in the tested samples. This finding is important because azo compounds are known to contain azo bonds (-N=N-) that are relatively stable and potentially hazardous when present in materials intended for food packaging or cosmetic applications (Saratale et al., 2011; Solís et al., 2019). The absence of significant absorbance peaks suggests that the cellulose-based bioplastic matrix effectively inhibited the persistence of azo dye residues, thereby improving product safety and environmental compatibility.

The optical behavior observed in the spectra was strongly influenced by the cellulose-to-starch ratio. Formulations containing higher cellulose concentrations, particularly F5 and F2, exhibited greater light transmittance and lower absorbance values, indicating higher transparency and improved film homogeneity. Cellulose possesses superior film-forming characteristics and promotes the development of compact polymer networks that reduce light scattering effects caused by structural irregularities (Peng et al., 2019; Folino et al., 2020). Consequently, films with elevated cellulose content appeared clearer and more optically stable than starch-dominated formulations.

Conversely, formulations with higher starch proportions, especially F3 and F4, tended to display reduced transmittance due to the heterogeneous distribution of starch granules within the polymer matrix. Similar observations have been reported by Aldabib and Edbeib (2020), who demonstrated that increasing starch content generally increases opacity and decreases optical uniformity in biodegradable films. Although the starch-rich formulations exhibited lower transparency, they potentially provided greater accessibility for microbial colonization because starch serves as a readily metabolizable carbon source for microorganisms (Sharma et al., 2021).

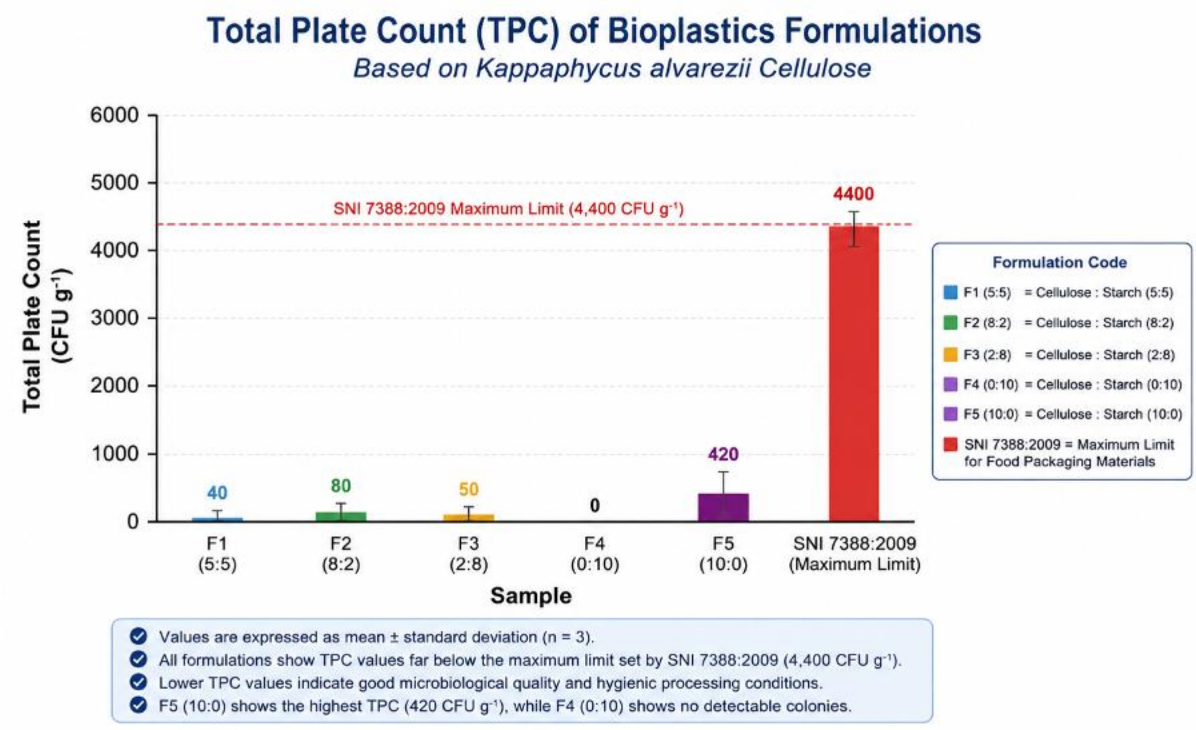
The UV–Vis results also provide indirect evidence of biodegradation processes. According to the Beer–Lambert law, reductions in absorbance correspond to decreases in dye concentration, indicating progressive degradation of chromophoric structures (Dio et al., 2021). The degradation process is likely facilitated by microbial enzymes capable of cleaving azo bonds, producing simpler aromatic intermediates that are subsequently mineralized into environmentally benign compounds (Katheresan et al., 2018; Saratale et al., 2011). Therefore, the optical analysis not only confirms the absence of hazardous azo residues but also supports the biodegradation potential of the developed bioplastic formulations.

Furthermore, the high transmittance observed in cellulose-rich formulations confirms compliance with safety requirements for materials intended for direct contact with food products. The absence of characteristic azo absorption bands within the critical wavelength range strengthens the evidence that the developed bioplastics meet safety expectations regarding chromophoric contaminants and synthetic dye residues (Intandiana et al., 2019; Huwaidi, 2022).

Total Plate Count (TPC) Analysis and Microbial Biodegradation Potential

The biodegradation performance of the bioplastics was further evaluated through Total Plate Count (TPC) analysis, which measured microbial abundance associated with each formulation. The results are presented in Figure 2.

Figure 2.
Total Plate Count (TPC) Results of Bioplastic Formulations



The TPC values ranged from 0 to 420 CFU g^{-1}. The highest microbial count was recorded in F5 (420 CFU g^{-1}), followed by F2 (80 CFU g^{-1}), F3 (50 CFU g^{-1}), and F1 (40 CFU g^{-1}), while F4 showed no detectable microbial colonies. Despite these differences, all formulations remained substantially below the maximum permissible microbial limit established by SNI 7388:2009 (4,400 CFU g^{-1}), indicating excellent microbiological quality and hygienic production conditions.

The relatively low microbial counts observed across all treatments suggest that the bioplastics possess sufficient resistance to uncontrolled microbial contamination while remaining susceptible to biodegradation under favorable environmental conditions. This characteristic is desirable because biodegradable materials should support controlled microbial decomposition without compromising product safety during storage and application (Folino et al., 2020; UNEP, 2023).

Interestingly, F5 exhibited the highest microbial population despite containing no starch. This observation suggests that cellulose extracted from *K. alvarezii* may provide a suitable substrate for specialized cellulolytic microorganisms capable of utilizing cellulose as an energy source. Similar findings have been reported by Sharma et al. (2021), who observed enhanced microbial colonization on cellulose-rich biodegradable polymers due to the availability of cellulase-producing bacteria and fungi.

The Kruskal–Wallis statistical analysis yielded an Asymptotic Significance value of 0.406 ($p > 0.05$), indicating that differences among formulations were not statistically significant. This result suggests that variations in cellulose and starch composition did not substantially affect the overall microbial population. Nevertheless, the presence of microbial growth in most formulations confirms that the developed bioplastics remain biologically degradable and environmentally responsive.

From an environmental perspective, the combination of low microbial contamination levels and measurable microbial activity is highly advantageous. Materials exhibiting excessive microbial growth may experience premature deterioration and pose safety concerns, whereas materials exhibiting no microbial activity may resist environmental degradation. The developed bioplastics successfully balanced these two aspects by maintaining microbiological safety while preserving biodegradability potential.

Relationship Between Azo Dye Degradation and Microbial Activity

The integration of UV–Vis and TPC analyses provides a comprehensive understanding of the biodegradation mechanisms occurring within the cellulose-based bioplastics. The absence of azo dye absorption peaks, combined with detectable microbial populations, indicates that microbial activity likely contributed to the degradation of azo compounds. Numerous studies have demonstrated that microorganisms produce azoreductase enzymes capable of cleaving azo bonds and transforming synthetic dyes into less toxic metabolites (Saratale et al., 2011; Solís et al., 2019).

Cellulose-rich formulations appeared particularly effective because they combined superior structural stability with sufficient microbial colonization to facilitate degradation processes. Meanwhile, starch-containing formulations may enhance biodegradation rates by providing readily available nutrients for microbial growth. Consequently, the synergistic interaction between cellulose, starch, and microbial communities contributes to both environmental degradability and functional performance of the bioplastic films.

Overall, the results demonstrate that bioplastics produced from *Kappaphycus alvarezii* cellulose possess desirable optical, microbiological, and environmental characteristics. The materials exhibited no detectable azo dye contamination, maintained microbial counts well below national safety limits, and supported biodegradation processes necessary for sustainable waste management. These findings reinforce the potential application of seaweed-derived cellulose bioplastics as eco-friendly alternatives to conventional petroleum-based plastics in food packaging, cosmetic packaging, and other environmentally sensitive applications.

CONCLUSION

Bioplastics produced from cellulose extracted from *Kappaphycus alvarezii* demonstrated favorable environmental and microbiological characteristics. UV–Visible spectrophotometric analysis confirmed that all formulations were free from detectable azo dye compounds, as evidenced by the absence of characteristic azo absorption peaks within the 300–500 nm wavelength range. Formulations containing higher proportions of cellulose exhibited superior optical transparency and structural homogeneity, indicating improved film quality.

The Total Plate Count (TPC) analysis revealed microbial populations ranging from 0 to 420 CFU g^{-1}, which were substantially lower than the maximum permissible limit established by SNI 7388:2009. These results indicate that the developed bioplastics possess excellent microbiological safety while remaining susceptible to biodegradation processes. Statistical analysis further demonstrated that variations in cellulose and cassava starch composition did not significantly affect microbial abundance ($p > 0.05$). Overall, the combination of low microbial contamination, absence of azo dye residues, and demonstrated biodegradation potential confirms that *Kappaphycus alvarezii*-based cellulose bioplastics can serve as environmentally friendly alternatives to conventional synthetic plastics. The material offers promising prospects for sustainable applications in food packaging, cosmetic packaging, and other biodegradable product sectors while contributing to the reduction of plastic pollution and supporting circular bioeconomy initiatives.

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