

# Implications of Preliminary Treatment Using *Effective Microorganisms* on the Chemical Composition of Daluga Starch Processing Waste (*Cyrtosperma merkusii*)

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## INFO ARTIKEL

Received 02 September 2025

Accepted 30 January 2026

Key word:

*Cyrtosperma merkusii*

Daluga

*Effective microorganism*

## ABSTRACT

Daluga (*Cyrtosperma merkusii*) starch processing yields waste with 70% of its total weight consist of organic biomass materials that can be utilized as advanced composite biomass materials, a potential base for reinforcing materials. Processing the Daluga waste (debris) requires stages of pretreatment and activations to eliminate inhibiting agents within the hydrolisis process (fermentation process). The purpose of this research is to understand the effects of biological pretreatments using the *Effective Microorganism* (EM4) bacterial consortium towards the chemical properties of Daluga debris. The chemical compositions of debris Daluga before and after each treatment were determined by NDF and Van Soest method for observing the cellulose, hemicellulose, lignin, fiber, ash content, crude protein, silicate and crude fat. These parameters were analysed following the methods outlined in the Indonesian National Standard (SNI 01-2891-1992) and while protein and carbohydrate content was measured with the Dumas combustion method. Results indicated that lignin concentration after delignification process decreased considerably to  $1.80 \pm 0.07\%$  although lignin content of these Daluga debris initially wasn't so high to begin with  $4.11 \pm 0.02\%$ . Herewith, hemicellulose also decreased giving an appearance for the increase and exposure of cellulose. Thus, providing a clear path to further processing the cellulose to advance cellulosic materials such as cellulose nano crystals (CNC).

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## Introduction

Giant swamp taro (*Cyrtosperma merkusii*), locally known as daluga, is an underutilized plant in North Sulawesi, Indonesia. Daluga is an endemic tuber of the outer islands of North Sulawesi, particularly Siau, Sangihe, and Talaud. Historically, daluga was not only endemic to North Sulawesi but was also distributed across Asia and the Pacific [1]. The tuber composition is dominated by carbohydrates (81–83%), with starch content reaching  $65.52 \pm 0.02\%$ . Amylose and

amylopectin contribute  $29.63 \pm 0.01\%$  and  $32.88 \pm 0.02\%$ , respectively, while fiber content reaches 18.55%. Rendering, daluga to have the potential not only as a reliable staple food source, but also as a functional food candidate due to its resistant starch ( $11.37 \pm 0.03\%$ ) and digestible starch ( $44.30 \pm 0.26\%$ ) contents [2].

Daluga is considered a promising starch source for both food processing and non-food applications such as bioplastics. Starch extraction produces approximately 70% by-products, consisting of cellulose-rich organic

biomass generally regarded as waste or debris. This debris contains hemicellulose, cellulose, lignin, and fiber, which can be metabolized by bacteria as carbon and inorganic nitrogen sources. Consequently, daluga debris can be utilized as stock materials for tilapia (*Oreochromis mossambicus*) natural feed, that potentially enhances their survival rate [3]. In addition, daluga debris has potential as a substitute for broiler chicken feed [4].

Lignocellulosic biomass from daluga debris can also serve as an organic material for soil amendments through composting or as a feedstock for bioethanol production. Furthermore, this biomass can be refined through mechanical, biological, or chemical pretreatments to produce nanoscale organic fibers such as cellulose nanocrystals (CNC) and cellulose nanofibers (CNF), which are valuable for eco-friendly composite materials [5]. For example, combining chemical pretreatments with physical size reduction using ultrafine grinding can yield CNC from daluga debris [6].

Daluga debris can thus be developed into advanced composite biomass, serving as a potential bio-reinforcement or filler material to improve mechanical, physical, barrier, biocompatibility, biodegradability, and reinforcement properties [7–9]. It can also be used to modify the rheology of cellulose-based materials [10]. Previous research has demonstrated that daluga debris can be used as raw material for bioplastic production reinforced with CNC [11–12].

Most organic waste consists of lignocellulose, which is difficult and slow to decompose naturally. Therefore, further utilization of debris requires pretreatments—whether chemical, physical, or biological—using microorganisms capable of breaking down complex compounds into simpler ones. Pretreatments are designed to remove or reduce inhibitory compounds such as wax and lignin [13].

Effective Microorganism-4 (EM4) is a microbial inoculant or consortium composed of *Lactobacillus*, yeast, photosynthetic bacteria, actinomycetes, and cellulose-degrading fungi, which can accelerate the decomposition of organic matter [14]. Fermentation with EM4 degrades cellulose and hemicellulose as energy sources, thereby altering the composition of Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), cellulose, and lignin. Since lignin, a phenolic polymer, binds cellulose chains and protects them from hydrolysis, the pretreatment of daluga debris is necessary to improve hydrolysis or fermentation efficiency. Accordingly, this study aimed to investigate the effect of biological pretreatment using the EM4 bacterial consortium on the chemical characteristics of daluga starch-processing waste (debris).

## **Materials dan Methods**

### **Material**

The raw material used in this study was the waste or debris from daluga starch processing. This debris is a biomass by-product generated during the starch extraction process, consisting of tuber peel and pulp residue. The pulp is produced when daluga tubers are crushed to extract starch; the starch-rich supernatant is then sedimented and collected as daluga starch, while the pulp is discarded. In this study, the discarded pulp was collected and used for further processing, with the ultimate aim of developing cellulose nanocrystals (CNC) [2].

### **Method**

This research was carried out at the Biology Laboratory, Faculty of Mathematics, Natural Sciences, and Earth Sciences, Manado State University. The chemical composition analysis of daluga debris was performed at the Nutrition and Animal Feed Laboratory, Faculty of Animal Husbandry, Brawijaya University, Malang. Daluga debris (peel and pulp residues) was first dried until a stable moisture content of approximately 14% was achieved. The dried

material was then ground into 100-mesh powder. A total of 500 grams of powdered debris was fermented using 1000 ml of the EM4 microbial consortium at a ratio of 1:2 for 48 hours. After fermentation, the daluga debris was oven-dried at 45 °C for 24 hours until fully dry. The chemical composition of daluga debris was determined using Neutral Detergent Fiber (NDF) analysis and the Van Soest method to measure cellulose, hemicellulose, lignin, fiber, protein, silicate, and fat. Ash content was determined according to Indonesian National Standard (SNI 01-2891-1992). Protein and carbohydrate contents were measured using the Dumas combustion method (DuMaster Buchi D-480, Switzerland). The results of EM4-pretreated debris were compared with those of untreated daluga debris [6]. Daluga debris' structural molecular integrity was then analysed with a S ATR-FTIR spectrometer (Bruker Invenio, Germany). Measurements were taken at room temperature (22-25 °C) in the 4000–400 cm<sup>-1</sup> range with 64 scans and 4 cm<sup>-1</sup> resolution. Ambient air served as the background, automatically removed via Opus 8.5 software. Triplicate scans of random sample portions showed uniform spectral profiles [15]

## Results and Discussion

The content of lignocellulose biomass generally consists of cellulose, lignin, and hemicellulose where cellulose is naturally bound by hemicellulose and protected by lignin, with the presence of this lignin binding compound makes lignocellulose biomass difficult to hydrolyze so pre-treatment is required [16]. Pretreatment is the most important process in lignocellulose biomass conversion where this process is needed to break the lignin bonds from the lignocellulose biomass structure and reduce the crystalline structure of cellulose so that it can increase the efficiency of the hydrolysis and saccharification process which is an advanced process in biomass conversion [17].

Delignification or the process of decomposition of lignin is one of the preliminary treatments that affects the bioconversion of lignocellulose biomass, especially the hydrolysis process and fermentation process, the process of changing the chemical structure of lignocellulose biomass with the aim of degrading lignin selectively so as to decompose its chemical bonds both in terms of covalent bonds, hydrogen bonds and van der Waals bonds, with other chemical components in lignocellulose biomass and These other components remain intact and reduce the degree of polymerization and crystalline properties of cellulose. The process of delignification can be done by heat, chemically and biologically [17, 18].

The pre-treatment process using a consortium of microbiological agents EM4 aims to remove lignin so that it makes it easier for the next treatment process. Biomass lignocellulose debris before and after treatment (Figure 1) while changes in debris content before treatment and after treatment using EM4 can be seen in Table 1.



**Figure 1.** Debris before and after treatment using EM4

**Table 1.** Biomass Content of Daluga Debris Before and After Treatment

| Components (%)  | Before Treatment [6] | After Treatment with EM4 |
|-----------------|----------------------|--------------------------|
| Dry Ingredients | 83,77 ± 0,01         | 86,03 ± 0,01             |
| Ash             | 11,71 ± 0,03         | 8,90 ± 0,05              |
| Protein         | 2,19 ± 0,05          | 3,29 ± 0,06              |
| Fiber           | 8,73 ± 0,06          | 10,02 ± 0,02             |
| Fat             | 0,57 ± 0,04          | 0,29 ± 0,04              |

|                               |              |              |
|-------------------------------|--------------|--------------|
| NDF (Neutral Detergent Fiber) | 60,69 ± 0,06 | 62,09 ± 0,02 |
| ADF (Acid Detergent Fiber)    | 10,36 ± 0,06 | 14,03 ± 0,02 |
| Hemicellulose                 | 50,33 ± 0,01 | 48,05 ± 0,04 |
| Cellulose                     | 6,01 ± 0,04  | 11,49 ± 0,37 |
| Lignin                        | 4,11 ± 0,02  | 1,80 ± 0,07  |
| Silicate                      | 0,24 ± 0,00  | 0,53 ± 0,00  |

Biological preliminary treatment using EM4 has an effect on increasing the content of dry matter, crude protein, crude fiber, NDF (*neutral detergent fiber*), ADF (*acid detergent fiber*), cellulose and silicate while preliminary treatment can reduce the content of ash, crude fat, hemicellulose and lignin (Table 1). This is in line with research from [19] which states that the delignification process using biological methods can reduce the levels of lignin, hemicellulose and starch levels of cassava peel starch biomass thereby increasing cellulose levels.

The increase in dry matter from  $83.77 \pm 0.01$  to  $86.03 \pm 0.01$  occurred due to the decomposition of various components that make up daluga debris by microorganisms contained in EM4. During the fermentation process, it is suspected that there is a growth of microorganisms and results in a decrease in the moisture content contained in the substrate, in this case daluga debris, resulting in an increase in dry matter. The reduction of water bond in the substrate is caused by the fermentation process that produces heat, where the heat causes some of the water to evaporate where the longer the fermentation time, the more substrate can be degraded by microorganisms so that at the end of fermentation the dry matter will increase [20].

The results of this study are different from the research conducted by [21], fermentation of sago pulp using EM4 can reduce the content of dry matter of sago along with the longer the incubation time. This

reaction occurs due to saturation of lack of oxygen, carbon, nitrogen and lack of substrate as nutrients. The increase in dry matter during fermentation is caused by EM4 is a consortium of microorganism of bacteria and fungi, namely *Saccharomyces cerevisiae* and *Rhodopseudomonas palustris* which can produce hyphae in the fermentation process so as to increase the content of dry matter [22], while research on the effect of EM fermentation on rice bran can increase dry weight, this is due to the formation of oligosaccharides into the shape of the cellulose structural components that cause an increase in dry matter content [23].

The increase in the protein content of debris during the fermentation process is caused by an increase in the number and activity of microbes in binding nitrogen as the basic material for protein synthesis, where an increase in nitrogen levels is very beneficial for microorganisms to grow and carry out activities optimally. The increase in crude protein is also due to the addition of proteins contributed by microorganism cells in EM4 resulting in single-cell proteins (SCPs) [24]. The high increase in crude protein is due to the presence of microorganisms in EM4 that can produce various enzymes such as cellulase enzymes and proteases that are able to break down protein bonds in addition, the increase in crude protein is due to single-cell proteins of microorganisms that develop during the fermentation process [25], this can be seen from the increase in debris protein from  $2.19 \pm 0.0$  to  $3.29 \pm 0.06$  after being given the preliminary treatment. The increase in protein levels is due to the synthesis of proteins by a consortium of microorganisms in EM4, the same thing happens in the fermentation process of sago pulp by the white oyster fungus (*Pleurotus ostreatus*) where the white oyster fungus can take nitrogen from the air. The ability of white oyster mushrooms to ferment in an aerobic atmosphere will support white oyster mushrooms to do nitrogen fixation from free air, after the growth of mycelium is

complete, the proteins from the substrate will be used for the formation of fruit bodies, so that the proteins on the substrate will decrease over time with fermentation time [26].

The coarse fiber content of daluga debris increased from  $8.73 \pm 0.06$  to  $10.02 \pm 0.02$ . The increase in coarse fiber is due to the formation of polysaccharides which are carbohydrate polymers with a high molecular weight of microorganisms and are affected by fluctuations in the moisture content of the substrate during the fermentation process [27]. This research is in line with the research from [28] where changes in moisture content will cause changes in the dry weight of the substrate so as to affect the coarse fiber content of the substrate, in addition to the increase in coarse fiber caused by the condition of the substrate, namely the availability of starch on the substrate in this case daluga debris and sago pulp so as to allow anaerobic microbes to degrade starch which is easier to digest than coarse fiber.

The preliminary treatment using EM4 increased the cellulose content from  $6.01 \pm 0.04$  to  $11.49 \pm 0.37$  and the silicate content from  $0.24 \pm 0.00$  to  $0.53 \pm 0.00$ . Biological treatment tends to increase the cellulose content of biomass waste, this is in line with research from [29], where biological treatment using fungal isolates increases the cellulose content but treatment using chemicals and physical treatments can increase the cellulose content, this is an indication that delignification treatment basically causes cellulose, which is the main target of the hydrolysis process to become more accessible [30]. The determining factor of cellulose decomposition activity depends on the type of biomass and its hydrolysis enzyme, in this case the microorganisms used in the fermentation process and the length of the fermentation process where research from [31] states that the longer the fermentation time using EM4 on corn cob biomass, the cellulose component will decrease. The increase in the

amount of cellulose in this study occurred due to a decrease in the content of hemicellulose from  $50.33 \pm 0.01$  to  $48.05 \pm 0.04\%$  and the degradation of lignin from  $4.11 \pm 0.02$  to  $1.80 \pm 0.07$  where cellulose, which is a component of plant cell walls, always binds to other components, namely lignin and hemicellulose, to form lignocellulose components [32].

The NDF component consists of cellulose, hemicellulose and lignin while the ADF component consists of cellulose and [26]. ADF degradation is related to NDF content and degradation of hemicellulose, where in this study there was an increase in the components of NDF and ADF always fermented, which were respectively  $60.69 \pm 0.06$  to  $62.09 \pm 0.02$  and  $10.36 \pm 0.06$  to  $14.03 \pm 0.02$ , this is different from the previous study conducted by [31] which stated that the longer the fermentation time of EM4 on corn cob flour can reduce NDF and ADF levels while the addition of the concentration of EM-4 and/or the addition of EM-4 with different durations of EM-4 fermentation has not had an effect on the decline of the ADF component. Another implication of this study shows that daluga debris fermented with EM4 for 48 hours is not appropriate to be used as a supplement to animal feed with the target of increasing the weight of livestock because the amount of fiber increases so that the digestibility of feed decreases so that it can reduce the body weight of livestock but daluga debris as much as 0.13% can be used as feed supplementation that can reduce blood glucose levels of broiler chickens [4]. In addition, daluga debris can be used as a source of carbon to increase the *survival rate* of mujair fish cultivated in biofloc culture [3].

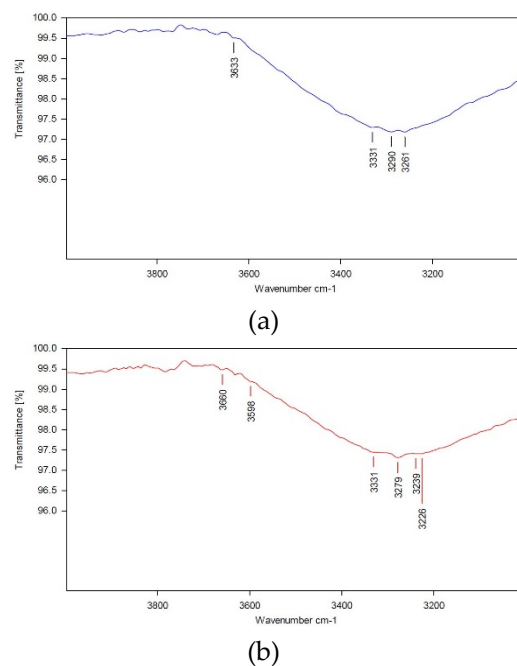
The lignin content during the fermentation process decreased from  $4.11 \pm 0.02$  to  $1.80 \pm 0.07$ , the decrease in lignin content during EM4 fermentation also occurred in studies from [31] where the fermentation time affected the amount of lignin decrease in corn cob flour. Microorganisms that play a role in the lignin degradation process are *Actinomyces*

and *Streptomyces* which are found in mixed cultures of EM4. The mechanism of lignin degradation by microbes is through the use of polysaccharides from lignin as a carbon source and energy source where the role of peroxidase and xylanase enzymes will break the lignin bonds so that they are degraded into sugars [33]. Delignification treatment can not only be carried out biologically using a consortium of EM4 microorganisms, but it can also use physical treatments such as heating or treatment using chemicals. Previous research on the degradation of daluga debris using the chemicals NaOCl and NaOH showed a decrease in lignin from  $4.11 \pm 0.02$  to  $0.86 \pm 0.03$  [6]. Chemical treatment shows the most decrease in lignin but is less recommended for large-scale use because it is toxic and corrosive and requires a dedicated reactor [34].

The results of the preliminary treatment in addition to reducing the lignin content also caused a decrease in the hemicellulose content from  $50.33 \pm 0.01$  to  $48.05 \pm 0.04$ . The decrease in hemicellulose levels after the treatment process indicates that the preliminary treatment carried out on the biomass of dalga debris waste, not only causes lignin degradation, but also occurs hemicellulose depolymerization [35, 36, 37], this is because hemicellulose is a carbohydrate compound that makes up lignocellulose biomass cells in addition to that hemicellulose has a lower degree of polymerization compared to cellulose. This decrease in hemicellulose content is also caused by the structure of hemicellulose, which is mostly soft or amorphous, so it is sensitive and easily degraded [38].

The preliminary treatment using EM4 had an effect on reducing the ash content from  $11.71 \pm 0.03$  to  $8.90 \pm 0.05$ , during the fermentation process of the debris there was an increase in organic matter due to the overhaul of the substrate by a consortium of microorganisms contained in *effective microorganisms* (EM4) so as to reduce ash

content where the less organic matter is degraded, the less ash content will be proportionally reduced [23]. The high and low levels of ash contained in a material can be related to the number of mineral elements where the high and low content of organic matter is caused by the influence of other components such as dry matter content and ash content [39]. The higher the amount of EM4 and the higher the concentration of microorganisms used in the fermentation process, the higher the decrease in the ash content of a material [22, 40].



**Figure 2.** FTIR of daluga debris, (a) untreated daluga debris, and (b) pretreated daluga debris with EM4.

FTIR spectroscopy was employed to evaluate the chemical modifications of daluga debris before the EM4 pretreatment (fig. 2a) and after 48 hours of treatment with an EM4 microbial consortium (fig. 2b). Both untreated (fig 2a) and treated (fig 2b) spectra exhibit broad O-H stretching bands within the 3600–3200  $\text{cm}^{-1}$  region, indicating the main characteristic of hydroxyl groups in cellulose and other polysaccharides [41]. However, the EM4-treated sample shows broader and more

pronounced O–H bands, extending toward higher wavenumbers (approximately 3598–3659  $\text{cm}^{-1}$ ). This change indicates increased exposure of hydroxyl functionalities due to partial depolymerization and deconstruction of the lignocellulosic matrix [6]. The O–H stretching region (3600–3200  $\text{cm}^{-1}$ ) provides critical insight into hydrogen bonding, cellulose accessibility, and supramolecular organization of lignocellulosic materials [41]. Notable differences were observed between untreated daluga debris (fig 2a) and EM4-treated debris (fig 2b), reflecting substantial biochemical and structural transformations induced by the EM4 consortium. In the untreated debris, the O–H stretching band appears as a broad but relatively heterogeneous envelope centered between approximately 3260 and 3330  $\text{cm}^{-1}$ . This broadness is characteristic of strong intermolecular hydrogen bonding, typical of intact lignocellulosic matrices where cellulose microfibrils are tightly embedded within hemicellulose and lignin networks [42, 43]. Meanwhile following 48 hours EM4 treatment, the O–H stretching region exhibits pronounced changes. The band becomes broader and more resolved, extending from approximately 3239  $\text{cm}^{-1}$  toward higher wavenumbers up to about 3659  $\text{cm}^{-1}$ . The emergence of higher-frequency O–H stretching components indicates weakened hydrogen bonding and increased hydroxyl group freedom [41]. The shift and broadening of the O–H stretching band from untreated to EM4 treated debris indicate a transition from a highly ordered, strongly hydrogen-bonded system to a more disordered and accessible cellulose structure [43]. The appearance of O–H stretching components at higher wavenumbers ( $>3500 \text{ cm}^{-1}$ ) is particularly significant, as these are typically associated with surface-accessible cellulose hydroxyls, which are highly relevant for CNC hydrolysis and surface chemistry [6].

In addition, preliminary treatment can reduce the crude fat content of daluga debris,

the decrease is caused by the overhaul of complex triglyceride bonds to simple bonds into fat and glycerol so as to cause the crude fat component to decrease, some of the fat will evaporate thus causing the loss of crude fat [44]. A decrease in fat content and an increase in protein content are one of the indicators of a well-running fermentation process.

Preliminary treatment can also be done by chemical methods such as the use of acidic chemicals such as  $\text{H}_2\text{SO}_4$  or the use of alkalis such as  $\text{NaOH}$ , the results of previous studies show that preliminary treatment of the same biomass, namely daluga debris, but using chemical treatment with the use of alkaline can reduce the levels of lignin and hemicellulose and increase the cellulose content [6], but the use of chemicals in preliminary treatment is less recommended for large-scale use because it is toxic and corrosive so it requires a special reactor [34]. Thereby, the implications of this study show that the preliminary treatment is simple and economical and short in time making it efficient. The biological method of fermenting debris using EM4 for 48 hours can break down the hemicellulose component and degrade lignin so that the number of cellulose increases, thus that daluga debris can be further extracted to obtain *nano crystal cellulose* as a *biofiller* component or bioplastic reinforcement made from daluga starch plastic [6, 11, 12].

### Acknowledgment

The author expressed gratitude to the LPPM UNIMA for providing Applied Research funds with contract number 418/UN41.9/TU/2025.

### Conclusion

The preliminary treatment of fermentation using EM4 on daluga debris is a simple, efficient and economical treatment because it can reduce the hemicellulose component and degrade the lignin component to  $1.80 \pm 0.07\%$  so that it can increase the cellulose component which is the target as the main component for the continued

use of cellulose biomass such as *cellulose nano crystal* (CNC) as a *biofiller* or *bioreinforcement* on bioplastics.

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