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*Original Research Article*

# Cellulose Hydrolysis of Mask Waste Using *Aspergillus niger* and Eco-Friendly Microwave Pretreatment

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## Abstract

The management of used medical mask waste has become a significant issue due to the increased volume of waste during and after the pandemic. Medical mask waste contains cellulose compounds that can be converted into derivatives such as glucose, which are then processed into bioethanol as an alternative energy source. This study aims to hydrolyse medical mask waste using cellulase enzymes from Aspergillus Niger to produce glucose. The cellulase enzyme composition was varied (5 ml, 15 ml, and 25 ml) to determine the optimal hydrolysis conditions. The glucose produced was measured using DNS reagent assay with spectrophotometry at a wavelength of 540 nm. The highest amount of glucose was obtained under optimal conditions with 25 ml of cellulase enzyme after 48 hours of hydrolysis, amounting to 88.16 ppm. Subsequently, the glucose from hydrolysis was fermented using Saccharomyces cerevisiae, and the fermentation product was analysed for ethanol concentration using GC-FID. The products of fermentation ware 0.017% ethanol concentration from mask waste fermentation. Hydrolysis is an environmentally friendly alternative solution for handling mask waste.

**Keywords:** *Aspergillus niger*; hydrolysis; mask waste

## 1. Introduction

In late 2019, the world was shocked by the announcement of a disease caused by an unidentified virus (Li et al., 2020). The World Health Organization (WHO) officially named this disease Covid-19 (Coronavirus Disease 2019) and the virus SARS-CoV-2 (Severe Acute Respiratory Syndrome) due to

Coronavirus 2), which can be transmitted through droplets or saliva when people talk, cough, and sneeze (Thevarajan et al., 2020; Wu et al., 2020). Subsequently, WHO also issued medical protocols, including the use of medical masks, which have been effective in gradually reducing the transmission of the Covid-19 virus. However, this protocol has also brought new problems to the world, one of which is environmental pollution due to the accumulation of medical mask waste (Pratama et al., 2021). The accumulation of mask waste is evident for example from the 5% increase in waste in several rivers in Jakarta, peaking between March and April 2020, with most of it being medical mask waste (Cordova et al., 2021).

Medical mask waste requires special handling because it is made from non-biodegradable materials, such as spunbond fiber layers or non-woven fiber layers like polypropylene, polyethylene, and cellulose (WHO, 2020). The accumulation of these non-biodegradable materials has led to significant environmental concerns, particularly the formation of microplastics. Microplastics are small plastic particles, typically less than 5 millimeters in diameter, that result from the breakdown of larger plastic debris in the environment. The improper disposal and widespread use of medical masks during the Covid-19 pandemic have exacerbated the microplastic pollution issue (Saadat et al., 2020). In the soil environment, microplastics can significantly alter its physicochemical properties, impacting soil health. These particles can disrupt soil structure, reducing porosity and water retention, which negatively affects plant growth. Additionally, microplastics can decrease microbial activity and alter crucial enzyme functions, impeding nutrient cycling. They can also adsorb harmful chemicals and heavy metals, acting as carriers for these contaminants, which can leach into groundwater or be absorbed by plants, posing further risks to the environment and human health (Sajjad et al., 2022; Ya-Di et al., 2022). The microplastics can furthermore enter aquatic ecosystems through surface runoff and improper waste management, ultimately impacting marine life and ecosystems. Once in the environment, microplastics can absorb and concentrate toxic pollutants, posing a threat to wildlife that ingests these particles. The ingestion of microplastics by marine organisms can lead to physical harm, chemical contamination, and disruption of the food chain, ultimately affecting human health through the consumption of seafood (Dharmaraj et al., 2021; Riaz et al., 2023). Additionally, microplastics can interfere with the reproductive and growth processes of marine life, leading to a decline in biodiversity and ecosystem health (Bowley et al., 2021; Sethia et al., 2024). This persistence and ubiquity of microplastics in the environment highlight the urgent need for effective waste management strategies and the development of biodegradable alternatives to conventional plastics.

Various approaches have been carried out in the disposal process of the mask waste material (named degradation) which can be grouped into chemical, physical, and microbiological processes. Thermochemical conversion, such as pyrolysis, involves heating polymeric mask components at elevated temperatures (350–500 °C) in the absence of oxygen to produce gaseous, liquid, and solid products that can yield useful hydrocarbons (Hussein et al., 2022; Sun et al., 2022). Catalytic cracking enhances degradation efficiency by using catalysts to break down plastics at lower temperatures, yielding more desirable products. For example, the Ketjenfine PR.9 catalyst has shown to improve liquid yields from plastic feedstocks (Dharmaraj et al., 2021). Alternative chemical processes such as the Photo-Fenton reaction combining UV light, hydrogen peroxide, and iron catalysts, have demonstrated high degradation efficiency, reducing polypropylene particle volume by over 95% within a week (Piazza et al., 2022). Subsequently, for physical degradation methods, mechanical shredding and thermal degradation are key approaches. Mechanical shredding breaks down masks into smaller pieces, increasing surface area and facilitating easier handling and further treatment while the thermal degradation such as incineration reduces mask waste volume by converting it into ash, gases, and heat, and thermochemical processes to achieve a significant mass reduction (Sari et al., 2022). These waste-to-energy processes demonstrate technical feasibility, but they rely on high temperature and specialized catalysts (and can still emit $CO_2$), highlighting the need for alternative low-impact approaches.

Beyond fuels, mask waste has also been upcycled into functional materials for environmental and energy applications. For instance, carbonized mask fibers (treated with acid and grafted with chitosan) yielded a porous activated-carbon adsorbent (CSMA) that bound uranyl-ions U(VI) with high capacity (1). Similarly, chemical functionalization of mask fiber mats produced superhydrophilic membranes that achieved >99.6% efficiency in oil–water separation at very high water flux (3). In another example, waste mask material coated with graphene and $Ca_3Co_4O_9$ formed solid-state electrodes for supercapacitors, reaching record performance for energy density and capacitance (2). These studies highlight the versatility of mask-derived materials, but they focus on remediation or energy storage rather than converting the carbon content into biofuels. Lastly, the microbiological degradation, a method involves the use of microorganisms that can break down polymers, offering a potentially sustainable and eco-friendly solution to plastic pollution. Various microorganism isolates, including *Bacillus*, *Pseudomonas*, *Chelatococcus*, and *Lysinibacillus fusiformis*, have been found capable of degrading those material. These bacteria utilize their enzymatic activities to break down the durable synthetic polymers, aiding in its decomposition. These microorganisms play a crucial role in the biodegradation process, breaking down complex polymers into simpler molecules that can be assimilated into natural biogeochemical cycles, thus mitigating the environmental impact of plastic waste (Dey et al., 2023).

One approach to enhance microbiological degradation is through bioconversion processes using specific microorganisms. Cellulose hydrolysis into glucose from medical mask waste could be carried out using cellulase enzymes from *Trichoderma reesei*, an aerobic mold species capable of producing polysaccharide-degrading enzymes (Hilakore et al., 2013). However, the results of this study were not optimal due to the low production of ß-glucosidase from *Trichoderma reesei*, despite it being able to produce up to 80% endo-ß-1,4-glucanase and exo-ß-1,4-glucanase. Therefore, alternative microorganisms that can produce higher levels of glycosides in the bioconversion process are needed. *Aspergillus niger*, a type of fungus that easily grows on various substrates and a versatile fungus, widely used in various biotechnological applications such as pectinase enzyme production and bioleaching for extracting metals from ores or waste materials, is considered suitable for this purpose (Sandri et al., 2013; Mulligan et al., 2004; Li et al., 2021). The *Aspergillus niger* can produce higher levels of glycosides as a catalyst for the hydrolysis of medical mask waste (Kodri et al., 2013; Puran et al., 2015). Despite these advances, few studies have integrated microwave pretreatment with enzymatic hydrolysis for the bioconversion of mask-derived cellulose. Microwave irradiation is known to disrupt the crystalline structure of lignocellulosic materials, enhancing enzyme accessibility (Sun *et al.*, 2022). Therefore, combining microwave pretreatment with *A. niger*-based enzymatic hydrolysis presents a novel and potentially efficient strategy to convert disposable mask waste into glucose for subsequent bioethanol fermentation. This study aims to investigate an integrated and eco-friendly approach for converting medical mask waste into value-added products. The process involves hydrolyzing cellulose from the mask material using cellulase enzymes produced by *Aspergillus niger*, followed by fermentation of the resulting glucose into bioethanol. Bioethanol, a renewable fuel, is commonly produced through microbial fermentation of sugars derived from biomass such as sugarcane and cassava (Ruswandi et al., 2018). By applying this method, the research seeks to offer a sustainable solution for managing disposable mask waste while contributing to circular bioeconomy through the production of bioethanol.

## 2. Experimental

### 2.1. Materials

The materials used in this study included yeast extract (*Saccharomyces cerevisiae*), Ammonium sulfate $(NH4)_2SO_4$, Monopotassium Phosphate ($KH_2PO_4$), Iron (II) Sulfate Heptahydrate ($FeSO_4.7H_2O$), Carboxymethylcellulose (CMC) 1%, Potassium Chloride (KCl), Sodium Hydroxide (NaOH), aquadest, *Aspergilus niger*, Etanol, and used medical masks waste.

### 2.2. Pre-Treatment of Mask Waste

The collected mask waste is first cut into pieces approximately 1 cm in size and dissolved in 2% NaOH to facilitate the decontamination process. Sterilization is performed in an autoclave at 135°C for 15 minutes to eliminate microorganisms on the masks. After decontamination, the mixture is cooled and then heated at a moderate temperature in a microwave for 40 minutes. The heating with NaOH and microwave is conducted to break the ester bonds between cellulose, hemicellulose, and lignin. The masks are then rinsed with water until the pH of the solution is neutral, followed by drying in an oven at 100 ± 5°C and grinding with a blender. After pretreatment, the samples were scanned by SEM to determine the changes in the structure of the constituents.

### 2.3. Hydrolysis of Cellulose Content in Mask Waste

After the pre-treatment, 0.1 g of yeast extract, 0.15 g of bacterial peptone, 0.14 g of $(NH_4)_2SO_4$, 0.2 g of $KH_2PO_4$, 0.005 g of $FeSO_4{\cdot}7H_2O$, and 0.5 ml of 1% CMC solution are mixed with 100 ml of citrate buffer solution. Then, 2 grams of ground mask waste is added and mixed thoroughly. The sample is then cooled and inoculated with a spore suspension of *Aspergillus niger*, followed by incubation at room temperature for 8 days. Enzymes are harvested by adding 100 ml of 1% Tween 80 solution and centrifuging at 4,000 rpm for 30 minutes. The supernatant is separated as the cellulase enzyme. The treated mask waste is weighed to 5 grams and varying amounts of cellulase enzyme (5, 15, and 25 ml) are added, diluted with citrate buffer pH 5 to a final volume of 150 ml. Hydrolysis is conducted at room temperature for 3 days with glucose measurements taken daily. The sugar content resulting from the hydrolysis is determined using the DNS reagent method.

The Dinitrosalicylic Acid (DNS) method is a chemical technique used to detect and measure reducing sugars, such as glucose, in a solution. The hydrolysed mask waste containing reducing sugars will react with the DNS reagent in a composition of 1 ml sample to 4 ml DNS reagent. The mixture is then heated over a Bunsen burner until the color changes from yellow to brown. In a strongly acidic environment (created by sulfuric acid), reducing sugars undergo oxidation reactions, turning the DNS reagent from blue to red. After the color change, the absorbance of the sample is measured using UV-vis at a wavelength of 540 nm. The absorbance value is then used in the glucose standard solution regression equation, y = 0.0043x - 0.1121 (Rachdianti et al., 2021).

### 2.4. Glucose Fermentation

After obtaining glucose from the hydrolysis process of mask waste, 12 ml of yeast extract solution is added, and fermentation is carried out on the hydrolysed solution at room temperature for 3 weeks. The most optimal glucose content is used as the basis for the fermentation process using Saccharomyces Cerevisiae. After the fermentation process is complete, ethanol in the solution is separated from secondary metabolites using a distillation apparatus at a vapor temperature of 68°C for 2 hours. The ethanol content is then tested using Gas Chromatography-Flame Ionization Detector (GC-FID) with a standard ethanol solution as a comparison for calculation.

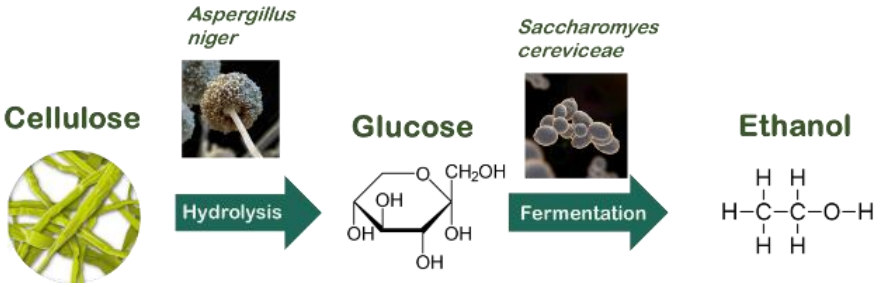
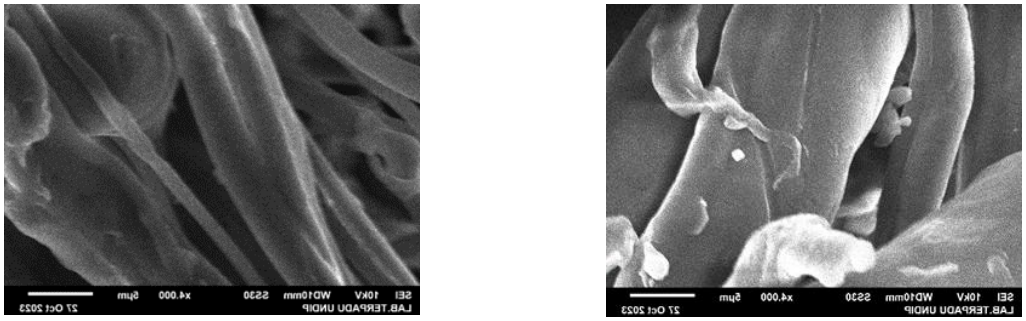



**Figure 1.** Visualization of Processes

# 3. Result and Discussion

## 3.1. Scanning Electron Microscope

Qualitative analysis was carried out with the Scanning Electron Microscope (SEM) to identify morphological changes that occur in masks that are not pretreated compared with masks that go through the pretreatment stage.



(a) (b)

**Figure 2.** SEM results (a) mask without pretreatment (b) mask sample after pretreatment

Based on the SEM test result in Figure 2, the pretreated mask will change its shape microscopically. The change in the image occurs due to the breaking of bonds that occur between lignin, polypropylene, and cellulose in the mask. Pretreatment is used to maximize the exposure of cellulose fibers. It is very important to destroy the solid structure before enzymatic hydrolysis. The purpose of pretreatment is to reduce the structural immunity of lignocellulosic biomass, such as breaking lignin bonds, dissolving hemicellulose, reducing the particle size of the material, and lowering the crystallinity of cellulose, thereby making it easier for cellulase enzymes to convert cellulose (Sun et al., 2022).

## 3.2. Dinitrosalicylic Acid Reagent

Qualitative analysis was carried out with the with the DNS reagent test to identify the content of reducing sugar compounds such as glucose in hydrolysed mask waste. The glucose content test uses the DNS (Dinitrosalicylic Acid) reagent method, the sugar reduction reaction with DNS reagent is a redox reaction where the aldehyde group which acts as a reducing agent will be oxidized to carboxyl, and DNS which acts as an oxidizing agent will be reduced to 3-Amino-5-nitrosalicylic acid. If there is a reducing sugar in the sample, the DNS solution which is initially yellow will react with the reducing sugar to form a reddish orange colour. This reaction takes place in an alkaline environment and at a temperature of 100°C (Ruswandi et al., 2018).

Microbes are important agents in genetic engineering, especially in industrial microbiology (Vitorino and Bessa, 2017). Microbes are widely used for the production of various biologically important molecules including enzymes, proteins, or other bioactive compounds (Abdel-Aziz et al., 2017). Enzymes produced by fungi are widely used in industry and the most prominent among them is the enzyme cellulase (Singh et al., 2021). This enzyme is very important due to its ability to break the beta 1-4 glycosidic bonds in cellulose. It is highly utilized in textile industry, pharmaceutical production, biofuel production and so on (Siva et al., 2022).

**Table 1.** Effects of cellulase enzyme volume variation and hydrolysis time on glucose content

| Cellulose enzyme volume (ml) | Glucose concentration (ppm) | | |
|---|---|---|---|
| | Day-1 | Day-2 | Day-3 |
| **5** | 40.95 | 44.67 | 50.44 |
| **15** | 35.37 | 44.67 | 50.49 |
| **25** | 47.70 | 88.16 | 55.37 |

In general, the longer the hydrolysis time, the higher the concentration (Sitompul and Putra, 2016). However, at an enzyme volume of 25 ml there was saturation on day two which caused the glucose concentration to decrease on day three. And the higher Cellulose enzyme volume can produce the higher glucose concentration (Aprilyanti et al., 2019), the highest glucose concentration was produced at a cellulose enzyme concentration of 25 ml. When reviewing the variation of samples, the concentrations of 5 ml and 15 ml have the highest glucose levels on the third day. However, in contrast to the 25 ml concentration, the optimum day to produce high glucose levels was on the second day and decreased glucose levels on the third day.

The increase in cellulase enzyme activity that occurred at 5 ml and 15 ml concentrations on days 1 to 3 and 25 ml concentration on days 1 to 2 indicates the high interaction between the cellulase enzyme and cellulose. This interaction forms an enzyme-substrate complex that produces glucose as the final product. Meanwhile, the decrease in cellulase enzyme activity at 25 ml enzyme concentration on days 2 to 3 indicates that the interaction began to decrease. This decrease occurred due to the accumulation of previously formed products, which caused inhibition of the cellulase enzyme. Cellulose hydrolysis products can act as inhibitors for cellulase enzyme activity. Glucose and cellobiose are enzyme inhibitors in the cellulose hydrolysis process. Cellobiose inhibits the work of exoglucanase or cellobiohydrolase enzymes in the cellulase enzyme complex, while glucose inhibits the activity of ß-glucosidase enzymes that play a role in hydrolyzing cellobiose (Idiawati et al., 2014).

Saccharomyces cerevisiae is the main yeast used in fermentation. The morphology of Saccharomyces cerevisiae is generally ellipsoid with a large diameter of 5-10 µm and a smaller diameter of about 5 µm. All yeasts are unicellular fungi characterized by an ultrastructure similar to that of higher eukaryotic cells. That is, they consist of a cell wall, nucleus, mitochondria, endoplasmic reticulum (ER), Golgi apparatus, vacuoles, micro bodies, and secretory vesicles along with a complex network of extracellular and intracellular membranes (Walker and Stewart, 2016). The use of Saccharomyces cerevisiae yeast is due to its ability to produce zymase enzyme to convert glucose into alcohol.

Saccharomyces cerevisiae can convert sugars contained in various raw materials into bioethanol. Saccharomyces cerevisiae is an attractive option for large-scale bioethanol production as it has the ability to survive at high ethanol concentrations. Fermentation uses Saccharomyces cerevisiae to ferment glucose into ethanol anaerobically (Fatima et al., 2024).

### 3.3. Gas Chromatography – Flame Ionization Detection

Qualitative analysis was carried out with the Gas Chromatography reagent test to identify the ethanol content of the glucose fermentation of the mask sample. GC-FID (Gas Chromatography with Flame Ionization Detection) test is a gas chromatography analysis technique that uses a flame ionization detector to identify and measure the concentration of organic compounds in the sample, in this research is the concentration of ethanol compounds (Mansur et al., 2022). Based on the results of the GC-FID test carried out on the distillation sample, there is 0.017% ethanol content as shown in Figure 3.

Figure 3 show the ethanol concentration obtained from the fermentation process (0.017%) was significantly lower than those reported in previous studies using lignocellulosic biomass, where yields ranged from 1% to 4% depending on fermentation efficiency and optimization strategies (Fatima et al., 2024; Yamakawa et al., 2023). This low yield may be attributed to several factors, including the extended fermentation duration of three weeks, which can lead to ethanol degradation or evaporation, and microbial competition that shifts metabolism toward higher alcohols such as propanol (Kunjapur & Prather, 2015). Optimizing fermentation conditions, such as reducing duration, maintaining anaerobic conditions, supplementing nutrients, and selecting engineered or co-culture microbial strains—may significantly enhance ethanol production (Sari et al., 2022; Singh et al., 2021). Furthermore, the current study lacks statistical analysis to support the reproducibility of results. Incorporating multiple replicates and reporting standard deviations or error bars would improve the scientific robustness and credibility of the data (Sajjad et al., 2022). Future studies should also utilize statistical methods such as analysis of

variance (ANOVA) to determine the significance of treatment differences in enzyme concentration or glucose yield (Hilakore et al., 2013).

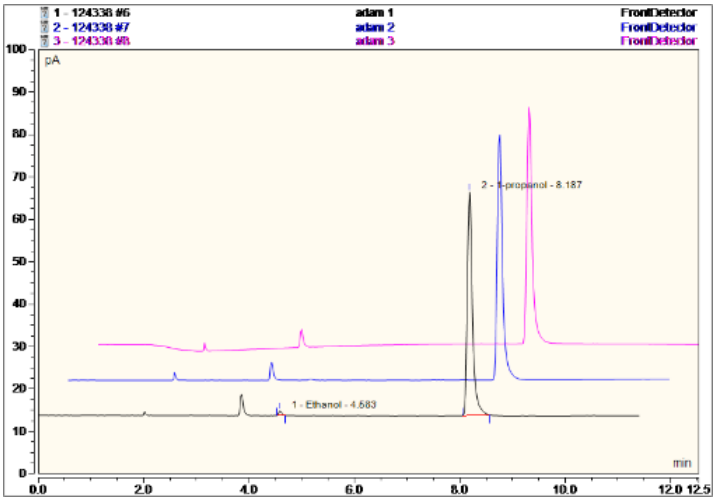



**Figure 3.** Peak graph of GC-FID test results

This study demonstrates the potential of converting disposable medical masks into bioethanol through enzymatic hydrolysis and fermentation. However, the feasibility of scaling up the process requires further analysis. Energy consumption, particularly during microwave-assisted pretreatment and long fermentation periods, poses significant economic challenges (Sun et al., 2022). A preliminary techno-economic analysis and life cycle assessment (LCA) are necessary to evaluate the cost-effectiveness and environmental impact of integrating this method into municipal waste management systems (Riaz et al., 2023). Although the study promotes an environmentally friendly degradation method, the fate of residual solid waste post-hydrolysis and fermentation remains unaddressed. These residues, potentially rich in microbial biomass and polymer fragments, may pose additional disposal challenges. Strategies such as composting, anaerobic digestion, or incorporation into construction materials could improve the environmental sustainability of the process (Cordova et al., 2021; Sethia et al., 2024). Future research should focus on valorising these by-products to achieve a circular economy framework in bioethanol production from polymeric waste.

## 4. Conclusion

In this study, enzymatic hydrolysis and fermentation processes were analyzed to understand the factors affecting glucose conversion efficiency and ethanol yield. The hydrolysis results indicated that while glucose production initially increased with longer reaction times, an unexpected decline was observed after the second day at an enzyme concentration of 25 mL, likely due to product inhibition, enzyme deactivation, or substrate depletion. Additionally, GC-FID analysis of the fermentation products revealed a higher proportion of propanol compared to ethanol, which contradicts the expected outcome of ethanol being the primary product. This anomaly can be explained by ethanol degradation over time, oxidation into acetic acid, microbial metabolic shifts favoring higher alcohol production, and possible volatilization losses due to prolonged fermentation. The findings highlight the importance of optimizing hydrolysis and fermentation conditions, particularly by controlling reaction duration and preventing ethanol degradation, to ensure maximum efficiency in bioethanol production. Future studies should focus on mitigating enzyme inhibition effects and improving fermentation stability to enhance ethanol yield and purity.

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