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Comparative Investigation on the Optimization of Hydrogel Derived from Cellulose of Banana Stem (*Musa x paradisiaca*), Cellulose and Carboxymethylcellulose (CMC) Crosslinked Using Citric Acid

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DECLARATION

I declare that this thesis entitled "Comparative Investigation on the Optimization of Hydrogel Derived from Cellulose of Banana Stem (*Musa x Paradisiaca*), Cellulose, and Carboxymethylcellulose (CMC) Crosslinked Using Citric Acid" is the results of my own research except as cited in the references.

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Kajian Perbandingan mengenai Pengoptimuman Hidrogel yang Dihasilkan daripada Selulosa Batang Pisang (*Musa x Paradisiaca*), Selulosa, dan Karboksimetilselulosa (CMC) yang Dirantai Silang Menggunakan Asid Sitrik

ABSTRAK

Kajian ini menyiasat asid sitrik sebagai agen pautan silang yang sesuai untuk pembangunan hidrogel yang berasal dari selulosa batang pisang, selulosa, dan karboksimetilselulosa (CMC). Ia membincangkan impak alam sekitar sisa batang pisang, mempertahankan pengekstrakan selulosa yang mampan daripada sisa pertanian untuk menangani pencemaran yang berpunca daripada pembakaran terbuka. Kaedah pengekstrakan berdasarkan alkali berjaya mengubah 43% serbuk batang pisang, dengan pertimbangan penting terhadap kepekatan Natrium Hidroksida (NaOH)/Natrium Hipoklorida (NaOCl), suhu pengekstrakan, dan tempoh penyingkiran lignin untuk mengoptimumkan hasil selulosa. Analisis morfologi berdasarkan mikroskopi menunjukkan sifat fibros selulosa batang pisang, membezakannya daripada CMC yang tidak fibros. Kajian penyelesaian menggunakan 8% NaOH dan 12% urea menyorot kepentingan keadaan penyelesaian yang optimum, mempengaruhi ciri-ciri hidrogel, dengan selulosa batang pisang menunjukkan kandungan fibros yang lebih tinggi daripada selulosa. Kepekatan asid sitrik membuktikan pentingnya dalam proses pautan silang, mempengaruhi ciri-ciri hidrogel. Hidrogel yang terbentuk dengan 40% berat asid sitrik menunjukkan pautan silang yang cekap, satu faktor penting untuk ciri-ciri yang dikehendaki. Analisis perbandingan, dengan menggunakan Spektroskopi FTIR dan imej SEM, membezakan Hidrogel Selulosa Batang Pisang (BSCH), Hidrogel Selulosa (CH), dan Hidrogel Karboksimetilselulosa (CMCH). Spektrum FTIR menunjukkan puncak yang berbeza yang berkaitan dengan kumpulan hidroksil, menyumbang kepada sifat hidrofil hidrogel. Imej SEM memperlihatkan struktur fibros dan berongga BSCH, menekankan potensinya dalam aplikasi seperti pembalut luka. Ujian antibakteria menunjukkan keberkesanan yang meningkat BSCH terhadap Escherichia coli, yang dapat diterangkan oleh kandungan asid sitrik. Kajian penyerapan menunjukkan kecekapan BSCH yang lebih tinggi dalam mengeluarkan pewarna metilena biru. Penyelidikan biodegradasi selama empat minggu menunjukkan kehilangan berat yang terkawal oleh BSCH sebanyak 91%, melampaui CH (89%) dan CMCH (100%). Struktur kompleks dan heterogen selulosa asli batang pisang, dengan komponen seperti hemicellulose dan lignin, menyumbang kepada proses degradasi yang terkawal berbanding struktur yang lebih seragam CMC. Penemuan ini menekankan potensi pelbagai aspek BSCH, menggabungkan aktiviti antibakteria, kecekapan penyerapan, dan biodegradasi yang terkawal. BSCH muncul sebagai bahan yang berjanji untuk pelbagai aplikasi, dari penjagaan luka hingga kepada pemulihan alam sekitar.

Kata kunci: selulosa batang pisang hidrogel, selulosa hidrogel, dan karboksimetilselulosa hidrogel

Comparative Investigation on the Optimization of Hydrogel Derived from Cellulose of Banana Stem (*Musa x Paradisiaca*), Cellulose, and Carboxymethylcellulose (CMC) Crosslinked Using Citric Acid

ABSTRACT

This study explores citric acid as a viable crosslinking agent for the development of hydrogels derived from banana stem cellulose, cellulose, and carboxymethylcellulose (CMC). It addresses the environmental impact of banana stem waste, advocating for sustainable cellulose extraction from agricultural waste to combat the pollution resulting from open burning. The alkaline-based extraction method successfully transforms 43% of banana stem powder, with critical considerations for Sodium Hydroxide (NaOH)/ Sodium Hypochloride (NaOCl) concentrations, extraction temperature, and lignin removal duration to optimize cellulose yield. Microscopy-based morphological analysis reveals the fibrous nature of banana stem cellulose, distinguishing it from non-fibrous CMC. Dissolution studies utilizing 8% NaOH and 12% urea highlight the importance of optimal dissolution conditions, impacting hydrogel characteristics, with banana stem cellulose exhibiting a higher fibrous content than cellulose. The concentration of citric acid proves pivotal in the crosslinking process, influencing hydrogel characteristics. Hydrogels formed with 40% citric acid demonstrate efficient crosslinking, a crucial factor for desirable properties. Comparative analyses, employing FTIR Spectroscopy and SEM imaging, differentiate Banana Stem Cellulose Hydrogel (BSCH), Cellulose Hydrogel (CH), and Carboxymethylcellulose Hydrogel (CMCH). FTIR spectra reveal distinct peaks associated with hydroxyl groups, contributing to the hydrophilic nature of the hydrogels. SEM images showcase BSCH's fibrous and porous structure, emphasizing its potential in applications like wound dressings. Antibacterial tests indicate BSCH's enhanced effectiveness against Escherichia coli, attributed to citric acid inclusion. Adsorption studies demonstrate BSCH's superior efficiency in removing methylene blue dye. Biodegradation investigations over four weeks reveal controlled weight loss, with BSCH losing 91%, outperforming CH (89%) and CMCH (100%). The complex and heterogeneous structure of natural banana stem cellulose, with components like hemicellulose and lignin, contributes to a regulated degradation process, distinguishing it from the more uniform structure of CMC. These findings underscore the multifaceted potential of BSCH, combining antibacterial activity, adsorption efficiency, and controlled biodegradability. BSCH emerges as a promising material for diverse applications, ranging from wound care to environmental remediation.

Keywords: Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, Carboxymethylcellulose Hydrogel, Citric Acid

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Hydrogel, also known as colloidal gel, was first invented in 1894 by Van Bemmelen (Aswathy et al., 2020). Hydrogels are a category of crosslinked polymers with the capacity to absorb water owing to their hydrophilic characteristics. When swollen, these materials exhibit moderate to high levels of mechanical, physical, and chemical stability (Wang et al., 2020). Therefore, the proper choice of initial processing methods and materials holds significance in the hydrogel formation process. In addition, hydrogels find diverse applications in wound healing, biosensors, tissue engineering scaffolds, and drug delivery systems. They are also commonly used in agriculture and environmental contexts. That are also particularly suitable for providing plants with water and nutrients due to their exceptional water-holding capacity. Originally used as potting material, they are now used as seed supplements and coatings for slow-release treatments. As soil conditioners, hydrogels significantly enhance water retention, leading to increased soil moisture content and reduced irrigation needs (Palanivelu et al., 2022).

Cellulose, derived from agricultural waste and plants, is a highly desirable natural polymer for hydrogel production due to its abundant availability. It is renewable, biocompatible, non-toxic, and biodegradable, making it environmentally friendly. Banana stems are a rich source of cellulose, providing structural components and imparting rigidity to plant cell walls. This characteristic enables them to maintain their shape and withstand external pressures. Cellulose is a long-chain polymer composed of a basic unit of glucose joined together by glycosidic bonds, as shown in Figure 1.1 below (Billah et al., 2018).

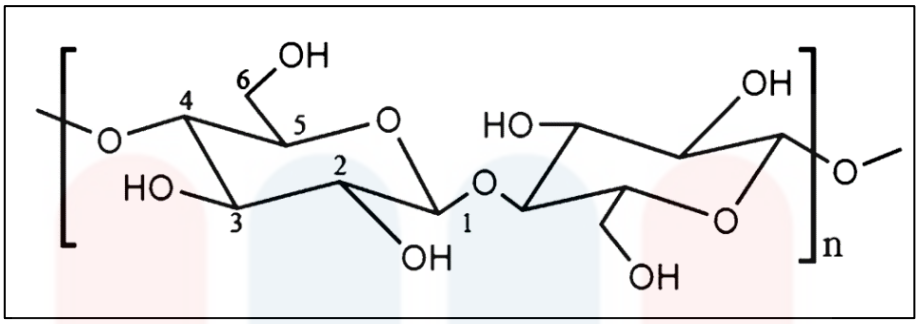

Figure 1.1: Chemical Structure of Cellulose
Source: (Billah et al., 2018)

The cellulose molecule consists of glucose units connected by β-1,4-glycosidic linkages. Each glucose unit has hydroxyl groups (-OH) attached to specific carbons. The glucose units are joined by β-1,4-glycosidic bonds, forming a linear, unbranched chain. The hydroxyl group arrangement alternates, creating a repeating pattern of up and down orientations along the cellulose chain. This unique structure allows for strong hydrogen bonding between adjacent cellulose chains (Harris et al., 2010).

Cellulose extracted from banana stems offers numerous environmental benefits due to its eco-friendly and biodegradable nature, minimizing its impact on the environment. Additionally, banana stem cellulose exhibits compatibility with various polymers and additives, thereby augmenting its adaptability in composite materials. Moreover, in contrast to synthetic counterparts, banana stem cellulose presents a cost-effective solution owing to its widespread availability and straightforward extraction process(Merais et al., 2022). Cellulose-based hydrogels are synthesized by modifying the hydroxyl groups in cellulose, forming a three-dimensional network structure. Functional groups like carboxymethyl are added to customize the hydrogel properties. These modifications enable tailored characteristics in the hydrogels, impacting their interactions with other substances (Seddiqi et al., 2021). Esterification, non-covalent alterations, and oxidation can all be conducted on cellulose's hydroxyl groups. These reactions enable the addition of additional functional groups or chemical bonds, which improve cellulose's characteristics and adaptability in various applications (Ma et al., 2016).

Carboxymethyl cellulose (CMC) is a cellulose-based hydrogel that becomes more water-soluble and hydrophilic when treated with carboxymethyl groups. This change increases its capacity to absorb water and swell. CMC viscosity is affected by molecular weight, degree of substitution, and concentration. Higher substitution degrees result in more excellent solubility and viscosity. CMC is widely utilized in the food,

pharmaceutical, and cosmetic sectors because of its various features, such as solubility, density, and swelling behaviour. It is an appealing material due to its biocompatibility and biodegradability. Hydrogels derived from banana stem carboxymethylcellulose (CMC) can be customized for bone tissue engineering. They mimic the bone's extracellular matrix, providing mechanical support and a favourable environment for cell adhesion, proliferation, and differentiation. The hydrogel properties can be adjusted to imitate the swelling and disintegration rates of natural bone tissue. It is biocompatible and can release bioactive compounds to aid in bone healing (Xue et al., 2022).

Agricultural residues, such as banana stems, sugarcane bagasse, rice straw, and maize stalks, are rich in cellulose and can be collected and processed to produce hydrogels, biodegradable polymers, and composite materials (Tibolla et al., 2018). Among these agricultural residues, the banana stem is distinguished by its high cellulose content, exceeding 50%. This cellulose is predominantly concentrated in the mature stem, rendering the banana stem a valuable and scientifically significant source of cellulose. Recently, there has been an increasing interest in extracting cellulose from banana stems due to its potential applications. Banana cultivation in Malaysia involves vast plantations covering 26,000 hectares, yielding 530,000 metric tonnes annually. The plant's life cycle lasts 10-12 months, and after harvesting the fruit, the tree is cut, leaving the lower stem and rhizome intact. This results in significant waste generation, producing approximately 4 tonnes of waste for every tonne of harvested bananas. The waste consists of various components, including rotten fruit, peels, stems, leaves, pseudo-stems, and rhizomes (Abdullah et al., 2014). Open burning of banana waste materials by local farmers is a common practice, leading to the substantial emission of smoke. This smoke poses substantial risks to both human health and the environment. Extracting cellulose from agricultural waste provides a promising solution to address these challenges by mitigating water and soil pollution associated with the combustion of such waste materials (Romruen et al., 2022).

Citric acid (CA) is used as a chemical crosslinker in the formation of both even cellulose-based hydrogels, banana stem cellulose hydrogels, and carboxymethylcellulose hydrogels since it is a naturally occurring organic acid obtained from citrus fruits (Salihu et al., 2021). Furthermore, CA is a tricarboxylic acid that, due to its ease of availability, low cost, and natural origin, has emerged as a sustainable and eco-friendly crosslinker for the development and production of biopolymeric materials. CA has been used to make a

wide range of materials, including films, composites, hydrogels, and membranes (Dudeja et al., 2023).

1.2 Problem Statement

The ineffective administration of wound dressings leads to excessive waste. Issues such as overuse, premature removal, improper sizing, and incorrect application contribute to unnecessary disposal and replacement of dressings. Improved practices are needed to minimize waste in wound care. Moreover, the variety of patient injuries presents a design problem for wound dressings. If wound dressings that are not adequately sized or chosen for a specific wound may not fit or adhere well, resulting in pain and poor covering. Thus, healthcare professionals may be forced to discard and replace these dressings, resulting in unnecessary waste (Rezvani Ghomi et al., 2019). Moreover, inadequate knowledge or training among healthcare providers regarding proper wound dressing techniques, selection, and usage can lead to preventable waste. An instance of this is when wound dressings encounter contaminants, such as blood, bodily fluids, or non-sterile surfaces, rendering them contaminated and requiring disposal. This situation can arise during dressing changes or because of improper handling practices.

Bananas are predominantly cultivated in tropical regions worldwide. Nonetheless, a substantial portion of banana biomass, approximately 60%, goes to waste following harvest. This amounts to a staggering 114.08 million metric tonnes of discarded bananas globally (Alzate Acevedo et al., 2021). This accumulation of waste exacerbates environmental concerns, particularly the emission of excessive greenhouse gases. The banana industry also generates substantial amounts of by-products and waste, with the banana pseudo stem (BPS) playing a notable role as a contributor. The BPS accounts for around 60.5% of the total mass of the banana plant, making it a significant source of waste within the industry.

Although the primary focus in banana cultivation is on the fruits, it is essential to address the considerable amounts of by-products and waste generated by banana farms (Padam et al., 2014). Disposing of the entire banana plant after fruit production raises environmental concerns (Subagyo & Chafidz, 2018). Removing the entire plant without proper soil management can lead to soil erosion, decreased fertility, and reduced agricultural yields. Banana plants require nutrients, and their removal without replenishing the soil can result in nutrient depletion. This often necessitates the use of

chemical fertilizers, leading to environmental degradation and pollution of groundwater resources. Therefore, sustainable farming practices and organic matter replenishment are crucial to preserve soil fertility and minimize environmental impact. Despite the challenges posed by waste management, these discarded materials possess significant industrial value due to their abundance in cellulose, hemicellulose, and natural fibers. Various methods can be employed to process these components and produce valuable products (Alzate Acevedo et al., 2021).

Hydrogels have substantial toxicity problems for biomedicine and drug administration due to their synthesis reactants, mechanical restrictions, and biocompatibility. Furthermore, it has a deleterious impact on unreacted components such as solvents and monomers, which can harm human health by causing neurotoxicity and carcinogenic effects (Nikolić et al., 2019). The expression of harmful effects on both the central and peripheral nervous systems caused by chemical, biological, or physical factors is referred to as neurotoxicity. It refers to the negative effects that these substances can have on the delicate functioning of the neurological system, which can result in a variety of negative results (Yadav et al., 2019).

1.3 Objectives

The objectives of this study are:

1. To extract cellulose from the banana stem by using alkaline-based extraction method.
2. To formulate hydrogel through crosslinking by employing citric acid as the crosslinking agent.
3. To compare the characteristics between banana stem cellulose hydrogel, cellulose hydrogel, and carboxymethyl cellulose hydrogel.

1.4 Scope of Study

This research aimed to synthesize hydrogel from banana stem cellulose, cellulose, and carboxymethyl cellulose, employing citric acid as the crosslinking agent for either chemical or physical crosslinking. The banana stems utilized in this study were sourced from the Agro Park of the University Malaysia Kelantan Jeli Campus. The stems were subsequently cut into small pieces, dried, and ground into a powdered form. The extracted cellulose from the banana stem was obtained through an alkaline-based extraction

method, using 8% sodium hydroxide (NaOH) to eliminate lignin content. The mixture was stirred on a hot plate for 3.5 hours at 100℃, and the resulting cellulose was dried in an oven at 55℃ until a constant weight was achieved. The cellulose powder underwent a bleaching process with 5% sodium hypochlorite (NaOCl) at 30℃ for 3 hours to achieve a white appearance. After drying again at 55℃ until a constant weight was reached, the banana stem cellulose was stored in a sample container for future use. Additionally, cellulose powder and carboxymethyl cellulose powder were obtained from the lab's chemical store.

Furthermore, the cellulose dissolution process involved using 8% NaOH and 12% urea to dissolve 5% of banana stem cellulose or cellulose. The mixture was stirred in an ice bath at -20℃ for approximately 1 hour, and to enhance dissolution efficiency, the solution was stored in a -20℃ freezer overnight. The following day, the dissolved banana stem cellulose and cellulose were treated with 40% citric acid at 70℃ for 3 hours. The resulting solvent was placed in a 60mm glass petri dish and frozen at -20℃ for 3 hours, followed by thawing at 30℃ for 3 hours. This freeze-thaw cycle was repeated twice to optimize the formation and structure of the hydrogel, which was then stored in a chiller for subsequent analysis.

To fabricate carboxymethyl cellulose hydrogel, 20% carboxymethyl cellulose was added to distilled water to form a CMC paste. This paste was molded into the shape of a 60mm petri dish. Subsequently, the CMC paste was immersed in a citric acid solution with a concentration of 6 mol/L for a predetermined time, resulting in the formation of the CMC hydrogel. To neutralize the pH of the CMC hydrogel and enhance its structure, it was immersed in distilled water for 2 days.

To comparison the hydrogel, by analyse the molecular structure and chemical interactions of the banana stem cellulose hydrogel, cellulose hydrogel and CMC hydrogel, Fourier Transform Infrared (FTIR) spectroscopy plays a vital role. It serves as an essential tool for examining the hydrogel's chemical composition of the hydrogel. FTIR spectroscopy enables the detection of functional groups, monitoring of chemical processes, and confirmation of successful the hydrogel production. Additionally, it allows for the assessment of hydrogel stability and structural integrity by monitoring changes in its infrared spectrum over time. The Scanning Electron Microscope (SEM) has been used for characterizing the microarchitecture of hydrogels, which are hydrophilic and non-conductive materials that pose challenges for imaging. SEM relies on sample

dehydration, including freeze-drying, and requires irreversible carbon or metal coating that could conceal finer surface details.

1.5 Significances of Study

The study investigates at the production of hydrogel from renewable materials, notably banana stem cellulose, cellulose, and carboxymethyl cellulose (CMC). This helps to produce novel and sustainable materials, answering the rising need for environmentally friendly alternatives in a variety of applications. Because banana stems are a prevalent sort of agricultural waste, they bring significant environmental benefits. Repurposing these stems reduces waste and promotes sustainable practices while minimising the environmental impact of traditional disposal methods such as landfills or incineration. This research emphasises the importance of appreciating and reusing previously rejected goods, demonstrating an environmentally friendly and resource-efficient technique.

Furthermore, the study investigates citric acid as a crosslinking agent, with optimisation efforts aimed at improving the hydrogel's water absorption capacity and biocompatibility. The implications of these optimisations hold the prospect of pushing hydrogel performance beyond what is now possible. The comparative dimension included within the study paradigm allows for a thorough deconstruction of hydrogels derived from various sources, including banana stem cellulose, cellulose, and CMC. Unravelling the subtle differences in quality throughout this range of hydrogels provides critical insights into the unique properties of each material, hence defining potential applications.

The present research addresses particular knowledge gaps and provides prospects for advancement in cellulose extraction and the fabrication of hydrogels using banana stems as a key raw material. The findings of this study are critical in addressing an important knowledge gap in the field of biomaterials in addition to various types of hydrogel research, notably by inspecting crosslinking procedures and conducting biodegradability tests, such as biodegradation testing. The study's results are set to provide critical insights and have a considerable influence on our understanding. Lastly, this study adds vital insights into the production, properties, and crosslinking techniques of hydrogels, which have the potential to shape and guide future studies in both biomaterials and hydrogel research of these areas, paving the path for future developments in biomaterials.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Hydrogels are a kind of polymer distinguished by its three-dimensional crosslinked structure. Hydrogels have the capacity to absorb huge amounts of water through sustained bonding to fill space with it while maintaining network structure when swollen (Bashir et al., 2020). Recent research show that hydrogels are used in a variety of industries, including agriculture, biomaterials, the food industry, medication delivery, tissue engineering, and regenerative medicine. Advances in hydrogel synthesis technologies are expected to broaden the field of applicability into new sectors. Despite differences in biodegradability and biocompatibility across hydrogels, these qualities may be fine-tuned by strategically modifying their functional groups or incorporating natural polymers, as demonstrated by the use of cellulose-based hydrogels (Ho et al., 2022).

A hydrogel based on cellulose is created through either physical or chemical cross-linking processes. The hydroxyl groups in cellulose, in combination with modified groups, are critical in promoting cross-linking to generate cellulose-based hydrogels. The formation of these hydrogels' interconnected network structure is tightly linked to their preparation. Mechanical chain entanglements, van der Waals contacts, hydrogen bonding, hydrophobic force aggregations, and electronic connections all contribute to this. Understanding the concepts and methods of hydrogel production is critical for creating biological materials that meet a variety of needs. This section gives a brief summary of hydrogel production methods, including physical and chemical cross-linking as well as interpenetrating networks (Zou et al., 2022).

Banana stems, as a by-product of banana cultivation, represent a significant agricultural waste stream that holds untapped potential (Nascimento et al., 2021). Because of its availability, renewability, and prospective uses, banana stem cellulose is a potentially sustainable raw material. The banana pseudo-stem, which is frequently thrown as trash, has a high cellulose content, ranging from 32.4 to 64.0 wt% (Nascimento et al.,

2023). Cellulose extraction from banana stem can be achieved through different methods, including chemical, mechanical, and environmentally friendly techniques. This extraction process offers an opportunity to obtain cellulose (Rahman et al., 2021).

Furthermore, Carboxymethyl cellulose (CMC) is a cellulose derivative that is an anionic polymer compound with a molecular weight of thousands and a white fibrous powder that is odourless, tasteless, and hygroscopic. CMC disperses in water to produce a clear colloidal solution, known as CMC gel. CMC hydrogels have piqued the interest of researchers due to their high biodegradability, biocompatibility, and low immunogenicity. CMC crosslinked hydrogels are very absorbent and have good physical and dynamic viscoelastic characteristics (W. Zhang et al., 2022).

Citric acid has gained popularity as a crosslinking agent for polysaccharides in recent years due to its ability to improve mechanical characteristics. Citric acid has FDA certification as a non-toxic organic weak tricarboxylic acid. It acts as a natural acid, forming covalent intermolecular di-ester linkages with hydroxyl groups inside polysaccharide structures, enabling cross-linking. This unique cross-linking method contributes to increased elasticity and mechanical strength, which is especially noticeable in the development of cellulose and CMC properties (Sharmin et al., 2022).

This review of the literature on hydrogels made from cellulose sources, such as banana stem cellulose, cellulose, and carboxymethyl cellulose (CMC), provides a thorough examination of optimisation methods and a direct comparison of their characteristics. While studies on individual cellulose sources exist, there is a research void that comprehensively explores the optimisation of hydrogels from different cellulose origins and assesses their respective qualities. The overarching goals of this research are as follows: first, to implement an alkaline-based extraction method for obtaining cellulose from banana stem; second, to develop hydrogels through chemical crosslinking, using citric acid as the crosslinking agent; third, to investigate the degradation behaviour of the resulting hydrogels; and finally, to characterise banana stem cellulose, cellulose, and carboxymethyl cellulose comprehensively. These goals aim to fill a gap in the literature by giving significant insights into the optimised synthesis and comparative features of hydrogels obtained from various cellulose sources.

2.2 Cellulose Extraction from Banana Stem

Cellulose, a polysaccharide found in nature, is abundant and consists of interconnected glucose units in a linear polymer structure. The extraction of cellulose from agricultural waste for sustainable products has gained attention. Its condensation polymer properties allow it to be broken down into glucose molecules. Cellulose and its derivatives have strong hydrogen bonding and rigid structures, resulting in high glass transition temperatures and melting points. The organized crystalline regions make cellulose insoluble in water, requiring potent alkalis for breakdown. Cellulose is readily available in agricultural waste and various plants as a component of plant cell walls (Seddiqi et al., 2021). To enhance the value and broaden the range of applications for cellulose, different chemical treatments and functionalization techniques have been employed to create a variety of cellulose derivatives. These derivatives have found extensive use in the biomedical industries.

2.2.1 Banana Stem as a Sustainable Source of Cellulose

Banana stems, known as pseudo stems, exhibit a complex composition and structure. Comprised of concentric layers, the outermost layer consists of fibrous sheaths that provide protection and support. Beneath the sheaths lies the epidermis, a thin layer that safeguards the underlying tissues (Ali & Mattsson, 2019). The critical component of the banana stem is its cellulose content. Cellulose, a complex carbohydrate, and the primary structural component of plant cell walls is abundant in the banana stem. It typically constitutes more than 50% of the total composition of the stem. This high cellulose content makes the banana stem an attractive and sustainable source of cellulose for various applications (Van et al., 2022).

Furthermore, Banana stem as an alternate source of pulp and paper has shown significant potential. Because of its high cellulose content, it was suitable for applications such as dissolving pulp, bio-composites, and industrial biofuel production. Compared to traditional wood sources, cellulose extraction from non-wood sources, such as banana stems, needed less chemical and energy use. This is primarily due to the reduced lignin percentage of non-wood sources, whereas wood generally comprised 40-50% cellulose and 18-35% lignin. A comparable degree of delignification in non-wood sources was

achieved with a lower chemical charge than was required for wood sources (Mohamad & Jai, 2022).

2.2.2 Methods for Cellulose Extraction

Various chemical treatments have been extensively explored for extracting cellulose from agricultural waste fibers, considering their potential impact on the crystalline structure of cellulose. These methods include chlorine bleaching, alkali treatment, and acid hydrolysis (Abdullah et al., 2021).

Alkaline treatment, also known as mercerization, is the process of immersing natural fibres in a sodium hydroxide (NaOH) solution to remove lignin, hemicellulose, wax, and oils, therefore enhancing the interface between the matrix and fibres for improved adhesion. Suboptimal treatment settings might result in problems such as fibre defibrillation and embrittlement. By removing less dense noncellulosic components, this procedure enhances fibre density. Fibres become yellowish after mercerization, separate, and vary in surface area, crystallinity, unit cell structure, and orientation. Cellulose's increased crystalline cellulose concentration strengthens fibres. By allowing charge transfer between the matrix and the fibre, alkaline-treated fibres increase composite mechanical characteristics. By eliminating leftover hydroxyl, neutralisation with acetic acid after washing assures that the process is permanently stopped (Ouarhim et al., 2019).

In fact, cellulose extraction comprises a number of methodical procedures, beginning with alkali treatment with NaOH, then bleaching, and finally acid hydrolysis. The alkali treatment is methodically carried out using a NaOH solution at reflux temperature, and the resulting solid is thoroughly washed to remove any remaining alkali residues. Following that, a thorough bleaching procedure employs sodium chlorite, acetate buffer, and water, which is repeated until the product acquires an exquisite white look. The last phase comprises acid hydrolysis, which is a critical step in the production of nanocrystalline cellulose. This methodical approach emphasises the significant potential for converting coffee husk waste into a profitable resource via a scientifically rigorous and properly conducted procedure (Chopra, 2022).

2.2.3 Chemical Modification of Extracted Cellulose

Cellulose extracted from banana stems can undergo various chemical modifications to enhance its properties and expand its applicability in different industries. One such modification involves the synthesis of cellulose ethers through a heterogeneous reaction. In this process, purified cellulose undergoes a nucleophilic substitution reaction at the interface between the solid cellulose and the liquid phase, facilitated by a base and an inert diluent. Alkylating reagents react with the hydroxyl groups of cellulose, transforming them into cellulose ethers (Shokri & Adibkia, 2013).

One of the cellulose derivatives of particular interest is carboxymethyl cellulose (CMC), which features carboxymethyl groups attached to its cellulose backbone. This unique molecular structure grants CMC exceptional suitability for the formulation of hydrogels, thereby bestowing favorable properties upon the resultant materials. The widespread recognition and utilization of CMC extend to various applications, including tissue engineering, drug delivery, wound dressing, and plant breeding. CMC's versatility and advantageous characteristics make it a highly valuable component in these fields, offering numerous benefits and driving advancements across diverse industries (Capanema et al., 2018).

2.3 Cellulose-Based Hydrogel

In the field of biomedical engineering, there has been a rising emphasis on cellulose-based hydrogels in recent years. These hydrogels have gotten a lot of interest because of their unique features including flexibility, reactivity to stimuli, biocompatibility, and degradability. They have great development potential since they have excellent mechanical characteristics and may be customized to particular tasks using various preparation procedures (Zou et al., 2022). Cellulose-based hydrogels are being studied intensively for a variety of applications, including medicinal applications, agricultural operations, smart materials, and other industrial uses. Their capacity to absorb and retain a considerable quantity of water, as well as respond to a variety of stimuli, makes them highly intriguing for biological applications (Guan et al., 2021).

2.4 Carboxymethylcellulose (CMC) Hydrogels

2.4.1 Overview of CMC Hydrogels

Carboxymethyl cellulose (CMC), known for its remarkable properties, demonstrates characteristics similar to those of superabsorbent hydrogels, enabling the absorption of substantial amounts of water. CMC surpasses the absorption capacity of conventional hydrogels by an impressive margin, exceeding 100 to 1000 times its own dry weight. This exceptional water absorption capability results in a significant increase in the water content encapsulated within the hydrogel matrix (Fekete et al., 2017).Carboxymethyl cellulose (CMC) stands as a notable cellulose derivative distinguished by the incorporation of a significant number of carboxymethyl groups that are covalently attached to the cellulose backbone. Its remarkable properties, including non-toxicity, water solubility, cost-effectiveness, and environmental friendliness, have propelled CMC to the forefront of hydrogel research and development. The widespread utilization of CMC in the realm of hydrogels can be attributed to its versatility and compatibility with various applications (Zheng et al., 2015).

2.4.2 Synthesis Methods for CMC Hydrogels

The synthesis of carboxymethyl cellulose (CMC) hydrogels involves the process of crosslinking, which refers to chemically or physically joining polymer chains to form a three-dimensional network structure. This crosslinking process is essential for imparting unique properties to the hydrogel, such as mechanical strength, stability, and controlled swelling behavior. Crosslinking agents can be incorporated during or after the hydrogel production to facilitate the crosslinking process and modify the hydrogel's characteristics for specific applications. By adjusting the type and concentration of crosslinking agents, as well as the crosslinking method employed, the mechanical and physical properties of the hydrogel can be tailored to meet specific requirements (Maitra & Shukla, 2014).

2.4.2 (a) Physical Crosslinking

Physically crosslinked hydrogels are formed through various interactions, including ionic and temperature effects, making them appealing for bone tissue engineering. Ionic interactions play a crucial role in the physical crosslinking process. Oppositely charged ions attract and interact with each other, forming crosslinks. The electrostatic interactions between ionizable groups in polymers or hydrogel precursors, such as carboxylate or amine groups, result in a three-dimensional network structure. This method of physical crosslinking offers the advantage of designing hydrogels without the need for toxic or potentially hazardous chemical crosslinkers (Bashir et al., 2020).

Cross-Linking		Advantages	Disadvantages
Physical	Self-assembly	Reversible mechanism [A] [52] Compatibility with biological systems [B] [3] Shear-thinning [2] Self-healing [2]	Additional post-cross-linking [C] [42] Poor mechanical properties [D] [52] Prolonged self-healing [2]
	Ionic interactions	[A,B] [3,52] Working under mild conditions [89]	[C,D] [42,52] Exhaustive of the number of ions [42]
	Thermal cross-linking	[A,B] [3,52] Rapid reassembly to hydrogel [2] Work under physiological conditions [89]	[C] [42] Precise temperature for cell viability [2]
	Stereo-complexation	[A,B] [3,52]	[C,D] [42,52]

Figure 2.1: Advantages and Disadvantages of Physical Crosslinking.
(Source: Naranjo-Alcazar et al., 2023)

Physical crosslinking offers several advantages and disadvantages. Firstly, it allows for reversibility, enabling the material to respond dynamically to external factors like temperature, pH, or mechanical stress, enhancing its adaptability. Secondly, physical crosslinking methods involve simple and moderate processing conditions, such as cooling or drying, without the need for complex chemical reactions or harsh environments. This simplicity facilitates ease of use. Thirdly, physical crosslinking can be performed under mild conditions, minimizing potential harm to delicate biological materials, making it suitable for biological applications. Lastly, the physical interactions responsible for crosslinking can be precisely adjusted, providing control over the material's mechanical properties, swelling behavior, and degradation kinetics (Naranjo-Alcazar et al., 2023).

2.4.2 (b) Chemical Crosslinking

The chemical crosslinking process typically involves using crosslinking agents, which are molecules capable of forming covalent bonds with the polymer chains. These compounds can be added or administered after the hydrogel's creation. When the

crosslinking agent is injected, it interacts with the polymer chains' functional groups, such as hydroxyl, amine, or carboxyl groups. This process creates a network topology by forming new chemical connections between the polymer chains. The concentration of the crosslinking agent can be adjusted to manage the crosslinking density or the number of crosslinks per unit volume (Parhi, 2017).

Chemical crosslinking offers advantages such as increased mechanical strength, stability, and control over degradation rate. It allows customization of material properties using different crosslinking agents. However, it has drawbacks. Complex reactions and harsh processing conditions can be harmful to sensitive materials and biological systems. Once crosslinked, the network becomes irreversible, limiting responsiveness to external stimuli and structural changes. Certain crosslinking agents may also pose biocompatibility challenges (Naranjo-Alcazar et al., 2023).

2.5 Properties and Characterization Techniques

2.5.1 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is a technique used to analyze molecular composition and structure by measuring the absorption of infrared light. In this study, FTIR is employed to investigate the chemical changes that occur in CMC during oxidation. Oxidation introduces functional groups, such as carboxyl groups, into the CMC structure, altering its characteristics for specific applications. By analyzing FTIR spectra, researchers can detect new peaks or absorption differences, indicating the integration of carboxyl groups and assessing the degree of oxidation. Additionally, FTIR can reveal changes in the hydrogen bonding network and molecular conformation of CMC (Anjali, 2012).

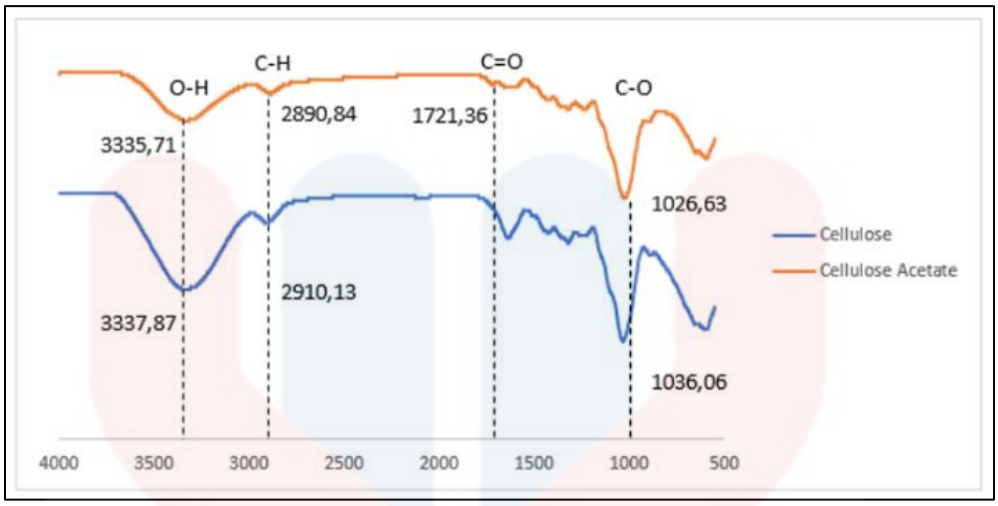

Figure 2.2: Graph of FTIR Cellulose and Cellulose Acetate EFB Analysis.
(Source: Tristantini & Yunan, 2018)

Figure 2.2 above shows an FTIR comparison of EFB cellulose and EFB cellulose acetate according (Tristantini & Yunan, 2018). There are absorption peaks for EFB cellulose at 3337 cm-1 (O-H), 1035 cm-1 (C-O), and 2910 cm-1 (C-H), but no C=O peaks. Four functional groups may be found in EFB cellulose acetate. The O-H signal at 3335 cm-1 indicates that acetylation was effective. The study's scope exceeds prior studies and is consistent with comparable findings. A distinct C=O signal at 1721 cm-1 indicates the presence of EFB cellulose acetate. This implies that the acetyl group replaces O-H, resulting in C=O. The company of these functional groups verifies the practical synthesis of EFB cellulose acetate.

2.5.2 Antibacterial Test

The use of antibiotics as the primary choice for antibacterial therapy has been effective in antibacterial therapy throughout the last few decades. In general, oral or injectable antibiotics are the most effective frontline therapies for fighting infections. Nonetheless, despite systemic treatment techniques, antibiotic use control creates problems, with potentially negative implications such as the development of bacterial resistance. The porous design of the hydrogel serves as a conduit for antibacterial agent loading. This allows for tailored antibiotic distribution precisely to the site of illness, decreasing cases of drug mishandling. This method optimises antibiotic use while maintaining a high level of biocompatibility (Tang et al., 2023).

To determine the effectiveness from different bacterial strains and to identify the most appropriate antibiotic for treating a bacterial infection chloramphenicol is used for the antibacterial test. Chloramphenicol is a broad-spectrum antibiotic that has been tested for antibacterial activity against a variety of bacteria including Escherichia coli and Staphylococcus aureus. Chloramphenicol, which is bacteriostatic, has bactericidal characteristics at high doses. Its medicinal uses include the treatment of a wide range of illnesses, including superficial eye infections, otitis externa, rickettsia disorders, and meningitis. Chloramphenicol's increased lipid solubility promotes rapid absorption in antibacterial testing, making it well-suited for integration into such tests (Singhal et al., 2023). Furthermore, due to variations in their processes, chloramphenicol has been shown to be the most effective antibiotic for Escherichia coli when compared to erythromycin (Akinyemi, 2020).

Evaluating the efficiency of antibacterial surfaces requires the use of several standardised procedures. The zone of inhibition test is a popular technique in which the substance is carefully placed near bacterial colonies. The absence of bacterial growth within the clear zone is visible evidence of its ability to inhibit proliferation. In addition, bacterial reduction assays measure the decrease in viable bacteria after contact with the antibacterial surface, providing a quantitative insight into its ability to reduce bacterial load. The Minimum Inhibitory Concentration (MIC) test examines the concentration of a drug required to inhibit bacterial growth, illuminating the material's intrinsic potency(Cunliffe et al., 2021). Furthermore, the agar disc diffusion test is a flexible, cost-effective, and highly reliable method for determining antifungal sensitivity to diverse fungal and yeast strains. This approach, which was developed in 1940, has withstood the test of time and acquired widespread recognition in clinical microbiology, obtaining certification from the Clinical and Laboratory Standards Institute (CLSI) and being well-standardized. When examining antifungal efficacy against Candida albicans, using the agar disc diffusion test with a fluconazole disc gives very excellent findings, making it a popular alternative for in-depth investigations in this context (Prusty, 2022).

2.5.3 Adsorption

The adsorption test for hydrogels evaluates their ability to remove compounds from wastewater such as dyes. A super-adsorbent hydrogel based on sodium styrene

sulfonate (NaSS) monomer was created in a recent study for the removal of dyes such as methylene blue (MB). The hydrogel, which was created by solution free radical polymerization, has a highly porous, three-dimensionally crosslinked polymer network, resulting in a swelling ratio of up to 27,500% (Salunkhe & Schuman, 2021). Therefore, hydrogel adsorption test is critical for determining their potential in wastewater treatment and environmental remediation. Researchers can create effective and sustainable methods for removing pollutants from water systems by better understanding the adsorption processes and capacity of hydrogels (Zhu et al., 2023).

2.5.4 Biodegradation Test

Biodegradation test for hydrogels is to evaluate the rate and extent of degradation of the hydrogel material under specific environmental conditions. Biodegradation is the breakdown of organic materials by microorganisms such as bacteria and fungus. When bacteria consume an organic substance as a source of carbon and energy, it results in material disintegration. This natural process is important for the ecosystem because it reduces the environmental effect of products and allows organic materials to degrade naturally without harming the environment (Poznyak et al., 2018).

2.6 Application

CMC hydrogels have extensive applications across multiple fields, including biomedical engineering, medication delivery, wound healing, tissue engineering, and environmental science. They possess desirable properties such as water absorption, biocompatibility, and adjustable gelation characteristics, making them suitable for diverse uses. In biomedical engineering, they serve as drug carriers, wound dressings, and tissue regeneration scaffolds. In environmental science, they contribute to water treatment, soil enhancement, and controlled pesticide release. The versatility and advantageous features of CMC hydrogels make them appealing for a wide range of industrial and biological applications (W. Zhang et al., 2022).

2.6.1 Wound Healing

The use of biomaterials and hydrogels in wound healing applications. It investigates the creation of scaffolds that facilitate wound healing and tissue regeneration utilizing diverse materials such as chitosan, alginate, and gelatine. Biocompatibility, controlled release of bioactive compounds, and the capacity to imitate the extracellular matrix are all desired qualities of these hydrogels. To improve wound healing characteristics, the study emphasizes the need to select suitable biomaterials and optimizing optimize the hydrogels' composition and structure (Kanikireddy et al., 2020).

2.6.2 Bone Tissue Engineering

The hybrid hydrogel is created by incorporating hydroxyapatite, a biocompatible and bioactive mineral that matches the composition of natural bone, into a carboxymethyl cellulose matrix. This material combination is expected to increase the hydrogel's mechanical characteristics, bioactivity, and biocompatibility for applications in bone tissue engineering. The carboxymethyl cellulose-hydroxyapatite hybrid hydrogel is synthesized and characterized by analyzing its physical, chemical, and mechanical properties. In addition, the hydrogel's ability to promote cell adhesion, proliferation, and differentiation is investigated to assess its potential for promoting bone regeneration (Pasqui et al., 2014).

2.6.3 Drug Delivery Systems

CMC hydrogels crosslinked with calcium chloride provide diverse drug delivery methods for various applications. They can provide medications via several routes, including oral administration, local implantation, and topical treatment, emphasizing two-dimensional hydrogel systems. Controlled release features of CMC hydrogel drug delivery systems for oral administration improve medication absorption and bioavailability. Local implantation allows for focused and prolonged drug release, which is advantageous for localized therapeutic applications. Topical hydrogel drug delivery methods, especially two-dimensional ones, attach to the skin or mucosal surfaces, allowing for delayed and prolonged drug release. They are used in dermatology for wound healing, transdermal medicine administration, and treating skin ailments.

These cross-linked CMC hydrogel systems provide flexible drug delivery with controlled release, which improves therapeutic results (Vigata et al., 2020).

2.7 Conclusions

In conclusion, this study thoroughly investigates the synthesis, characteristics, and uses of hydrogels produced from cellulose sources, with a particular emphasis on banana stem cellulose, cellulose, and carboxymethyl cellulose (CMC). The ability of hydrogels to absorb water, along with their distinct structural properties, positions them as flexible materials with uses in agriculture, biomaterials, food, pharmaceutical administration, tissue engineering, and regenerative medicine. Notably, the study highlights the need of optimising hydrogel synthesis from various cellulose sources and undertaking a comparison investigation of their relative properties, therefore filling a significant vacuum in present research.

The following parts construct into cellulose-based hydrogels and CMC hydrogels in particular, offering an overview of synthesis methods, properties, and characterization methodologies. The contrast between physical and chemical crosslinking techniques is investigated, with a thorough examination of their benefits and drawbacks. Furthermore, the review of the literature looks at important characterisation techniques like as Fourier Transform Infrared (FTIR) Spectroscopy, antimicrobial testing, adsorption tests, and biodegradation tests, emphasising their importance in assessing hydrogel characteristics and performance.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials that were used in this study are Banana Stems from the species *Musa x paradisiaca*, which was harvested from the Jeli region of University Malaysia Kelantan (UMK). 5 kg of banana stems will be used as the primary raw material for the project. The stems will be selected and harvested from local banana plants in the Argo Park UMK Jeli.

3.1.1 Chemical

In this study, the chemicals that was used are Sodium Hydroxide (NaOH), Sodium Hypochlorite (NaOCl), Cellulose, Carboxymethyl Cellulose (CMC), Urea, Citric Acid (CA), These chemicals were employed for various purposes and processes throughout the study.

3.2 Methods for formation of Banana Stem Cellulose and Cellulose-Based Hydrogel

3.2.1 Collect and Prepare Banana Stem

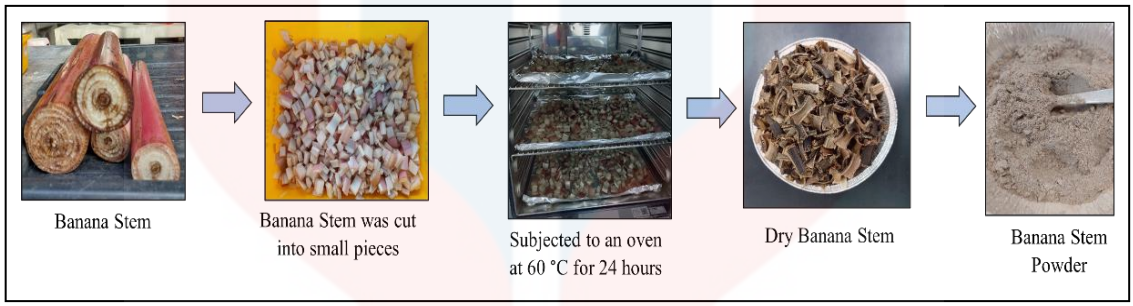

Figure 3.1: Procedural for the preparation of Banana Stem Powder.

The Banana Stem was collected from the Banana Plantation situated at Agro Park in Jeli, UMK. Upon collection, the Banana Stem was thoroughly rinsed with water, followed by cutting it into small slices. Subsequently, the sliced stems were placed in an oven and dried at 60 °C for a period of 24 hours. After thorough drying, the Banana Stem underwent processing in a blender to achieve a 250-mesh size, resulting in a powdered form. This powder was stored in a zipper bag until it was needed for future use (Mulyatno et al., 2017).

3.2.2 Extraction of Cellulose from Banana Stem

The research article titled “Synthesis and Characterization of Sodium Carboxymethylcellulose from Cavendish Banana Pseudo Stem (Musa Cavendishii LAMBERT)” described a detailed extraction method for obtaining cellulose from banana stem. The method involved a series of steps aimed at achieving high purity and desired properties of the extracted cellulose. The article describes the method for treating cellulose powder with 8% NaOH at a cellulose-to-solvent ratio of 1:20 (w/v). The result is that the mixture is heated to 100°C for 3.5 hours, producing a black sludge. The slurry is then filtered and rinsed with distilled water. It was then bleached with 5% NaOCl for 3 hours at 30°C. The bleached cellulose is extensively washed with distilled water until the

remaining hypochlorite odour is eliminated. Finally, the treated cellulose is dried at 60 °C in a cabinet drier to obtain the purify cellulose. (Adinugraha & Marseno, 2005).

3.2.2 (a) Lignin Removal

To initiate the process, 25 grams of Banana Stem powder were introduced into a 1000 ml beaker along with 500 ml of 40 grams of NaOH with a concentration of 8%. The mixture was subjected to heating by using hot plate at 100°C for a duration of 3.5 hours. The purpose of this step was to eliminate the lignin content present in the Banana Stem. Through this thermal treatment, the non-cellulosic components broke down and dissolved in the solution, leaving behind purified cellulose.

3.2.2 (c) Bleaching Process

Next, in order to eliminate any remaining lignin and impurities and achieve a white appearance, the washed cellulose powder was subjected to bleaching. According to the findings presented in the study by Aridi et al. and Brahma & Ray, it was determined that the utilization of a 5% NaOCl solution proved to be the most effective bleaching agent for the removal of lignin and hemicellulose from the fiber (Aridi et al., 2020) (Brahma & Ray, 2024). The selection of NaOCL instead of other bleach chemicals is because NaOCl is an effective oxidising agent capable of had effectively oxidising and degrading lignin, a complex organic polymer responsible for plant cell wall colour and structural support. It might additionally interact with hemicellulose, another component of plant cell walls, eliminating it (Aridi et al., 2021). This involved placing the cellulose powder in a 1000 ml beaker along with 500 ml of 5% NaOCl solution and subjecting it to the hot plate and stir at 30°C for 3 hours. After the bleaching process, the cellulose was washed again with distilled water and filtered using vacuum filtration to separate the cellulose powder, which was retained on the filter paper. The purpose of this method was to further purify the washed cellulose powder by removing any remaining lignin and impurities and achieving a white appearance.

3.2.3 Dissolve Banana Stem cellulose and cellulose

The procedures for dissolving Banana Stem Cellulose and Cellulose were identical. This involved dissolving 0.8 grams of NaOH and 1.2 grams of urea in distilled water and adjusting the solution volume by adding distilled water until the total weight

reached 10 ml. Following the complete dissolution of NaOH and urea in distilled water, 0.5 grams of Banana Stem Cellulose or Cellulose was dissolved by agitating the viscous solution vigorously in an ice bath for approximately 1 hour. The objective of combining NaOH and urea was to formulate a solvent suitable for cellulose dissolution. The synergistic action of NaOH and urea facilitated the breakdown of the cellulose structure, rendering the cellulose or banana stem cellulose water-soluble (Golor et al., 2020).

3.2.4 Forming of Banana Stem Cellulose or Cellulose-Based Hydrogel using Citric Acid

When the Banana Stem Cellulose or Cellulose had been completely dissolved in the solvent. Then, the different amount of CA (10,20,30 and 40%) (Banana Stem Cellulose/ CA 40) or (Cellulose/ CA 40) percent by mass (m/m) was then loaded into the dissolved pulp and then stirred with magnetic stirrer for 18 minutes at 70°C for 10ml of solution. Additionally, the stirring rate during the hydrogel formation process plays a crucial role in influencing the final characteristics. A higher stirring rate can lead to accelerated solvent evaporation, potentially impacting the ultimate texture of the hydrogel. Conversely, a slower stirring rate may result in a more gradual solvent evaporation, affecting the successful formation of the hydrogel due to extended evaporation times. The meticulous control of stirring rates is therefore imperative in ensuring optimal hydrogel formation and desired characteristics. Citric acid was incorporated into banana stem cellulose or cellulose solution to affect the characteristics, and CA acted as a crosslinker for the creation of hydrogel. After that, the mixture went through two freeze-thaw cycles to produce the hydrogel. The cycle was freezing at -20°C for 3 hours on freezer and thawing at room temperature for 3 hours. The purpose of freeze-thawing was to promote gelation and cross-linking in the banana stem or cellulose solution, resulting in the formation of a hydrogel structure (Golor et al., 2020).

3.3 Methods for the formation of Carboxymethylcellulose (CMC) Hydrogels

3.3.1 Forming of CMC Hydrogels using Citric Acid

The CMC powder was admixed with distilled water containing a 20% CMC content in the initial phase, which culminated in a CMC paste that was molded into a predetermined shape after being placed in a mould and subjected to pressure to eliminate entrapped air, although the polymer chains of CMC remained uncross-linked at this point. Following that, the moulded CMC paste was immersed in a citric acid solution with concentrations ranging from 6 mol/L to saturation for approximately 12 hours. This immersion time aided in the creation of a CMC hydrogel. The immersion technique caused physical cross-linking within the CMC paste while also encouraging the formation of hydrogen bonds between the polymer chains, contributing to the formation of the CMC hydrogel. In the last stage, the produced CMC hydrogel was immersed in distilled water for two days. This equilibration step in distilled water was critical in stabilising and optimising the CMC hydrogel's characteristics. The hydrogel reached a state of equilibrium in terms of structural and distinctive properties at this stage (Zheng et al., 2015).

3.4 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

Fourier Transform Infrared (FTIR) Spectroscopy was used to systematically discern and juxtapose the distinct features inherent in the infrared spectra, with a particular emphasis on the characteristic peaks corresponding to the various functional groups inherent in banana stem cellulose powder, cellulose powder, and carboxymethyl cellulose powder. Furthermore, to investigate minor modifications in the FTIR spectra, specifically shifts in certain peaks caused by the crosslinking process triggered by citric acid inside the hydrogel matrices. The goal of this comprehensive method was to uncover subtle molecular changes and structural variations associated with cellulose and its derivatives, revealing information on the delicate interplay of chemical components during hydrogel formation and crosslinking.

3.5 Antibacterial Culture Preparation and Isolation Methods

3.5.1 Nutrient Agar Formulation and Sterilization

To initiate the process, the culture media, specifically nutrient agar, was prepared by dissolving 28g of nutrient agar in 1 liter of distilled water. In order to foster microbial development, this phase aimed to generate a well-mixed, nutrient-enriched medium. The prepared medium had been sterilized in an autoclave set to 121°C and 15 psi of pressure for 15 minutes. Here, the main goal was to remove all possible pollutants in order to create a sterile environment that would support good microbial development.

After that, the nutrient agar medium was aseptically transferred into sterilised Petri plates inside a laminar flow hood after sterilisation. This painstaking procedure was designed to preserve the media's sterility and reduce the possibility of contaminants entering during the transfer procedure. The Petri plates were allowed to cool down to ambient temperature for 24 hours following their solidification. The purpose of this incubation phase was to further stabilize the nutrient agar, enabling it to set and fostering a microbiological growth environment. The idea was to create a regulated atmosphere that would encourage the growth of microbes (Bakht et al., 2011).

3.5.2 Aseptic Transfer and Incubation, and Single Colony Isolation

The technique employed for isolating single colonies, known as streaking, for Escherichia coli (E. coli), is conducted to facilitate the isolation of individual bacterial colonies. While this method proves efficacious in the isolation of single colonies, the purpose of quantifying the total cell count within a culture is not served by it. During the operation, an inoculum of bacteria is streaked across one side of an agar plate using an inoculating loop. Following the first streak, a sterilized loop is inserted through the initial streak, and then a new section of the plate is streaked. In order to enhance the isolation of individual colonies, this sequential procedure is repeated at least once more. (Elbing & Brent, 2019). The streaked agar plate is then placed in an incubator set at a temperature of 37°C overnight. This temperature corresponds to the recognized optimum growth temperature for E. coli, which is around 37°C. The purpose of maintaining this temperature during incubation is to precisely simulate the bacterium's natural

developmental conditions. At 37°C, E. coli experiences accelerated growth, enabling rapid multiplication of bacterial cells within a short incubation period. This rapid growth is crucial for generating a substantial bacterial population necessary for subsequent experiments.

3.5.3 Nutrient Broth Preparation and Sterilization

Subsequently, a nutrient broth comprising 1.3g dissolved in 100ml of distilled water was meticulously prepared in conical flasks. Approximately 20 ml of the nutrient broth per test tube was dispensed. The media underwent sterilization in an autoclave at 15 psi and 121°C for 15 minutes. Following autoclaving, the nutrient broth was allowed to cool to room temperature. A singular colony, potentially housing a pure E. coli culture, was judiciously chosen from a pre-established petri dish. Employing aseptic techniques, the selected colony was then inoculated into the test tube containing the nutrient broth, instigating the culture with a controlled introduction of bacterial cells into the liquid medium. The inoculated test tube was subsequently placed in an incubator shaker, adjusted to the optimal growth temperature for E. coli, typically around 37°C. The culture underwent incubation for a predetermined duration, during which bacterial cells underwent multiplication, resulting in an increase in culture density (Bakht et al., 2011).

3.5.4 Disc Diffusion Methods for Antibacterial Test

In this procedure, nutrient agar media plates were inoculated with 18 to 24 hour cultures of microbial inoculums. Positive control filter paper discs, measuring 3 mm in diameter, were prepared. Antibiotic discs containing Chloramphenicol (3.5 mg each) were aseptically placed on the left side of the media using sterile forceps to serve as the positive control. Subsequently, three types of hydrogels (Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, and Carboxymethylcellulose Hydrogel) were applied to the discs. The media plates were incubated at 37°C for 18 to 24 hours in an incubator. During incubation, the chemicals diffused from the discs into the agar media, inhibiting the growth of E. coli in the surrounding area, known as the zone of inhibition. On the subsequent day, the zones of inhibition resulting from each treatment were calculated and subjected to analysis (Bakht et al., 2011).

3.6 Biodegradation Test

To examine the breakdown of three different hydrogel samples (Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, and Carboxymethyl Cellulose Hydrogel), several characteristics such as weight loss, morphological changes, and microbial activity were monitored. Placed strategically in separate pots holding 100 grams of soil, all different types of hydrogels began to degrade at the same time. After a 7-day interval, exact weight measurements of the hydrogel samples were taken. Subsequent observations were made on the 14th, 21st, and 28th days after planting to evaluate the ongoing biodegradative process. The precise selection of time intervals in our research is critical since it allows for the identification of differences in the deterioration rate, whether accelerated or decelerated. Additionally, these precise time intervals are also consistent with the necessity to capture early, middle, and advanced phases of degradation, allowing for a thorough study of the materials' reactions to microbial activity and environmental circumstances across time.

3.7 Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) is the most widely used method for studying the microarchitecture of hydrogel. This sophisticated imaging approach provides a detailed representation of nanoscale characteristics on the surface of hydrogels. As the name implies, SEM works on the basis of electron interaction, in which a high-energy beam guides primary electrons towards a metal- or carbon-coated material. This mechanism causes the emission of secondary and backscattered electrons. Secondary electrons emphasise the specimen's shape and topography, whilst backscattered electrons help to discern regions with different chemical compositions. To avoid gas interference, SEM imaging requires a high vacuum condition. SEM microphotographs of hydrogels are extremely useful in identifying important properties such as pore size, pore distribution, and porosity %. In addition, this approach offers information on factors such as fibre thickness and orientation. Before SEM observation, the three types of hydrogel samples had to be lyophilized. The samples (2 mm × 2 mm × 2 mm) were frozen at -20°C for three hours before being freeze-dried for 24 hours at -90°C. Following this, the dried samples were secured to an aluminium specimen holder using conductive adhesive tape.

These prepared samples were then placed into the SEM microscope and evaluated under high vacuum conditions, using a secondary electron detector at a voltage of 10 kV. During SEM analysis, the magnification circumstances for banana stem cellulose, cellulose, and CMC were set at 500x and 750x, respectively. In contrast, the examination of hydrogels generated from banana stem cellulose, cellulose, and CMC used magnifications of 500x, 1000x, 1500x, and 2000x. The selection of these several magnification levels is critical for a thorough evaluation of the hydrogel's characteristics. Lower magnifications, such as 500x and 750x, offer a broad view of surface morphology, but higher magnifications, ranging from 1000x to 2000x, provide finer details that help in the identification of microstructural characteristics. The ability to examine surface features, porosity, and any structural anomalies in hydrogels allows for a more in-depth knowledge of their properties and prospective uses.

3.8 Adsorption of Methylene Blue Dye Test

The adsorption isotherm was carried out by preparing a series of bottles, each holding 0.05 L of methylene blue at different concentrations. Standard curve values ranged from 2, 4, 6, 8, 10 mg/L, whereas hydrogel adsorption experiments used concentrations of 25, 50, 75, 100, 125, and 200 mg/L. Methylene blue was diluted from a concentrated dye stock solution of 500 mg/L. Each concentration was then divided into three sets, with 5ml of each put to a 10ml volumetric flask. Three varieties of hydrogel, Banana Stem Cellulose, Cellulose Hydrogel, and Carboxymethylcellulose Hydrogel were freeze-dried at -20 °C for 24 hours. Next, the dry hydrogel blocks (±0.05 g) were added to each volumetric flask. The adsorption procedure lasted 24 hours at room temperature. Equation 3.1 was used to compute the equilibrium quantity of adsorbed dye (QE, mg/g).

$$QE = \frac{(Ci - CE)}{m} \, x \, V \qquad \text{Equation 3.1}$$

where Ci and CE are the starting and final methylene blue concentrations in mg/L, m is the hydrogel mass in gram, and V is the total volume in litres. The goal of this research was to explore the adsorption capacity of three distinct kinds of hydrogels for methylene blue at various concentrations, which would provide important insights into their possible uses in wastewater treatment and dye removal procedures.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Extraction of Banana Stem Cellulose Fibre by using Alkaline Based Methods

In the process of extracting cellulose from banana stem powder, NaOH acts as a hydrolyzing agent, weakening the hydrogen bonds between hemicellulose and cellulose. This chemical reaction has the potential to aid in the breakdown of the amorphous component of cellulose (R.-Y. Zhang et al., 2022). The use of NaOH originates from its strong alkaline features which allow for the efficient breakdown of lignin and other impurities found in banana stem powder, which is an essential part of the lignocellulosic structure. Lignocellulosic biomass, such as plant wood, is principally made up of extractives, hemicellulose, cellulose, and lignin. Lignin, a cross-linked polymer, fills intercellular gaps inside the cell wall, connecting cellulose, hemicellulose, and pectin components and playing an important role in the movement of water and aqueous nutrients in plant stems. Furthermore, due to its hydrophobic nature, lignin functions as a natural glue, binding cellulose and hemicellulose components together (Shah et al., 2022). Thus, NaOH's role in the extraction process is to dissolve and remove this critical component, allowing for the separation of cellulose from plant material.

4.1.1 Effect of NaOH on Lignin Removal

To isolate cellulose from banana stem powder, the delignification process employs an 8% sodium hydroxide (NaOH) solution. This methodology is instrumental in achieving a banana stem powder with a reduced lignin content. As elucidated in studies by Adinugraha and Marseno (2005) and Mulyatno et al. (2017), the 8% NaOH concentration has been identified as optimal for cellulose extraction from banana stem (Adinugraha & Marseno, 2005; Mulyatno et al., 2017). The extensive study provided in these publications demonstrates the deliberate choice of this exact NaOH concentration, indicating its position as the preferred agent in the complex pretreatment process. Furthermore, lower concentrations of NaOH may result in insufficient lignin removal from the banana stem powder. This might result in a greater lignin concentration in the extracted cellulose, influencing its purity and possible uses. In contrast, an extremely high concentration of NaOH may cause cellulose molecules to degrade, which leads to a decrease in the quality and structural integrity of the recovered cellulose.

Figure 4.1: Banana Stem powder dissolved with NaOH.

The solvent becomes dark brown after heating at 100°C for 3.5 hours, indicating increased concentration due to lignin dissolution as shown in Figure 4.1. It is because of the NaOH contributes in the breakdown of lignin, therefore removing lignin during extraction may cause the solvent's colour to become dull. The colour shift may be more evident if the banana stem has a lot of lignin (Vardhini et al., 2019). Furthermore, changes in solvent colour during the extraction process can be used as a qualitative indication of the advancement of certain chemical processes or the extraction's effectiveness. Monitoring these variations may aid in optimising process conditions for extracting desired components from the banana stem.

The addition of NaOH to the banana stem powder for lignin removal results in a rise in solvent content, which can be attributed to the chemical process known as alkaline hydrolysis. Moreover, in the organic chemistry the alkaline hydrolysis is a form of nucleophilic substitution process in which the attacking nucleophile is a hydroxide ion. Therefore, the reaction products are mainly water-soluble salts and the matching phenol, which are less likely to be harmful to the environment due to its use less energy than traditional cremation, making it a more ecologically friendly alternative by comparing with other extraction methods (Bengtsson et al., 2022). The reaction can be represented in Figure 4.2 below:

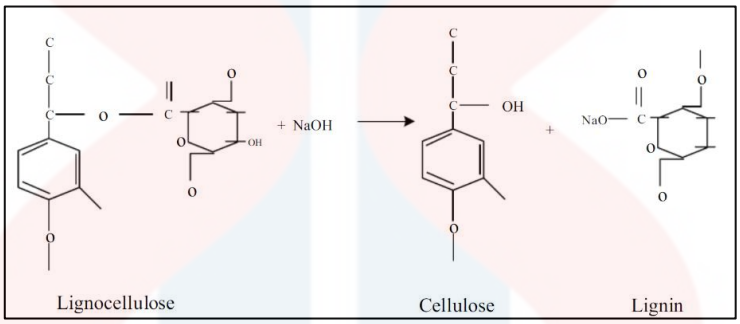

Figure 4.2: The mechanism interaction between lignocellulose and NaOH.

In this reaction, the NaOH serves as a strong base, reacting with the ester bonds found in the lignin structure to break down the lignin-carbon bonds, resulting in lignin dissolution in the NaOH solution. The lignin is then transformed into soluble lignin derivatives, increasing the concentration of solvent, and producing a dark brown colour.

After a 3.5-hour duration, the solvent underwent filtration with distilled water employing a vacuum filtration process until neutralization of the residual banana stem powder was achieved. The duration of powder immersion in the solvent and the elevated concentration of the solvent directly correlated with an extended filtration process, thereby yielding a diminished recovery of cellulose powder from the banana stem material. Consequently, a considerable volume of distilled water was requisite during the filtration process to enhance efficiency and mitigate cellulose degradation. The heightened concentration of distilled water within the solvent facilitated a more facile filtration through filter paper.

4.1.2 Optimal Parameters for Pre-treatment Bleaching Process

The primary objective of the bleaching procedure is the meticulous removal of impurities, encompassing hemicellulose, lignin, and other non-cellulosic components, from Banana Stem Cellulose. This comprehensive purification process extends to the removal of colorants, fundamentally transforming the cellulose material into a visually pleasing and aesthetically neutral state, particularly crucial for applications necessitating a pristine, white, or colorless final product. Moreover, the bleaching methodology is tailored to eliminate contaminants such as residual lignin, hemicellulose, and extractives, known to compromise the quality and inherent characteristics of cellulose. The systematic design of this process involves the breakdown and elimination of these contaminants, thereby elevating the overall quality of the cellulose and ensuring its compliance with specified standards across a diverse array of applications. Thus, the outcome of this meticulous procedure manifests in the development of a brighter Banana Stem Powder.

Figure 4.3: Bleaching treatment by using NaOCl.

Furthermore, due to impurities had the potential to cause colouring in Banana Stem Cellulose, Sodium Hypochlorite (NaOCl) was used in the bleaching stage to remove the colour from the cellulose powder obtained from the Banana Stem. Furthermore, NaOCl was composed of hypochlorite ions, which are powerful oxidizing agents capable of cleaving the ether bonds within the lignin structure, simultaneously enhancing the white brightness of the banana stem powder cellulose (Sayakulu & Soloi, 2022). Besides, to improve the brightness of Banana Stem Cellulose Powder, a 5% sodium hypochlorite (NaOCl) solution was prepared from a stock solution containing 10% accessible chlorine and heated to 30°C for 3 hours. The working solution contains 5% NaOCl, which is calculated based on the available chlorine in the initial sodium hypochlorite solution. This

precise treatment condition was chosen in order to maximise the brightening impact of the Banana Stem Cellulose Powder during the bleaching process.

4.1.3 Yield of Banana Stem Cellulose

To calculate the yield of Banana Stem Cellulose after the alkaline treatment which is by using 8% of NaOH and bleaching process which is by using 5% of NaOCl to extract the cellulose from the banana stem. The cellulose content was calculated using the formula as below:

equation 4.1

$$Yield\ (\%) = \frac{Weight\ of\ Banana\ Stem\ Cellulose}{Weight\ of\ Initial\ of\ Banana\ Stem\ Powder} \times 100$$

$$= \frac{8.778\ grams}{20.003\ grams} \times 100 = 43.883$$

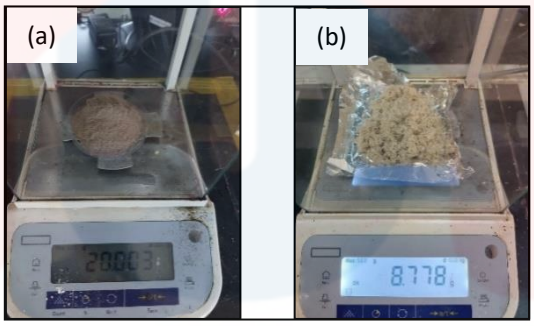

Figure 4.4: Weight before and after the alkaline treatment process (a) Initial weight of Banana Stem Powder (b) Final weight of Banana Stem Cellulose Powder.

The yield % is calculated by dividing the weight of the resulting Banana Stem Cellulose by the original weight of the Banana Stem Powder, then multiplying by 100. The formula (Weight of Banana Stem Cellulose) / (Weight of Initial Banana Stem Powder) × 100 measures the effectiveness of extracting cellulose from the raw material. Essentially, it estimates the amount of cellulose effectively extracted from the original Banana Stem Powder, providing a useful indicator for evaluating the extraction process's efficacy.

Hence, 43% of the initial banana stem powder was successfully transformed into cellulose. The yield of cellulose varied based on the extraction method, with the percentage concentration of NaOH/NaOCl, the extraction process temperature, and the

duration for lignin removal being pivotal. This variability stemmed from the fact that a high concentration of NaOH, when used for cellulose extraction, could potentially impact the crystalline structure of cellulose, making it susceptible to easy degradation. Similarly, cellulose degradation could occur at elevated temperatures. Therefore, maintaining an optimal extraction temperature is crucial to prevent cellulose breakdown. The duration for lignin removal also played a crucial role; excessive time might lead to cellulose degradation, while insufficient time could result in incomplete removal of lignin, affecting cellulose purity.

4.2 Morphological Characterization of Banana Stem Cellulose, Cellulose and Carboxymethyl Cellulose (CMC) through Microscopy

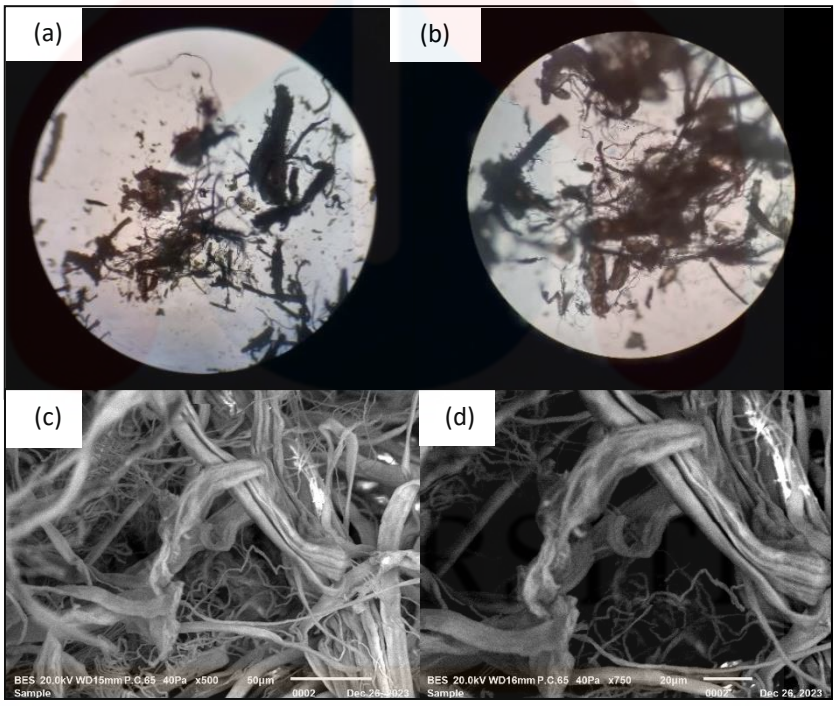

Figure 4.5: Optical Microscopy and SEM micrographs of Banana Stem as a cellulose source. (a) 4x magnification from optical microscope (b) 10x magnification from optical microscope (c) 500x magnification from SEM (d) 750x magnification from SEM.

In the observed of banana stem cellulose in Figure 4.5 under 4x and 10x magnification had reveals the different traits. At a moderate 4x magnification, the cellulose forms a thick, interwoven network fibres with random orientation, at this magnification. Ironically, because of its intrinsic poor resolution, this level of

magnification makes it difficult to properly detect individual fibres. However, when the magnification increases to 10x, a more complex image of cellulose fibres appears. The strands of fibre develop considerably clearer, revealing delicate striations, branching patterns, and tiny kinks, giving essential insights into cellulose's distinct structure. Thus, the increased magnification not only sharpens the visual features but also highlights the complex linkages between fibres in the network. This thorough investigation at different magnifications adds to a better understanding of the complicated structures found in banana stem cellulose.

Furthermore, switching to the scanning electron microscopy (SEM) at 500x and 750x magnification presents a more detailed perspective. At 500x magnification, cellulose fibres appear as distinct strands with a rough surface roughness that includes pits, grooves, and ridges. The integrated network of cellulose stays evident, with each fibre orientation growing more defined. Further magnification at 750x enhances surface characteristics, displaying visible striations, pits, and interior hollowness in fibres, emphasising the cellulose's distinguishing traits that are important for scenarios such as lightweight composites. In general, the optical microscope results demonstrate the effective extraction of the cellulose fibres from banana stems, which highlighting their fibrous shape, varying diameters, and rough surface roughness. Higher magnification SEM reveals internal complexities, indicating prospective applications where fibre properties, large surface area, and specialised internal structures are favourable.

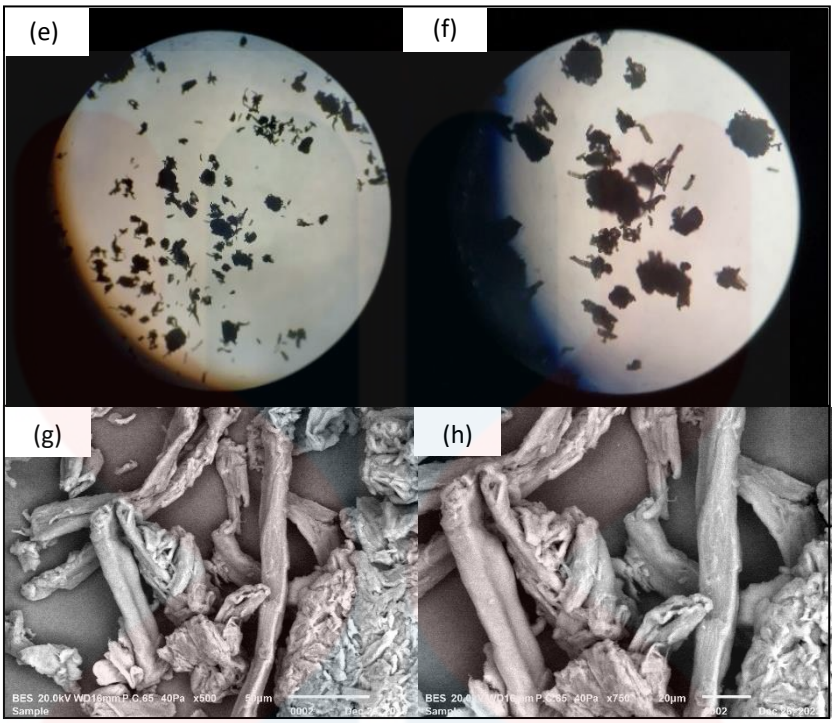

Figure 4.6: Optical Microscopy and SEM micrographs of Cellulose powder. (a) 4x magnification from optical microscope (b) 10x magnification from optical microscope (c) 500x magnification from SEM (d) 750x magnification from SEM.

The investigation of cellulose in Figure 4.6 under optical microscopy at both 4x and 10x magnification reveals different properties that give insight into the composition of its structure. At 4x cellulose appears as a thick which the substances almost amorphous with no discernible fibre structure. Although faint graininess may be visible, individual fibres remain elusive. This picture predominantly shows the presence of cellulose, implying a non-fibrous form, such as clumping together or particles. Furthermore, at 10x magnification, more features regarding the cellulose structure are not well seen. The majority of it remains non-fibrous, with sort more obvious striations. Some particles' edges may look smoother, but individual particle size remain unknown. This supports the fact that commercial cellulose may not adhere to a typical fibrous form, implying a processed condition, such as microcrystalline cellulose, in which the fibrous structure has been changed.

A considerable improvement in detail may be seen when using Scanning Electron Microscopy (SEM) at 500x. While fibres are still not visible, the cellulose mass has a more uneven and fractured structure, with sharp edges, platelet-like forms, or

agglomerates of smaller particles. The surface texture seems rough and perhaps porous. This picture supports the non-fibrous character of commercial cellulose, implying a microcrystalline in which cellulose chains are reorganized and compacted. Magnification to 750x highlights surface characteristics, revealing a more visible broken and uneven structure. Pores, cavities, and internal fissures are visible inside the particles, and the surface texture seems rougher and more complex. Individual particle diameters are further defined. This high magnification photograph repeatedly confirms the non-fibrous character of commercial cellulose. The internal characteristics and rough surface indicate a processed or modified cellulose form, which may be suitable for applications needing high surface area or precise particle size distributions.

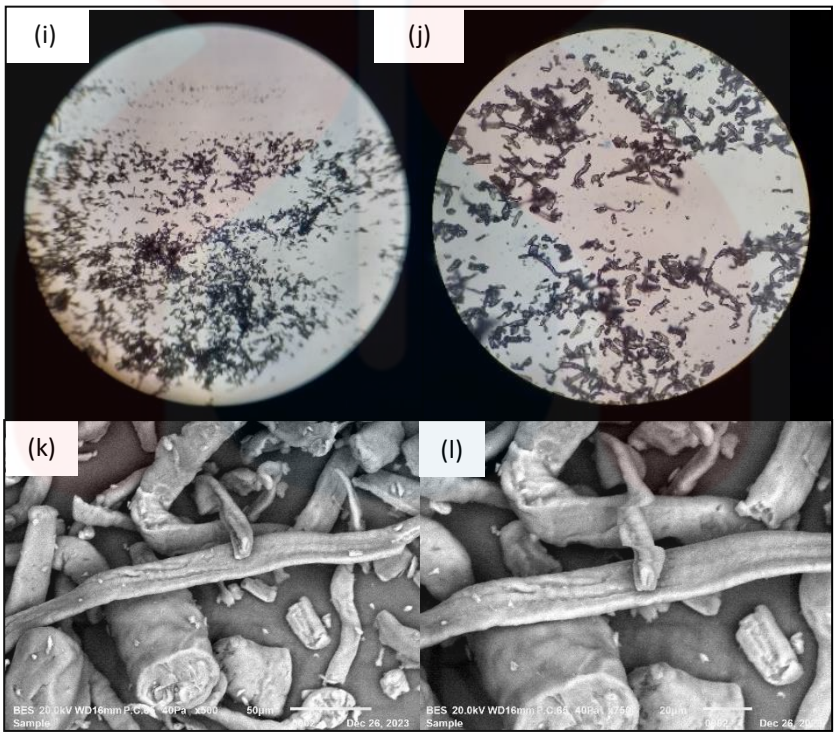

Figure 4.7: Optical Microscopy and SEM micrographs of CMC. (a) 4x magnification from optical microscope (b) 10x magnification from optical microscope (c) 500x magnification from SEM (d) 750x magnification from SEM.

In Figure 4.7 show that the CMC powder has specific properties when seen under optical microscope at 4x and 10x magnifications. At 4x magnification, the powder appears as a thick collection of irregular particles with no discernible fibrous structure, slightly uniform in size but without the characteristic elongated shape of cellulose fibres. Collapse patterns may be visible inside the particles and determining individual particle diameters is difficult owing to aggregation. This figure shows the existence of cellulose,

although the CMC may be in a non-fibrous, particle state due to the chemical modification process. When magnification is increased to 10x in Image j, more details about the CMC particles are lost. They remain non-fibrous, though delicate granular characteristics become increasingly evident. While certain particle edges look sharper, determining individual particle diameters remains challenging. This image supports the fact that the CMC in this sample lacks a characteristic fibrous shape, suggesting that the carboxymethylation process most likely influences the original cellulose fibre structure.

Furthermore, the image k shows a considerable improvement in detail when using Scanning Electron Microscopy (SEM) at 500x. While individual fibres are still not visible, the CMC powder has a more uneven and fractured structure. Sharp edges, platelet-like forms, or agglomerates of smaller particles are visible, and the surface texture seems rough and perhaps porous. This picture validates the CMC's non-fibrous nature and shows a broken cellulose fibre structure caused by carboxymethylation. The large surface area and possibly porous nature are helpful for certain applications such as thickening, suspending, and binding. The image l magnification to 750x emphasizes surface details, presenting an even more obvious fractured and uneven structure. Pores, cavities, or internal fissures may be seen inside the particles, making the surface texture rougher and more complicated. Individual particle size are resolved at this magnification, and some may be less than 1 micron. This high magnification image confirms the CMC's non-fibrous nature, providing more proof of the modified cellulose structure, which may be tuned for specific attributes like as water-holding capacity, biodegradability, and reactivity.

Optical microscopy (OM) and scanning electron microscopy (SEM) reveal unique properties between cellulose powders generated from banana stem, normal cellulose, and carboxymethylcellulose (CMC). Notably, banana stem cellulose powder exhibits a detectable fibrous network structure, emphasising its natural composition, whereas ordinary cellulose powder and CMC look non-fibrous in both OM and SEM examinations. In terms of surface roughness, all three powders have a rough exterior, but CMC stands out with a significantly rougher and more porous surface, indicating a changed structure caused by the carboxymethylation process. Furthermore, SEM investigation revealed common internal characteristics in all three powders, including pits, grooves, and ridges. However, CMC exhibits additional internal properties such as fractures and voids, indicating a more disturbed cellulose structure. In conclusion, OM

and SEM investigations give detailed insights into the shape and internal properties of these cellulose powders. The comparison of their visual characteristics yields diverse findings. Banana stem cellulose powder preserves its original fibrous structure, providing options for applications that require strength and reinforcement. Standard cellulose powder appears processed and non-fibrous, making it suitable for applications where fibrous properties are not critical; carboxymethylcellulose powder has a significantly altered morphology with a large surface area and distinct internal features, making it ideal for thickening, gelling, and binding.

4.3 Comparing and optimizing the Production of Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, and Carboxymethyl Cellulose Hydrogel Synthesis

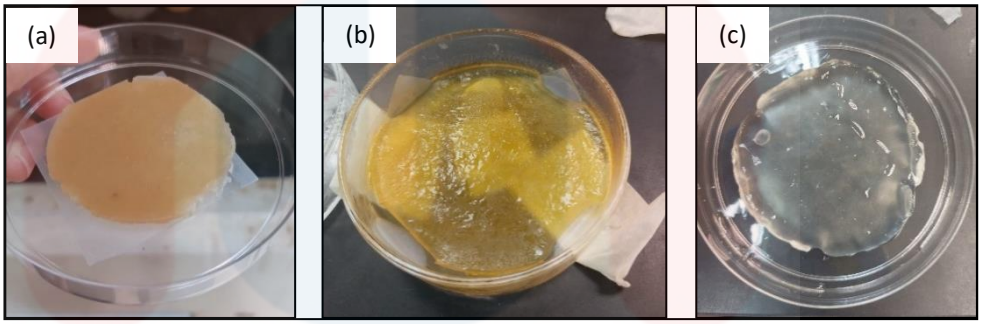

Figure 4.8: The synthesis of hydrogel involves the preparation of cellulose from a distinct variety, employing citric acid as the crosslinking agent. (a) Banana Stem Cellulose Hydrogel (b) Cellulose Hydrogel (c) CMC Hydrogel.

The hydrogels made from banana stem cellulose, generic cellulose, and carboxymethyl cellulose (CMC) have different visual qualities that reflect their distinctive capabilities in Figure 4.8. The figure labelled "(a)" on the left depicts the banana stem cellulose hydrogel. This hydrogel has a little cloudy brownish look and a smooth texture. These visual signals indicate that the hydrogel was successfully formed, since they are consistent with the predicted results of well-crosslinked banana stem cellulose hydrogels. The centre figure, labelled "(b)," appears to represent the cellulose hydrogel. When compared to the banana stem cellulose hydrogel, this hydrogel has more transparency and may have a slightly softer appear. These characteristics are congruent with the expected properties of cellulose hydrogels, demonstrating the variety of visual effects that might occur from the hydrogel synthesis process. Ultimately, the figure on the

right labelled "(c)" depicts the CMC hydrogel. It retains translucency, as do the other hydrogels, but it has more elastic comparing with the other hydrogel. This visual property is consistent with the predicted properties of CMC hydrogels, demonstrating the presence of carboxymethyl groups in the hydrogel structure. The subtle changes in appearance between these hydrogels demonstrate the specificity and variety possible with bespoke hydrogel production procedures.

4.3.1 Optimizing the dissolution of untreated banana stem cellulose powder and untreated cellulose powder

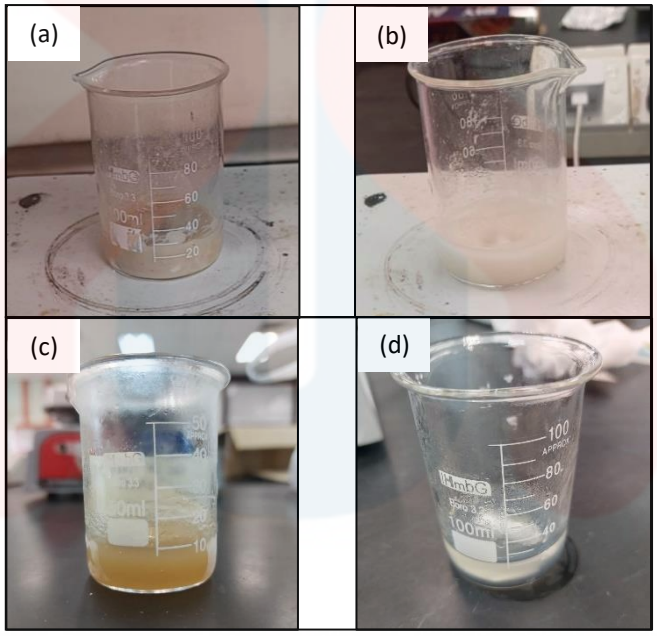

Figure 4.9: Texture observed pre- and post-dissolution of cellulose (a) before banana stem cellulose dissolve in the solvent (b) before cellulose dissolve in the solvent (c) untreated banana stem cellulose (d) untreated cellulose.

Figure 4.9 illustrates the discernible transformation occurring before and after the dissolution process of untreated banana stem cellulose powder and commercial cellulose powder. The labelled segments (a) and (b) depict the initial states of banana stem cellulose powder and commercial cellulose powder, respectively, undergoing dissolution with 8% NaOH and 12% urea. Conversely, labelled (c) and (d) represent the subsequent states after the banana stem cellulose powder has undergone dissolution through exposure to freezing conditions at -20°C for a duration of 24 hours.

Upon close examination, the untreated banana stem cellulose exhibits a solution with a brownish hue, in stark contrast to the nearly transparent and clear solution observed in the untreated cellulose. This discrepancy can be attributed to the less pristine nature of the banana stem cellulose, resulting from a less rigorous bleaching process during its extraction. In contrast, the untreated cellulose originates from commercially sourced cellulose, characterized by a higher degree of purity when compared to banana stem cellulose. This disparity in purity levels becomes evident in the contrasting visual representations of the solutions, underscoring the scientific nuances associated with the dissolution processes and the inherent characteristics of the cellulose samples under investigation.

4.3.2 Optimizing Citric Acid Concentrations for Banana Stem Cellulose and Cellulose in the Synthesis of Hydrogels

The concentration of citric acid as an essential factor in determining the characteristics of the resulting hydrogel during the crosslinking process. Inadequate citric acid concentrations can result in weak gels with reduced swelling ability, whilst excessive amounts might inhibit gel formation or produce too brittle hydrogels. Obtaining the correct concentration requires thorough testing, depending on the exact cellulose variety used.

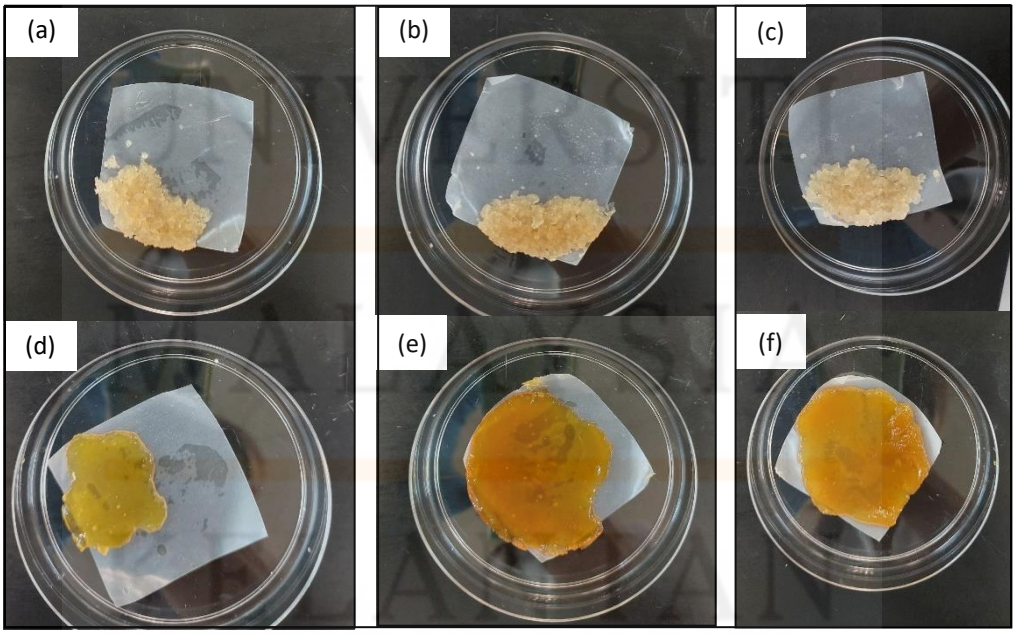

Figure 4.10: Optimal parameters for the formation of hydrogel through the utilization of citric acid. (a) BSC-CA10% (b) BSC-CA20% (c) BSC-CA30% (d) Cellulose-CA10% (e) Cellulose-CA20% (f) Cellulose-CA30%.

As figure 4.10 shown the unsuccessful of forming of the hydrogel where labelled a, b and c show that the different percentage of the formation of the banana stem cellulose hydrogel by using 10%, 20% and 30% of the citric acid for the forming of the hydrogel respectively. However, labelled d, e and f show that the different percentage of the formation of the cellulose hydrogel by using 10%, 20% and 30% respectively.

4.4 Comparative Analysis of Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, and Carboxymethylcellulose Hydrogels

4.4.1 Fourier Transform Infrared (FTIR) Spectroscopy

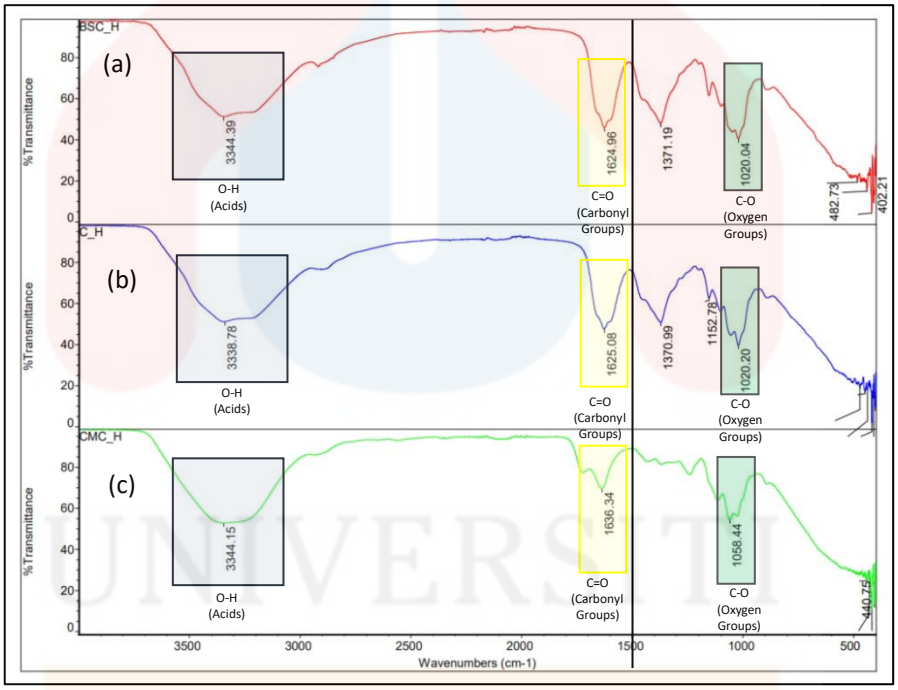

Figure 4.11: Fourier transform infrared spectroscopy (FTIR) spectra: (a) BSC Hydrogel (b) Cellulose Hydrogel (c) CMC Hydrogel.

Figure 4.11 shows the FTIR starch spectrum of three type of hydrogel which are Banana Stem Cellulose Hydrogel, Cellulose Hydrogel and Carboxymethylcellulose Hydrogel which label as BSC_H, C_H and CMC_H respectively at figure above. Figure 4.11 depicts the FTIR spectrum of the hydrogel with a strong absorption peak at 3344.39 cm-1 for BSC_H, 3338.78 cm-1 for C_H and 3344.15 cm-1 for CMC_H, which are assigned to the hydroxyl group of the polysaccharide chain, -OH stretching. Furthermore, they are slightly different as the peak on the BSC_H and C_H has showed the sharp peak

at 1624.96 cm-1 and 1625.08 cm-1 respectively for the carbonyl group (C=O), however, CMC_H show the moderate peak which is 1636.34 cm-1 on the C=O. Cellulose is a polysaccharide that consisting of glucose units linked by β-1,4-glycosidic bonds. Therefore, the C=O stretching vibration in the cellulose or the carboxymethylcellulose structure can be observed around this wavenumber.

Moreover, other differences between the hydrogel on the FTIR analysis was the methylene group as the BSC_H and C_H show that the strong sharp adsorption peak on 1371.19 cm-1 and 1370.99 cm-1 respectively and which has corresponded to the bending motion of the CH2 group. In contrast, CMC_H has showed the very weak peak on this methylene group by comparing with the BSC_H and C_H. Furthermore, a significant stretching C-O vibration of primary ethers and alcohols in the cellulose backbone of the cellulose chain was observed at 1020.04 cm-1, 1020.20 cm-1 and 1058.44 cm-1 on the BSC_H, C_H and CMC_H respectively.

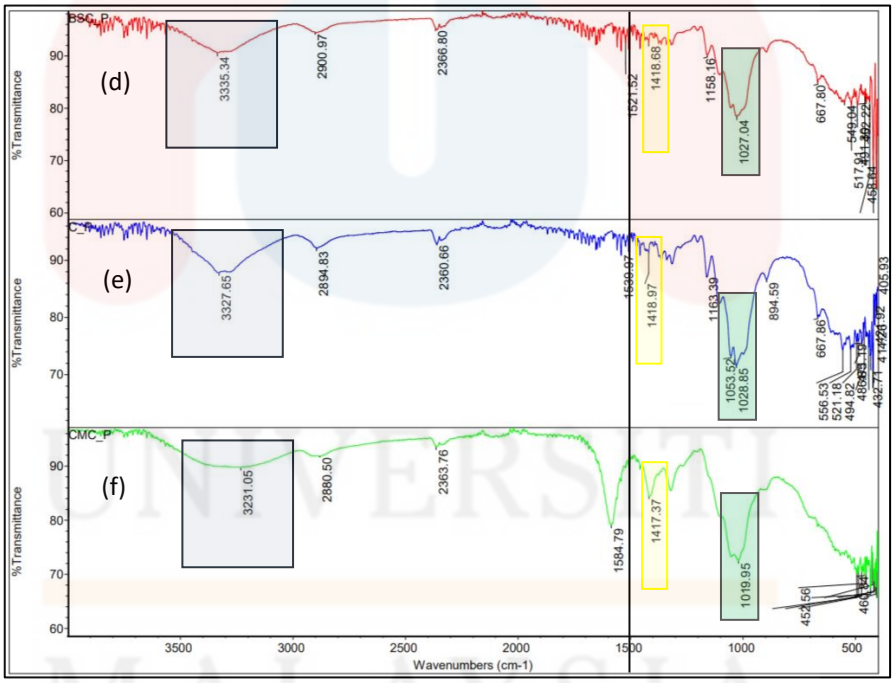

Figure 4.12: Fourier transform infrared spectroscopy (FTIR) spectra: (d) BSC Powder (e) Cellulose Powder (f) CMC Powder.

Figure 4.12 depicts the FT-IR infrared spectroscopy analysis of Banana Stem Powder (BSC_P), Cellulose Powder (C_P), and Carboxymethyl Cellulose Powder (CMC_P). FTIR is a valuable tool for discerning cellulose composition through the examination of absorption bands in these three powder types. The results in Figure 4.x

reveal two prominent absorption regions: one characterized by a low wavenumber in the range of 950-1150 cm^{-1} and another exhibiting a higher wavenumber around 3000-3500 cm^{-1}. These regions demonstrate considerable similarity across all three powder types.

Upon comparing the IR spectra of BSC_P and C_P, both belonging to the cellulose category, it is observed that the IR spectrum of CMC_P exhibits slight variations. Specifically, BSC_P and C_P display broad peaks at 3335.34 cm^{-1} and 3327.65 cm^{-1}, respectively, attributed to the hydroxy (-OH) groups. In contrast, CMC_P presents a broader peak at 3231.05 cm^{-1} originating from the hydroxy (-OH) groups. The -OH groups are associated with hydrogen bonds, involving interactions among the OH groups and surrounding molecules or ions. These distinctions highlight subtle structural differences among the cellulose powder variants.

Furthermore, the absorption band at 2900.97 cm-1 and 2894.83 cm-1 in the BSC_P and C_P spectra revealed that the peak is moderately intense when compared to the CMC_P 2880.50 cm-1 peak, which was slightly intensity from the vibration of the methylene group (-CH2-) on the C-H bonds. Following that, BSC_P and C_P have a weak peak with moderate absorption at 1418.68 cm-1 and 1418.97 cm-1 for the methylene (-CH2-) band, respectively, but CMC_P has a strong peak with moderate intensity at 1417.37 cm-1 for the methylene (-CH2-). Ultimately, the strong bands at 1027.04 cm-1, 1028.85 cm-1, and 1019.95 cm-1 from the ether group (COC) at BSC_P, C_P, and CMC_P have showed a comparable peak when compared to other peaks.

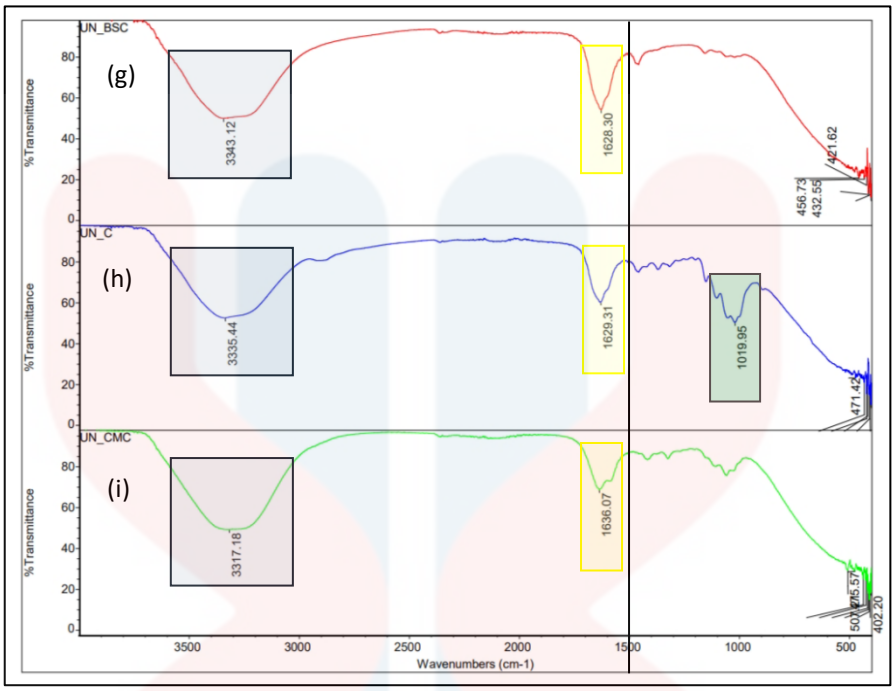

Figure 4.13: Fourier transform infrared spectroscopy (FTIR) spectra: (g) Untreated BSC (h) Untreated Cellulose (i) Untreated CMC Powder.

Figure 4.13 illustrates the FTIR starch spectrum of three distinct hydrogels: Untreated Banana Stem Cellulose (UN_BSC), Untreated Cellulose (UN_C), and Untreated Carboxymethylcellulose (UN_CMC), as labeled in the figure above. In Figure 4.x, the FTIR spectrum of the hydrogel unveils a robust, broad absorption peak at 3343.12 cm-1 for UN_BSC, 3335.44 cm-1 for UN_C, and 3317.18 cm-1 for UN_CMC, attributed to the hydroxyl group of the polysaccharide chain and corresponding to -OH stretching. Notably, slight variations are observed in the sharp peak at 1628.30 cm-1 for UN_BSC, 1629.31 cm-1 for UN_C, and 1636.07 cm-1 for UN_CMC, indicating a diminishing intensity for the carbonyl group (C=O). Given that cellulose is a polysaccharide composed of glucose units linked by β-1,4-glycosidic bonds, the C=O stretching vibration in the cellulose or carboxymethylcellulose structure is typically observed around this wavenumber. Additionally, a prominent stretching C-O vibration of primary ethers and alcohols in the cellulose backbone of the cellulose chain is evident at 1019.95 cm-1 specifically for UN_C, which is notably absent in UN_BSC and UN_CMC. This comparative analysis provides insights into the distinctive chemical signatures of these untreated hydrogels, shedding light on their structural variations at the molecular level.

4.4.2 Scanning Electron Microscope (SEM)

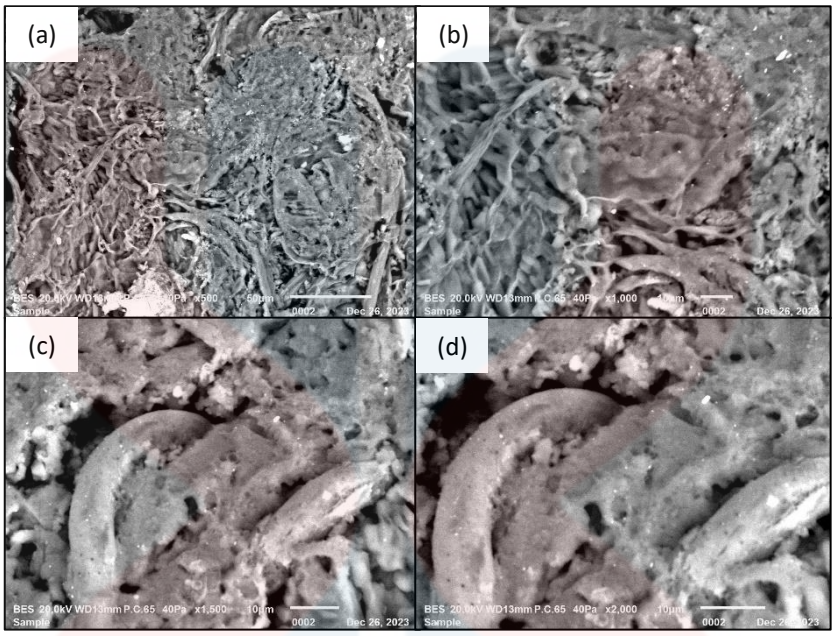

Figure 4.14: SEM micrographs depicting hydrogels prepared from banana stem as the source of cellulose.

Scanning electron microscopy (SEM) illustrations at various magnifications offer a detailed understanding of the structural properties of banana stem cellulose hydrogel at Figure 4.14. Image (a) at 500x magnification shows a comparatively smooth surface with identifiable holes, most likely caused by the elimination of lignin and other non-cellulosic components during hydrogel formation. As we approach to greater magnifications, Image (b) at 1000x reveals a complex network of cellulose fibers that comprise the hydrogel. These fibres are interconnected, producing the backbone that provides strength and structure to the hydrogel. At 1500x magnification in Image (c), a closer look emphasizes the rough surface of the cellulose fibres, which include characteristic striations owing to the crystalline structure present in cellulose. At the greatest magnification of 2000x, image (d) shows the individual cellulose microfibrils that constitute up the fibres. When taken as a whole, these SEM pictures clarify that banana stem cellulose hydrogel is a porous substance with a web of linked cellulose fibres. The hydrogel's strength, form, and unique features are attributed to its crystalline structure and rough fibre surface, which makes it a viable material for a variety of applications.

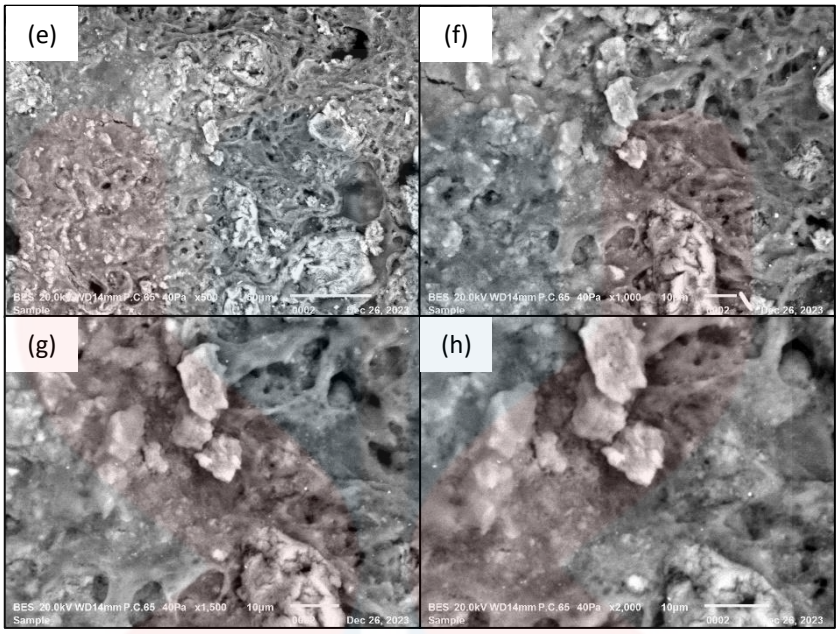

Figure 4.15: SEM micrographs depicting hydrogels prepared from cellulose.

Figure 4.15 illustrates micrographs portraying hydrogels derived from cellulose. Under SEM at 500x magnification €, the surface exhibits a relatively smooth appearance with discernible pores, indicative of gaps between individual cellulose fibers within the hydrogel network. The asymmetry in structure suggests a potential influence of additives in the commercial product beyond cellulose. Upon closer examination at 1000x magnification (f), the cellulose fiber network reveals intricately interwoven strands that contribute to the overall architecture of the hydrogel. Depending on the processing methods employed in commercial hydrogel production, the fibers exhibit varying textures, appearing either somewhat rough or smooth. Further magnification to 1500x (h) exposes the cellulose fiber surface, highlighting striations that signify the crystalline nature of the material. In comparison to non-crosslinked hydrogels, the fiber network densifies due to the evident crosslinking action of citric acid. Even at the maximum 2000x magnification (i), distinct cellulose microfibrils with diameters ranging from 2 to 5 nm become visible. The impact of citric acid as a crosslinking agent is evident, leading to a more organized and aligned arrangement of these microfibrils compared to hydrogels crosslinked with alternative agents.

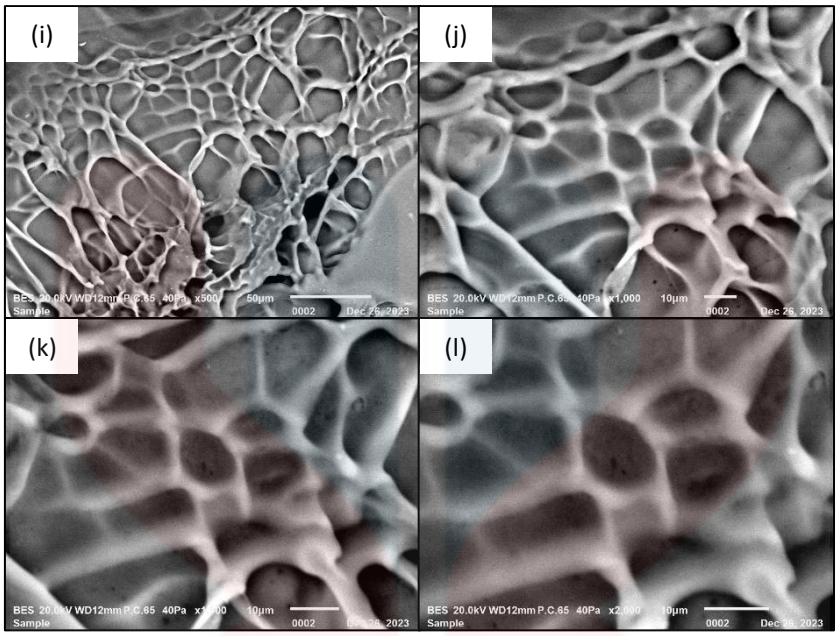

Figure 4.16: SEM micrographs depicting hydrogels prepared from CMC.

Figure 4.16 shows that the hydrogel's surface has a smooth texture and visible pores that are thought to be the gaps between the chains of carboxymethylcellulose (CMC) when seen at a 500x magnification (i). The structure seems a little asymmetrical, which might be caused by extra ingredients or results of crosslinking. A more in-depth picture of the CMC network is seen at 1000x magnification (j), emphasising the interwoven chains that make up the hydrogel's structure. Because of the carboxymethyl groups, the CMC chains are smoother than ordinary cellulose fibres and can be distinguished. Finer features become visible at 1500x magnification (k), including the denser network of citric acid crosslinking sites and striations that represent the crystalline structure of cellulose in CMC chains. A close-up of individual CMC molecules may be seen at the greatest magnification of 2000x (l), which displays long, linear chains with carboxymethyl groups attached.

4.4.3 Antibacterial Test

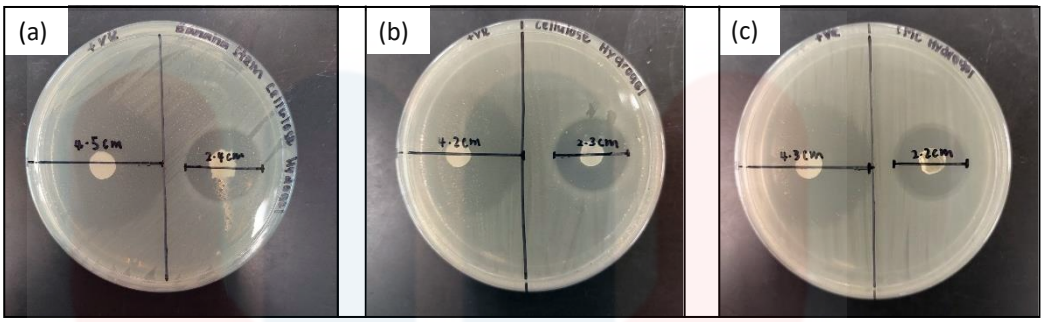

Figure 4.17: Antibacterial assessment against Escherichia coli employing the disc diffusion method, encompassing (a) Banana Stem Cellulose (BSC) Hydrogel, (b) Cellulose Hydrogel, and (c) Carboxymethyl Cellulose (CMC) Hydrogel.

Escherichia coli (E. coli) was chosen as the model organism for antibacterial testing in hydrogel evaluations for a variety of scientific reasons. E. coli is a Gram-negative bacteria distinguished by its unique cell wall construction, which includes an outer membrane. This makes it especially important for assessing the efficacy of antibacterial treatments such as hydrogels, as Gram-negative bacteria frequently present unique obstacles due to the existence of this extra membrane layer. Furthermore, E. coli is not only a well-known model organism, but also a common bacteria found in people and animals' intestines, as well as in a variety of environmental conditions. Its widespread availability and ease of culture make it an ideal candidate for laboratory experiments. As Figure 4.17 above show that the antibacterial test by using disc diffusion method for Banana Stem Cellulose Hydrogel, Cellulose Hydrogel and Carboxymethyl Cellulose Hydrogel by using E. coli as the bacterial and chloramphenicol as the positive control on the antibacterial test.

In the antibacterial testing of hydrogels, the inclusion of a positive control, such as chloramphenicol, is very scientific. Chloramphenicol is a well-known and extensively used antibiotic that has broad-spectrum action against a variety of bacteria, including E. coli. Researchers set a standard for antibacterial efficiency by using chloramphenicol as a positive control. It provides as a benchmark with an established antibacterial profile against which the hydrogel's performance may be measured. The relevance of chloramphenicol as a positive control stem from its ability to decrease bacterial protein synthesis, making it an efficient and dependable standard in antibacterial experiments.

The comparison to chloramphenicol not only verifies the antibacterial test technique, but it also sheds light on the hydrogel under investigation's relative effectiveness.

The efficient incorporation of citric acid into the structure of banana stem cellulose resulted in the formation of a hydrogel, paving the door for a thorough investigation of its antibacterial properties. The accurate measurement of inhibition zones, defined as locations where bacterial growth finds substantial resistance, revealed fascinating differences among the hydrogel variations under investigation. The banana stem cellulose hydrogel outperformed its predecessors with a broad inhibition zone extending 24mm. In contrast, the cellulose hydrogel, while respectable, had a slightly smaller but significant inhibitory zone spanning 23mm. Following closely, the carboxymethylcellulose hydrogel showed an approximate reduced inhibitory zone of 22mm. This sophisticated research emphasises the critical importance of the banana stem cellulose hydrogel, which stands out as the pinnacle of increased antibacterial activity. The observable expansion in the inhibitory zone can be due to the judicious addition of citric acid, which forms a symbiotic alliance and strengthens the hydrogel's defence against microbial penetration. This finding not only explains the efficient incorporation of citric acid into the cellulose matrix, but it also highlights the banana stem cellulose hydrogel's potential as a robust antibacterial agent.

The results indicate that the banana stem cellulose hydrogel, when combined with citric acid, outperformed both the cellulose and carboxymethylcellulose hydrogels in terms of antibacterial activity. The greater inhibition zone indicates a stronger antibacterial activity, highlighting banana stem cellulose's potential as a very efficient antibacterial material. This increased antibacterial activity might be ascribed to the natural qualities of banana stem cellulose, along with the synergistic effect of citric acid, which is known for its antimicrobial properties. Moreover, the border inhibition zone on banana stem cellulose hydrogel is due to the modulation of pH that leads in an adverse acidic environment that breaks bacterium membranes. Furthermore, citric acid's detoxifying characteristics contain critical metal ions required for the growth of bacteria, inhibiting their metabolic activities.

4.4.4 Adsorption Test

Table 4.1: Absorbance reading of each concentration.

Concentration (mg/L)	Absorbance Reading 1	Absorbance Reading 2	Absorbance Reading 3	Average
2	0.274	0.278	0.288	0.280
4	0.659	0.648	0.644	0.650
6	0.915	0.925	0.908	0.916
8	1.189	1.198	1.203	1.197
10	1.445	1.455	1.449	1.450

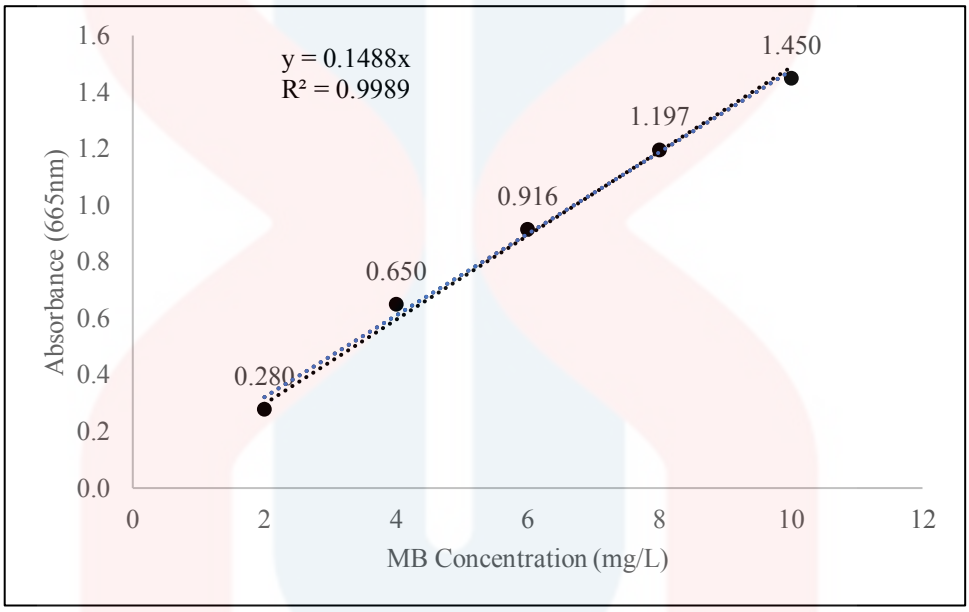

Figure 4.18: Standard Curve for the Methylene Blue (MB).

The graph at Figure 4.18 depicts the relationship between absorbance levels (y-axis) and methylene blue concentration (mg/L) (x-axis), with discrete data points at 2, 4, 6, 8, and 10 mg/L. This graph includes a precisely fitted straight line labelled "Standard Curve: Methylene Blue (665nm)," which provides more contextual information. The obtained equation, y = 0.1488x, not only validates the absorbance-concentration connection, but it also emphasises the calibration process's accuracy. In practice, a y-intercept of 0 indicates that the absorbance at a concentration of 0 mg/L of methylene blue should likewise be zero. This theoretical equation simplifies the calibration curve by emphasising that the absorbance is exactly proportional to the concentration of methylene blue. The equation y = 0.1488x states that for every unit increase in concentration (x-axis), the absorbance (y-axis) rises by the slope value of 0.1488. The slope, shown by 0.1488, demonstrates the calibration's sensitivity, allowing for the detection of even minute variations in methylene blue concentration via absorbance changes. The R^2 result

of 0.9989 indicates a strong match, providing confidence in predicting unknown concentrations in adsorption test samples.

Table 4.2: The amount of Dye Adsorb for each concentration of three type of hydrogel.

Concentration (mg/L)	Dye Adsorb, Qe (mg/g)		
	Banana Stem Cellulose Hydrogel	Cellulose Hydrogel	Carboxymethylce-llulose Hydrogel
25	2.051	2.234	2.261
50	4.013	4.406	4.505
75	6.478	6.818	6.900
100	8.772	8.978	9.238
125	11.233	11.256	11.608

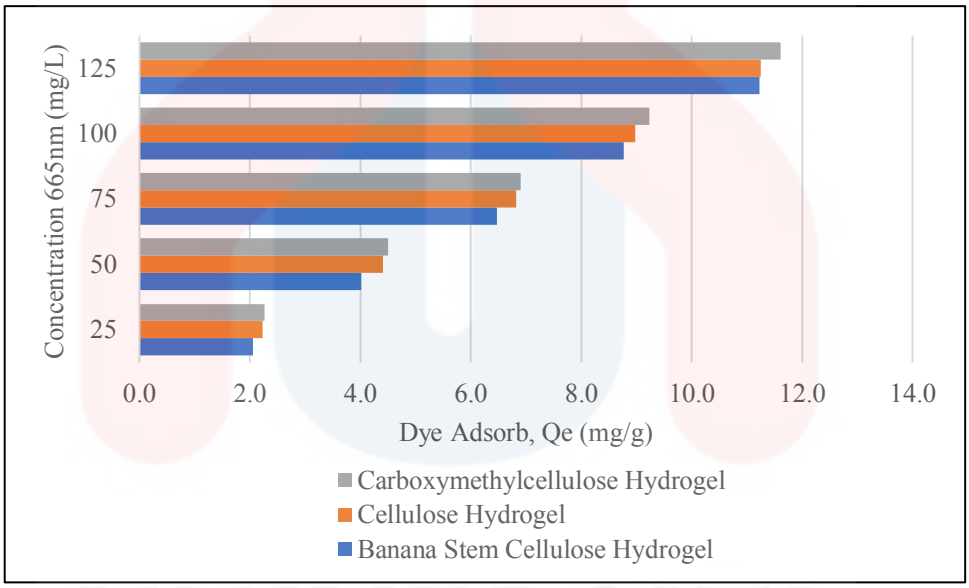

Figure 4.19: Compare the quantitative bar graph depicting the dye adsorption capacity (Qe, mg/g) for three different types of hydrogels.

Figure 4.19 depicts a complete bar graph of the adsorption performance of three different hydrogels: Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, and Carboxymethylcellulose Hydrogel at changing dye concentrations (25, 50, 75, 100, and 125 mg/L). The x-axis corresponds to concentration levels, while the y-axis displays Dye Adsorption (Qe) values in mg/g, which represent the quantity of dye adsorbed per gram of hydrogel. Each hydrogel is distinguished by color-coded bars, with the green bar showing dye adsorption in Carboxymethylcellulose Hydrogel, the red bar representing Cellulose Hydrogel, and the blue bar representing Banana Stem Cellulose Hydrogel. This

graphical depiction allows for a clear and visual comparison of their relative dye adsorption efficiencies.

In the adsorption investigation, Banana Stem Cellulose Hydrogel (BSC Hydrogel) showed substantial effectiveness, adsorbing 2.051 mg/g of dye at 25 mg/L. The Q e values progressively increased to 11.233 mg/g at 125 mg/L. The accompanying bar graph depicts the increasing trend in dye adsorption capacity, demonstrating the hydrogel's efficacy in collecting dye molecules. Cellulose Hydrogel performed similarly, adsorbing 2.234 mg/g at 25 mg/L and raising Q e values to 11.256 mg/g at 125 mg/L. The bar graph for Cellulose Hydrogel shows a similar rising trend, showing equivalent dye adsorption efficiency to BSC Hydrogel. Carboxymethylcellulose Hydrogel (CMC Hydrogel) showed good adsorption, beginning at 2.261 mg/g at 25 mg/L and gradually increasing to 11.608 mg/g at 125 mg/L. The related bar graph for CMC Hydrogel reflects the climbing pattern seen in the other hydrogels, indicating a consistent capacity to adsorb dye molecules.

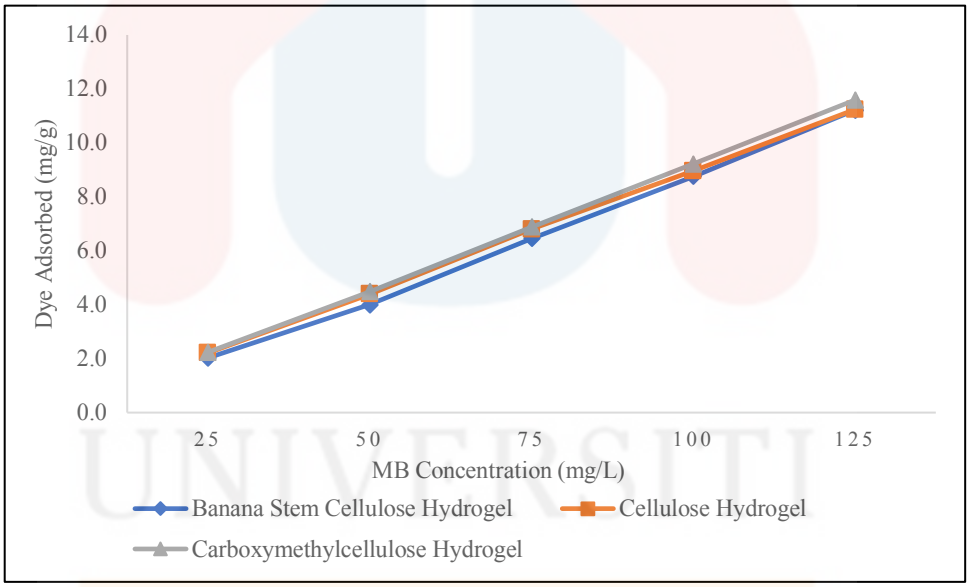

Figure 4.20: Comparison Line graph of the treats of dye adsorbed for three type of hydrogel.

The presented line graph depicts the dye adsorption performance of Banana Stem Cellulose Hydrogel (BSC Hydrogel), Cellulose Hydrogel, and Carboxymethylcellulose Hydrogel at various concentrations (25, 50, 75, 100, and 125 mg/L), with each hydrogel denoted by a color-coded line - green for Carboxymethylcellulose Hydrogel, red for Cellulose Hydrogel, and blue for Banana Stem Cellulose Hydrogel. Beginning at 2.051 mg/g at 25 mg/L, BSC Hydrogel exhibits a continuous rising trajectory in dye adsorption capacity, peaking at 11.233 mg/g at 125 mg/L. Cellulose Hydrogel exhibits parallel

development, starting at 2.234 mg/g and reaching 11.256 mg/g at 125 mg/L. Carboxymethylcellulose Hydrogel's dye adsorption begins at 2.261 mg/g at 25 mg/L and gradually increases to 11.608 mg/g at 125 mg/L. These observed trends represent proportional and equivalent increases in dye adsorption capabilities over the concentration range for all three hydrogels

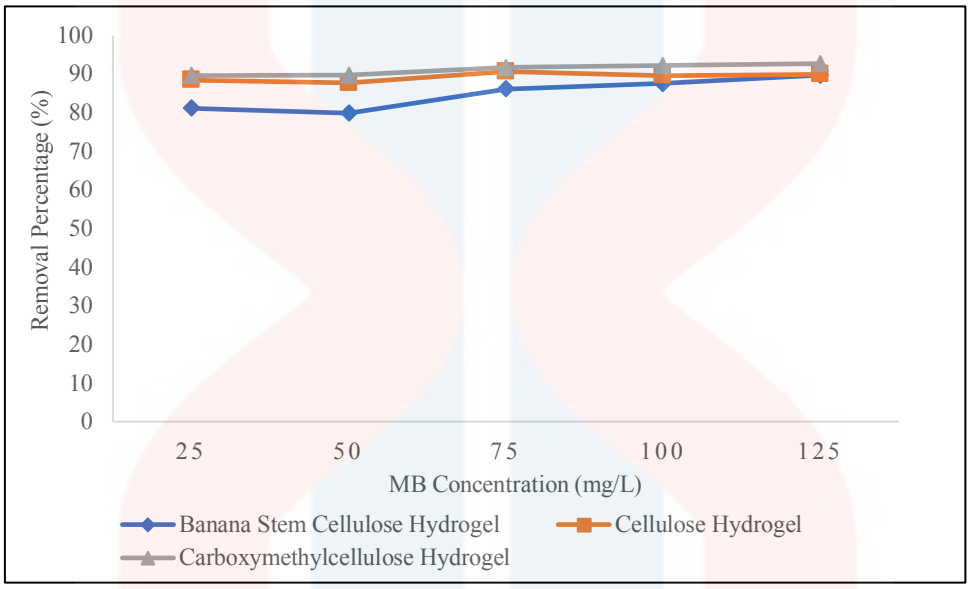

Figure 4.21: Comparison line graph of the removal percentage for three type of hydrogel.

The comprehensive analysis of the line graph at Figure 4.21, illustrating the removal percentage of dye by Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, and Carboxymethylcellulose Hydrogel at varying concentrations (25, 50, 75, 100, 125 mg/L), exposes distinctive trends for each hydrogel. Banana Stem Cellulose Hydrogel exhibits a fluctuating removal percentage, starting at 81.19% at 25 mg/L, decreasing slightly to 79.87% at 50 mg/L, and then experiencing an upward trend reaching 89.72% at 125 mg/L. This fluctuation implies an initial adjustment period at lower concentrations, followed by an increasing efficiency in dye removal. Conversely, Cellulose Hydrogel demonstrates a relatively consistent removal percentage, initiating at 88.47% at 25 mg/L and maintaining stability through subsequent concentrations, concluding at 89.90% at 125 mg/L. This stability indicates a consistent and efficient dye removal capability across the entire concentration range. Carboxymethylcellulose Hydrogel displays a steady increase in removal percentage, starting at 89.59% at 25 mg/L, progressing consistently, and reaching a peak of 92.70% at 125 mg/L. This ascending trend signifies an incremental

improvement in dye removal efficiency with increasing concentrations. In summary, the collective analysis unveils nuanced patterns in the removal percentages of these three hydrogels, offering valuable insights into their respective performances in dye removal across different concentration levels.

4.4.5 Biodegradation Test

The findings result come from a four-week investigation on the effectiveness of the biodegradation from three type of hydrogel which are Banana Stem Cellulose Hydrogel, Cellulose Hydrogel, Carboxymethylcellulose Hydrogel. The comprehensive of the study looked for the ability of the different phases of biodegradation demonstrated by comparing the three different hydrogels, with an emphasis over the long observing period. The following table show the data that gathered over this prolonged investigation of the biodegradation test for these three types of hydrogels and the graph for the percentage of weight loss during the 4 weeks of the investigation.

Table 4.3: The quantification of biodegradation for each consecutive week.

Type of Hydrogel	Initial Weight (g)	Biodegradation (g)				Weight loss (%)
		1st Week	2nd Week	3rd Week	4th Week	
Banana Stem Cellulose Hydrogel	1.175	0.704	0.529	0.469	0.106	91
Cellulose Hydrogel	1.073	0.522	0.377	0.307	0.115	89
Carboxymethylcellulose Hydrogel	1.867	0.914	0.400	0.199	-	100

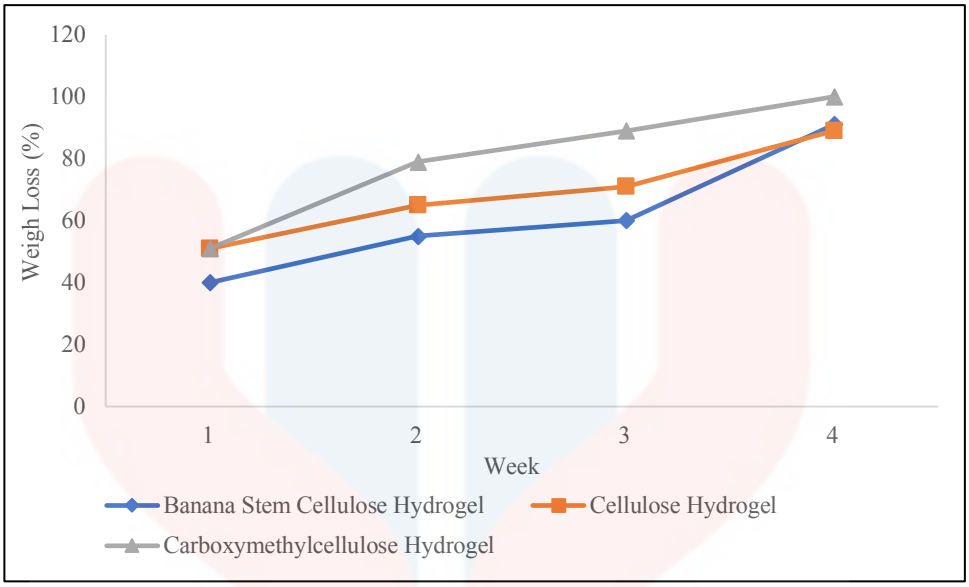

Figure 4.22: Comparative line graph showcasing the weight loss during the biodegradation process for three different types of hydrogels.

As shown in Figure 4.22, the weight loss % for Carboxymethylcellulose (CMC) Hydrogel increased significantly and rapidly when compared to the two kinds of hydrogel. The percentage of weight loss for CMC Hydrogel increased significantly throughout the biodegradation test, rising from 51% in the first week to 79% in the second, 89% in the third, and finally 100% by the fourth week. Nonetheless, the weight loss evolves seen for the Banana Stem Cellulose Hydrogel and Cellulose Hydrogel followed distinguish patterns. During the first week of the biodegradation test, the former and latter lost 40% and 51% of their body weight, respectively. In the second week, these values increased to 55% and 65%, followed by further rises to 60% and 71% in the third week. Intriguingly, by the end of the fourth week, the Banana Stem Cellulose Hydrogel had a weight loss percentage of 91%, outperforming the Cellulose Hydrogel, which had a weight loss of 89%.

Banana Stem Cellulose Hydrogel and Cellulose Hydrogel are derived from pure plant sources, and the harvested cellulose is directly used to make the hydrogel. Furthermore, the unaltered form of cellulose has a crystalline structure due to the arrangement of its chains. Additionally, the durability of banana stem cellulose is strengthened by its natural, unaltered condition, which has a well-defined crystalline structure and contains other components such as hemicellulose and lignin. Carboxymethylcellulose has a more amorphous structure and may be less stable than

native cellulose, particularly under certain climatic conditions. The stability of any material is determined by its individual application requirements and intended use in various circumstances.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Eventually, this study addressed the common issues connected with wound dressing waste and investigated the sustainable use of banana plant byproducts. The goal was to improve hydrogels made from cellulose taken from banana stems, cellulose, and carboxymethylcellulose (CMC) crosslinked with citric acid. The major goal was to address concerns connected to the enormous waste created by wound dressings while also utilizing the significant byproducts of banana growing. The use of an alkaline-based extraction technology permitted the separation of cellulose from banana stems, reducing waste and providing an ecologically favorable alternative. The use of citric acid to chemically crosslink hydrogels showed promise in wound care applications. The study thoroughly characterized the generated materials and assessed their degrading behavior. It also emphasized the environmental benefits of using banana byproducts and shone light on the toxicity issues connected with traditional hydrogels. Antibacterial and biodegradation experiments revealed that these hydrogels have potential use in both medicine and the environment. Scanning electron microscopy (SEM) revealed important details about the microarchitecture of the hydrogels, including pore size and distribution. Furthermore, the study looked at the hydrogels' adsorption ability for methylene blue dye, indicating possible uses in wastewater treatment. In conclusion, this comparative study provides critical knowledge for promoting sustainable wound care methods and harnessing agricultural byproducts, paving the way for future research and practical applications in biomedicine and environmental remediation

5.2 Recommendations

5.2.1 Further Exploration of Hydrogel Properties

The optimisation of hydrogel synthesis, especially the use of citric acid as a crosslinking agent, has shown encouraging results, demonstrating the potential for improving material qualities. However, in order to gain a more nuanced knowledge of the crosslinking process and its complex impact on hydrogel performance, a detailed investigation of the effect of various citric acid concentrations is required. A thorough analysis of various citric acid concentrations is expected to show concentration-dependent differences in hydrogel physical qualities such as tensile strength, porosity, and swelling behaviour. This comprehensive analysis contains the key to determining the ideal citric acid concentration that determines certain hydrogel properties, allowing for exact customisation of hydrogels adapted to specific uses.

5.2.2 Comprehensive Biodegradation Studies

The initially collected information from the biodegradation experiments described in the study present an early indication of the hydrogel environmental impact. While these findings are useful, a more thorough and nuanced knowledge of the long-term consequences demands a more extensive and diverse approach to biodegradation research under varied environmental situations. To gain a comprehensive view, it is important to perform a longer-term exploration that includes a variety of environmental elements. This technique will allow to examine the hydrogel behavior over time and under a variety of circumstances, such as temperature, humidity, and microbial activity. By studying how hydrogels respond to these conditions, that may acquire a better knowledge of their degradation processes, stability, and possible environmental ramifications.

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APPENDIX A

Table A.1: Initial UV-Vis Absorption Readings for Banana Stem Cellulose Hydrogel

Banana Stem Cellulose Hydrogel	Time (hrs)	Reading Control	Reading 1	Reading 2	Reading 3	Average	Standard Deviation
1	24	1.651	0.682	0.681	0.680	0.681	0.001
2	24	1.819	1.458	1.458	1.457	1.458	0.001
3	24	1.869	1.507	1.509	1.508	1.508	0.001
4	24	1.883	1.809	1.800	1.804	1.804	0.005
5	24	1.950	1.881	1.838	1.864	1.861	0.022

Table A.2: Initial UV-Vis Absorption Readings for Cellulose Hydrogel

Cellulose Hydrogel	Time (hrs)	Reading Control	Reading 1	Reading 2	Reading 3	Average	Standard Deviation
1	24	1.675	0.418	0.417	0.417	0.417	0.001
2	24	1.848	0.892	0.891	0.887	0.890	0.003
3	24	1.886	1.015	1.017	1.018	1.017	0.002
4	24	1.933	1.511	1.506	1.507	1.508	0.003
5	24	1.982	1.816	1.841	1.826	1.828	0.013

Table A.3: Initial UV-Vis Absorption Readings for Carboxymethylcellulose Hydrogel

Carboxymethyl Cellulose Hydrogel	Time (hrs)	Reading Control	Reading 1	Reading 2	Reading 3	Average	Standard Deviation
1	24	1.679	0.379	0.376	0.376	0.377	0.002
2	24	1.780	0.752	0.748	0.740	0.747	0.006
3	24	1.866	0.903	0.893	0.901	0.899	0.005
4	24	1.945	1.132	1.135	1.130	1.132	0.003
5	24	1.957	1.320	1.322	1.320	1.321	0.001

APPENDIX B

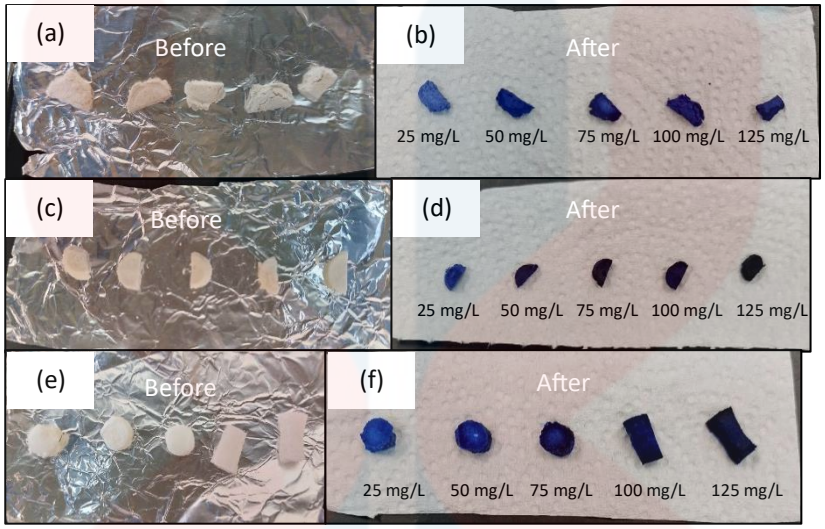

Figure B.1: Hydrogel before and after adsorption periods with methylene blue dye. The images show: (a) Banana Stem Cellulose Hydrogel before adsorption, (b) Banana Stem Cellulose Hydrogel after adsorption, (c) Cellulose Hydrogel before adsorption, (d) Cellulose Hydrogel post-adsorption, (e) Carboxymethylcellulose Hydrogel before adsorption, and (f) Carboxymethylcellulose Hydrogel after adsorption.