UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Encapsulation of probiotics to enhance survival of shrimps.

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniería Biomédica

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Dedicatoria

Dedico este trabajo a mi mamá Gladys, quien a través de su amor, trabajo y dedicación me ha ayudado a labrar este camino tan importante en mi vida.

A mis hermanos.

A las grandes amistades que he formado en esta universidad.

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Luis Armando Taipe Chanalata

Resumen

Las cápsulas de celulosa tienen una variedad de aplicaciones en farmacología, cosmetología y la industria alimentaria debido a sus características únicas, como alta capacidad de encapsulación, renovables, económicas y biodegradables. Los interesados en el diseño químico de materiales de base biológica están cada vez más enfocados en la fabricación de productos a base de celulosa. Hay muchos tipos de celulosa extraída, que dependiendo de la fuente, pueden tener diferentes propiedades, pero no todas son adecuadas para crear perlas de celulosa. En este contexto celulosa de fuente alternativas, como la celulosa de los desechos orgánicos ordinarios, son apreciadas. Sin duda alguna la microencapsulación ha sido de relevancia para la atención de la salud, la higiene, la farmacia, la alimentación, el tratamiento de aguas residuales, el papel, la industria química y en muchos otros campos como la acuicultura, debido a los beneficios obtenidos por su incorporación. Particularmente, en el campo de la acuicultura, podría usarse como vehículo para agentes de liberación controlados de microorganismos como probióticos. El uso de este material es ilimitado, especialmente cuando están elaborados a partir de recursos renovables y biodegradables, como la celulosa. Sin embargo, apenas se ha informado de perlas preparadas a partir de celulosa no sustituida debido a la insolubilidad de la celulosa en soluciones acuosas. Esta investigación tiene como objetivo desarrollar perlas probando la celulosa extraída de varios desechos orgánicos para probar su eficiencia de encapsulación de probióticos marinos. Para ello, la celulosa fue extraída y procesada para formar diferentes perlas, caracterizadas estructuralmente y composicionalmente utilizando técnicas estándar como difracción de rayos X (XRD), espectroscopía infrarroja por transformadas de Fourier (FT-IR), Microscopía electrónica de barrido (SEM), y finalmente pruebas de encapsulación. Se sintetizaron nuevas perlas de celulosa mediante un método de "tres pasos" a partir de celulosa, disuelta directamente en una solución acuosa. Este trabajo proporciona una forma rápida y sencilla de preparar perlas ecológicas a partir de celulosa sin sustituir. Todavía no se puede utilizar para encapsular probióticos debido a su sensibilidad al cambio de pH.

Palabras Clave:

Perla, Celulosa en acuicultura, Encapsulación, Probióticos marinos, Caracterización de celulosa.

Abstract

Cellulose beads have a variety of applications in pharmacology, cosmetology, and the food industry due to their unique features such as high encapsulation capacity, renewable, inexpensive, and biodegradable**.** Cellulose is the most common organic polymer. The concerned in the chemical design of biobased materials are increasingly interested in the manufacture of cellulose-based products. There are many types of extracted cellulose that may have different properties depending on the source; however, not all of them are suitable for the production of cellulose beads. In this context, alternative types of cellulose, such as cellulose from ordinary organic wastes, are appreciated. Microencapsulation has undoubtedly been of relevance in health care, hygiene, pharmaceutics, food, wastewater treatment, paper, chemical industry, and in many other fields such as aquaculture, which is increasingly growing. In aquaculture, it could be used as a carrier for probiotics-controlled release agents. The use of this material is unlimited, especially when they are made from renewable and biodegradable resources, such as cellulose. However, beads prepared from unsubstituted cellulose have been scarcely reported because of the insolubility of cellulose in aqueous solutions. This research aims to develop beads by testing cellulose extracted from various organic wastes to test their marine probiotic encapsulation efficiency. For this purpose, the cellulose was extracted and processed to form different beads, characterized structurally and compositionally using standard techniques such as X-ray diffraction (XRD), Fourier transforms infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM), and finally encapsulation tests. Novel cellulose beads were synthesized using cellulose's "three-step" method, dissolved directly in an aqueous solution. This work provides a fast and straightforward way for preparing eco-friendly beads from unsubstituted cellulose. It cannot yet be utilized to encapsulate probiotics due to their sensibility to pH change.

Key-words:

Beads, Cellulose in aquaculture, Encapsulation, Marine probiotics, Cellulose characterization.

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1 INTRODUCTION – JUSTIFICATION

1.1 Global Shrimp Industry

Shrimp production is one of the most significant growth markets worldwide, with an estimated value of USD 19.5 billion (CEDIA, 2021). Shrimp farming is defined as the production of marine shrimp impoundments by (i) stocking shrimp, (ii) controlling water quality, (iii) adding fertilizers to increase primary and secondary productivity, and (iv) applying pelleted feeds to increase productivity (Villalon & Preis, 1993)**.** Most worldwide aquaculture sites (90 percent) are small-scale and located in Asia, where aquaculture has increased by roughly eightfold since 1950 (FAO, 2016)**.** During 2015, approximately 76.6 million tonnes of aquaculture food products were collected globally, with farmed crustaceans accounting for 9.6% of the total. The primary outcome of crustacean farms and fisheries is euryhaline shrimp, with two penaeid species dominating farming: Pacific whiteleg shrimp *(Penaeus vannamei)* and giant tiger prawn *(Penaeus monodon)*. These penaeids are the two most valuable supplies in the international seafood trade, accounting for $19 billion and $5 billion in yearly market sales, respectively (FAO, 2017)**.** Even though most crustacean farming occurs in Asia and Central America, the main consuming regions are the United States, Japan, and Europe, supporting the industry's globalization and prosperity. Governments in underdeveloped nations that can cultivate shrimp have taken advantage of this opportunity to reduce poverty on a local level (Millard et al., 2020).

1.1.1 Relevant Problems

Accelerated increases in the shrimp industry and intensification of farming methods have coincided with the appearance of deadly diseases caused by intricate interactions between the host, pathogen, and environment. Diseases produced by pathogens *(Table 1)* have been projected to cause significant losses of annual marine and euryhaline shrimp harvests, posing a severe limitation on present and future production (Timothy W. Flegel, 2019; Lightner, 2011). Viruses are thought to be responsible for 60% of illness losses in shrimp farming, whereas bacterial pathogens are responsible for 20%. (vibriosis). Fungi and parasite losses, on the other hand, have been minimal (Timothy W. Flegel, 2012)**.**

Table 1. OEI listed crustacean diseases as of 2006 and those being considered for listing (Lightner, 2011)

Disease name	Pathogen type	Pathogen name & acronym	Principal host group
Taura syndrome	ssRNA virus	Taura syndrome virus (TSV)	Penaeid shrimp
White spot disease	dsDNA virus	White spot syndrome virus (WSSV)	Penaeid shrimp
Yellowhead disease	ssRNA virus	Yellow head virus (YHV) & Gill-associated virus (GAV)	Penaeid shrimp
Tetrahedral baculovirosis	dsDNA virus	*Baculovirus penaei*, BP	Penaeid shrimp
Spherical baculovirosis	dsDNA virus	Monodon baculovirus, MBV	Penaeid shrimp
Infectious hypodermal and hematopoietic necrosis (IHHN)	ssDNA virus	IHHN virus, IHHNV	Penaeid shrimp
Infectious myonecrosis (IMN)[a]	dsRNA virus	IMN virus (IMNV)	Penaeid shrimp
Necrotizing hepatopancreatitis (NHP)[a]	bacteria	NHP-bacterium (NHP-B)	Penaeid shrimp
Crayfish plague[b]	fungus	*Aphanomyces astaci*	Freshwater crayfish
White tail disease[b]	ssRNA virus	*Macrobrachium* nodavirus (MrNV)	*Macrobrachium rosenbergii*
Hepatopancreatic parvovirus disease[b]	ssDNA virus	Hepatopancreatic parvovirus (HPV)	Penaeid shrimp
Mourilyan virus disease[b]	ssRNA virus	Mourilian virus (MOV)	Penaeid shrimp

[a] Listing of this disease is under study by the OIE.
[b] Listed as emerging diseases by the OIE.

Shrimp virus illnesses account for seven of the nine crustacean diseases identified by the World Animal Organization (OIE). *(Table 1)* (including White Spot Disease, Yellow Head Disease, and Taura Syndrome) because of their socioeconomic significance to shrimp farming. Five of the seven penaeid shrimp viral infections are native to the Americas or have become enzootic as a result of their introduction (T. W. Flegel, 2006; Lightner, 2011)**.** The white spot syndrome virus (WSSV) is a major cause of production losses in crustacean aquaculture. Global losses from this disease have previously approached $3 billion per year, having a devastating impact on a $19 billion-per-year global sector (Millard et al., 2020).

Viruses aren't the only pathogens that can cause serious problems in the shrimp industry; bacterial diseases caused by vibrios are the leading cause of death in shrimp hatcheries, especially at the very early larval and juvenil stage (Kumar, Roy, Meena, & Sarkar, 2016; Ramirez et al., 2021). Bacterial diseases such as shrimp acute hepatopancreatic necrosis disease (AHPND) appeared in China in 2009. Since then, AHPND has resulted in significant decreases in shrimp production by up to 20 percent and financial losses for commercial producers worldwide (Hong, Lu, & Xu, 2016)**.**

Viruses and bacteria cause the central part of disease losses for shrimp growers. It has been concluded that future sustainable shrimp aquaculture will depend on developing more efficient biosecurity production. Effective pathogen monitoring and disease prevention approaches (Novel probiotics) are critical for establishing and managing aquaculture (Domínguez-Borbor et al., 2019; Timothy W. Flegel, 2019). Moreover, leading scientists are particularly concerned about these viruses and bacteria because it threatens the global food supply in the future, as aquaculture is one of the most significant food sources for meeting the growing need of a growing global population (Stentiford et al., 2012)**.**

1.2 Importance of shrimp Industry in Ecuador

The commercial shrimp *(Penaeus vannamei)* production industry in Ecuador represented an export value and subsequent generation in foreign revenues approaching U.S. $ 3.9 billion in 2020 (*Figure 1)* (CNA, 2020). The analysis provided by the Observatory of Economic Complexity (OEC) segments crustaceans as a cluster of productive specialization, which farmed shrimp and prawns dominate. According to the OEC, as of 2019, Ecuador ranks as the second-largest shrimp supplier globally, with India being the first producer. Within the Ecuadorian economy, the shrimp sector has become increasingly important, particularly concerning foreign trade. Ecuador's production represents 25% of local consumption, while the other 75% is exported. The main destination markets for shrimp are Asia, 60%; European Union, 20%; the United States, 18% and the rest of America, approximately 2% *(Figure 2)* (CEDIA, 2021).

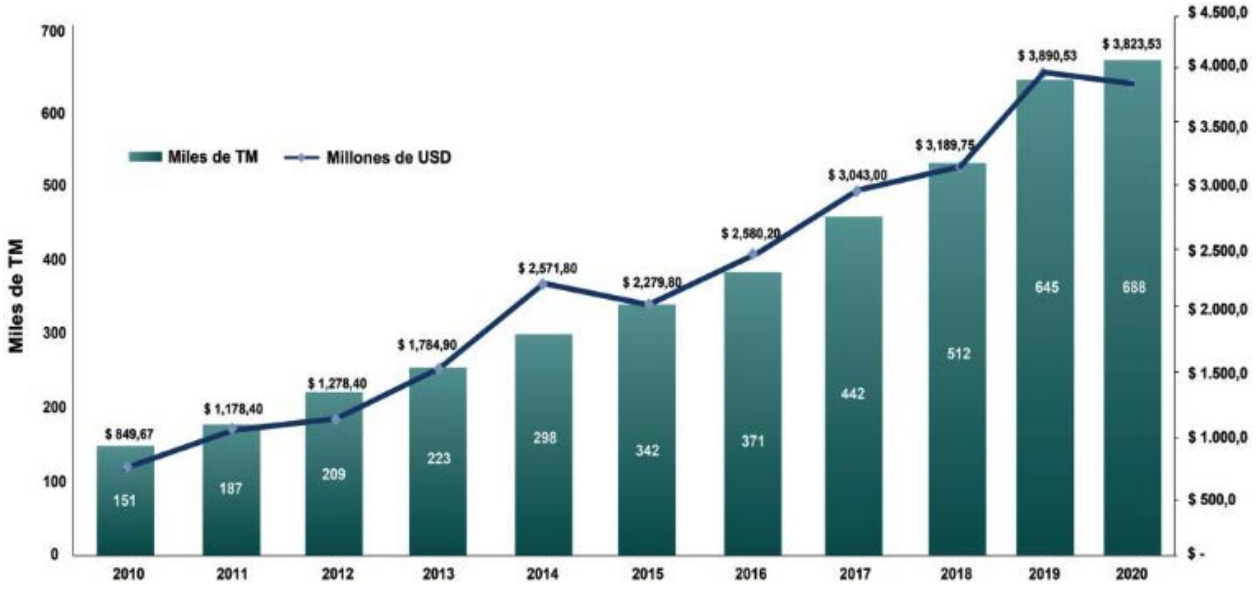

Figure 1: Shrimp - Total Ecuadorian Exports Report 2020 (CNA, 2020)

For two decades, Ecuador has considered the exported value of shrimp as an item worth taking into account; It should be noted that the product is among the primary ones (among which stand out: crude petroleum, bananas, coffee, cocoa, abaca, wood, tuna, fish, natural flowers and others); the industrialized is as a complement to primary products. It is interesting to compare the exported value in shrimp with the value that comes in from bananfa exports; thus, in 2015, it was 81%, and in 2020 it was 104%; In other words, in 2020, more profits were generated from the sale of shrimp to other countries than from the sale of bananas, when, since the middle of the last century, bananas were the product that produced the most profits (CEDIA, 2021). As a result, research aimed at

enhancing and safeguarding the Ecuadorian shrimp industry, primarily through the creation of novel, sustainable, and biocompatible products, is essential for the socioeconomic stability of the country.

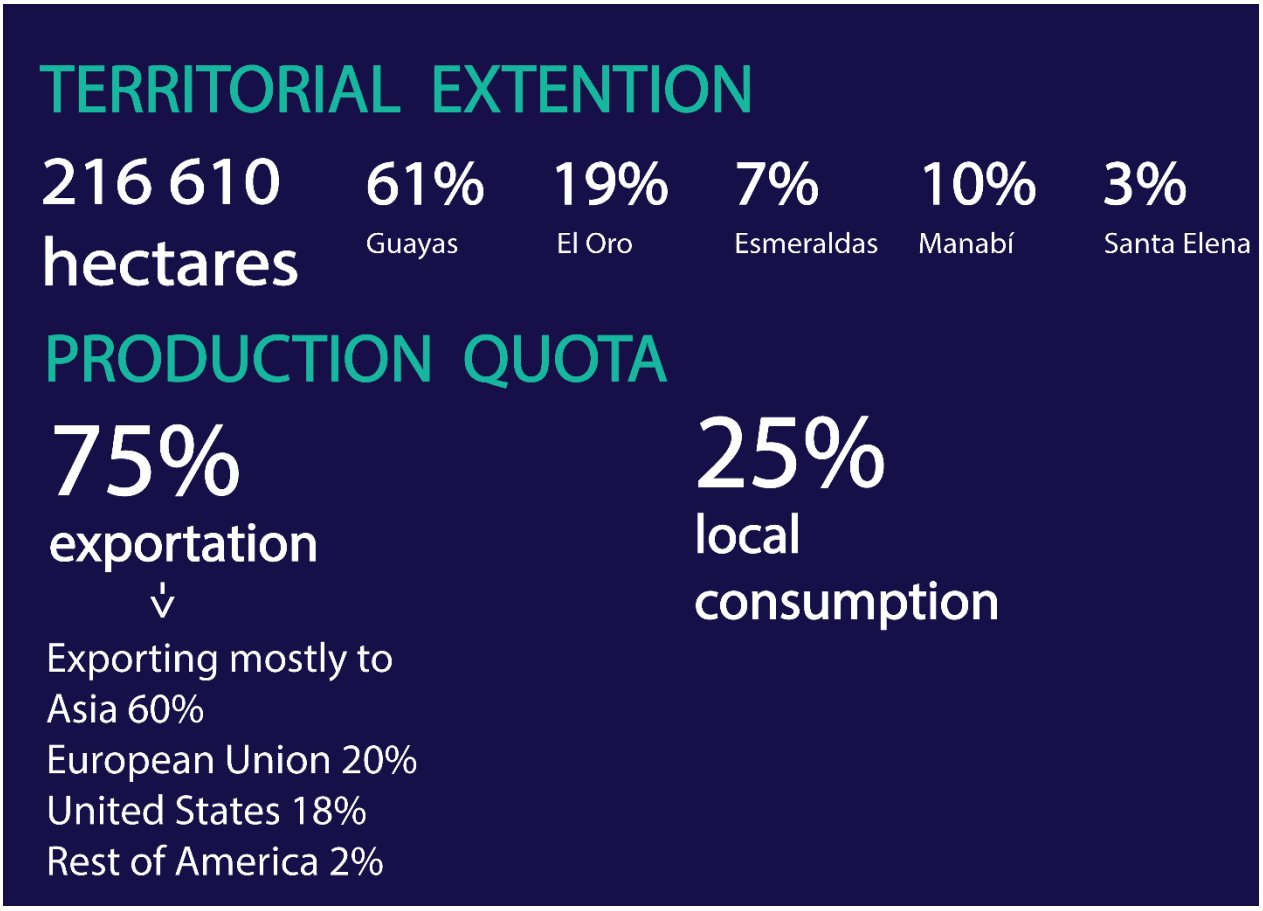

Figure 2: Overview Of Productive Sector Shrimp In Ecuador 2021 (CEDIA, 2021)

1.3 Cellulose Base Beads

Cellulose is the most prevalent organic polymer, accounting for around 1.5 x 10^{12} tons of total yearly biomass production, and is regarded as an essentially limitless source of raw material for the growing need for environmentally friendly and biocompatible products (Klemm, Heublein, Fink, & Bohn, 2005). Researchers in chemistry, chemical engineering, biology, and a variety of other fields who are involved in the chemical design of biobased materials are increasingly interested in manufacturing cellulose-based products. The biopolymer can be chemically changed in various ways to produce derivatives with varying characteristics, ranging from hydrophilic to hydrophobic, noncharged to anionic or cationic (Gericke, Trygg, & Fardim, 2013).

Cellulose can be formed into well-defined structures such as fibers of various geometries, films, sponges, or spherical particles known as cellulose beads. Cellulose beads are identified as (i) sphere material with microscale to millimeter-scale dimensions (ii) that are prepared in three stages: dissolution, shaping, and regeneration of the polysaccharide (iii) cellulose beads are primarily composed of cellulose and are fixed in their spherical form by restoring the hydrogen bonding structure and maintaining normal cellulose-cellulose connections (Gericke et al., 2013; Sescousse, Gavillon, & Budtova, 2011). Moreover, several techniques have been developed, with the primary differences being the solvent used and the methodology used to generate spherical particles.

Cellulose beads have been manufactured in various ways, including dropping, jet cutting, spinning drop atomization, spraying, and dispersion *(Figure 3)*(Ganesan et al., 2018; Gericke et al., 2013; Poshadri, 2010; Suganya & Anuradha, 2017). Producing spherical drops of a polysaccharide solution and consolidating them in a mold can produce beads in a nonsolvent coagulation bath. When the combined forces of gravity and pressure used for ejection reach a specific value determined by the surface tension of the solution and capillarity at the surface, a droplet is formed when the solutions are pressed through a thin aperture, such as a syringe needle (Ganesan et al., 2018).

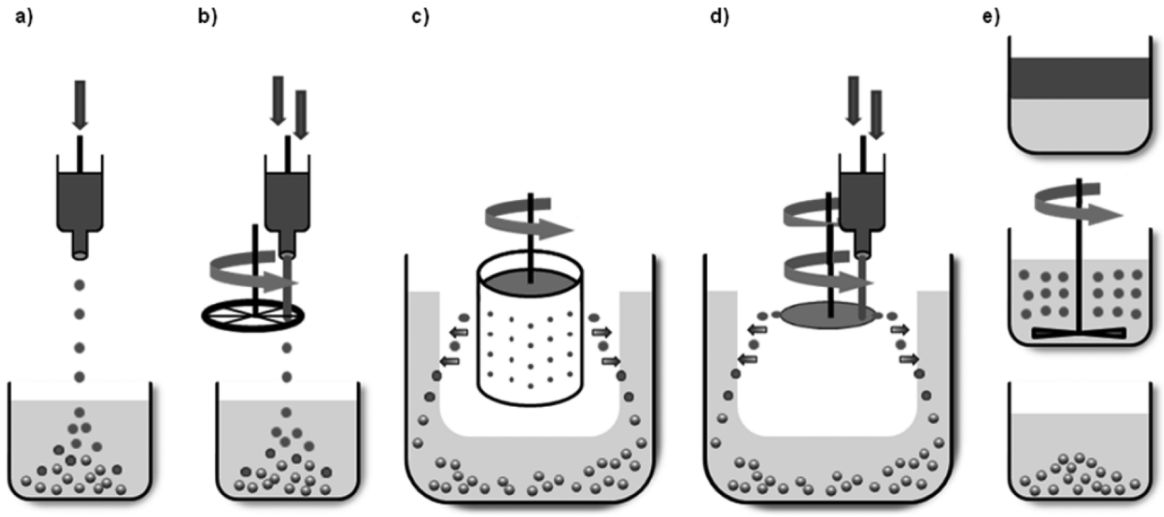

Figure 3: Dropping (a), jet cutting (b), spinning drop atomization (c), spinning disc atomization (d), and dispersion (e) are graphic representations of different operations for the fabrication of cellulose beads by different techniques (Gericke et al., 2013).

Due to cellulose's insolubility in water and conventional organic solvents, it is probably as a result of the development of a powerful inter and intramolecular hydrogen bonding network, several alternative solvents for dissolving, shape, and chemical derivatization have been documented

(Jarvis, 2003; Lue & Zhang, 2010). Figure 4 shows three solvent systems that can be identified*:* (i) Instead of chemically converting cellulose's hydroxyl groups, non-derivatizing solvents break it down through physical interactions. Disrupting these linkages, such as coagulation in an abundant of protic nonsolvent, allows polysaccharide reformation to occur. (ii) in most cases, derivatizing solvents change cellulose into derivative compounds that are only unstable under dissolution circumstances. By splitting the intermediate derivatives, which can be activated by adding water or changing the pH or temperature, cellulose can be regenerated and shaped into beads. (iii) commercially accessible stable cellulose derivatives that are soluble in conventional organic solvents. Following particle formation, the derivative is regenerated by coagulation or evaporation of the solvent in the presence of a nonsolvent (Gericke et al., 2013)**.**

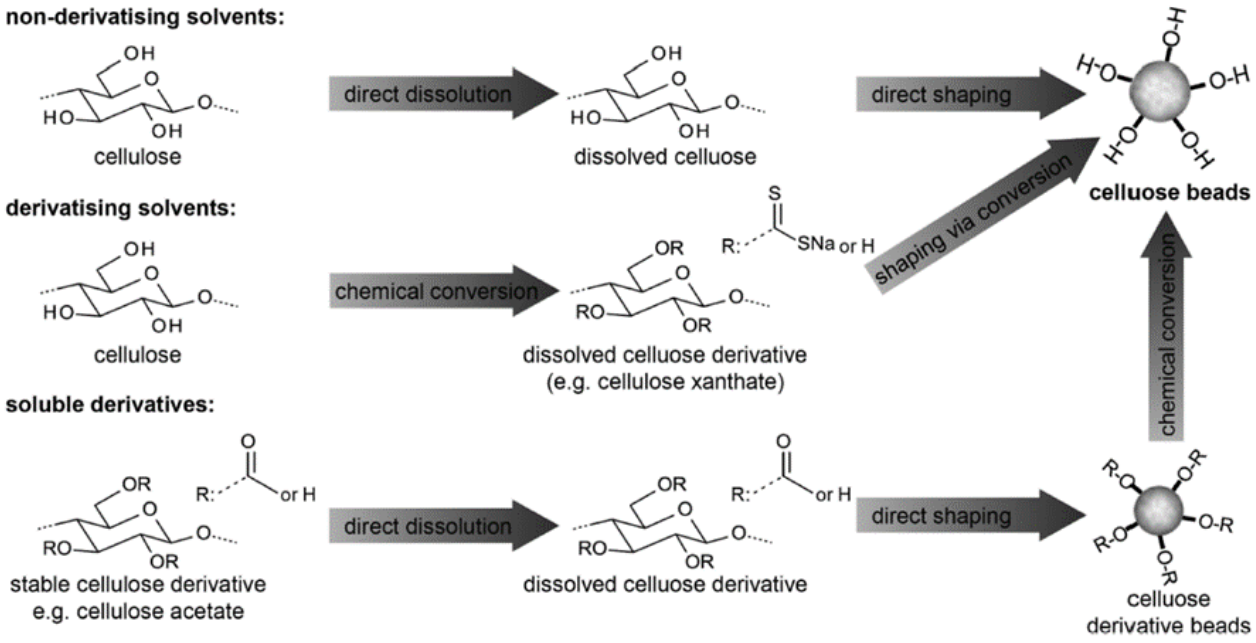

Figure 4: Dissolving cellulose and molding it into beads can be done in a variety of ways. (Gericke et al., 2013).

Novel cellulose solvents have gained substantial attention in the field of biopolymer research in recent years, supporting investigation on the manufacture of cellulose beads. In this context, aqueous NaOH solutions in combination with various chemicals that prevent gelation, such as Urea, thiourea, or ZnO, have sparked a lot of interest (Egal, Budtova, & Navard, 2008; Zhang, Ruan, & Gao, 2002).

Cellulose beads are valuable materials for a wide range of applications, particularly when the performance of cellulose beads is adjusted for a specific application by various physical properties such as size, shape, morphology, and chemically modified to improve their performance (Gericke

et al., 2013). Since their spherical shape, they are suitable filling materials for chromatographic columns because they can resist high flow rates. As a result, cellulose beads could be used as a stationary phase in size exclusion chromatography as well as a selective adsorbent for biological molecules such proteins, endotoxins, and viruses (Luo & Zhang, 2010; Xia, Lin, Wang, Chen, & Yao, 2008). Moreover, encapsulation of a large number of core materials like live cells, oils, pharmaceuticals, etc.

1.4 Encapsulation Beads

Encapsulation is a rapidly growing technology that surrounds or coats tiny droplets or particles of liquid or solid material with a continuous film of polymeric material. Encapsulation is used to turn liquids into solids, alter colloidal and surface properties, protect the environment, and manage the release characteristics of various coated materials. Live cells, adhesives, flavors, agrochemicals, enzymes, medicines, and other essential components can all be encapsulated (Suganya & Anuradha, 2017)**.**

Such particles can be utilized as a carrier or binder material in tables or as targeted/controlled drug release agents in pharmaceutical products (Bacakova et al., 2019; Volkert, Wolf, Fischer, Li, & Lou, 2009; Voon, Pang, & Chin, 2017). They can be employed as antibacterial agent carriers for deodorizing effects, scrubbing or peeling, or moisture binding in hygienic products. They can be utilized to entrap (heavy) metals, adsorb other contaminants, or act as ion exchangers in wastewater treatment (Fan, Liu, & Liu, 2010; Hirota, Tamura, Saito, & Isogai, 2009; Peng, Meng, Ouyang, & Chang, 2014). There are a variety of uses in chemistry and analytical procedures as well. These materials have virtually limitless applications, especially when manufactured from renewable and biodegradable resources such as cellulose or other natural polysaccharides. Food, healthcare, pharmaceutical, and hygiene items, in particular, must meet high purity criteria and be chemically inert and harmless (Rosenberg, Rom, Janicki, & Fardim, 2008).

1.5 Probiotic Encapsulation

Cell encapsulation has been shown to enhance probiotic bacteria' resistance to harsh environments and reduce cell losses in hydrogels matrices. Various probiotic encapsulation techniques are currently in use, with particles of multiple characteristics being generated. Extrusion, emulsion,

spray drying, spray chilling, and the fluidized bed is some of the most used methods for encapsulating probiotic organisms (Kailasapathy, 2002)**.**

For the stability and properties of the developed particles, selecting the suitable material for encapsulating microbial cells is fundamental. The encapsulating material must not be harmful, as it directly impacts the particle's shape, diameter, and permeability. It should also preserve microbial cells from external influences and perform well in controlled release situations *(Table 2).*

Table 2: Recent reports about effects of encapsulating materials used to entrap probiotic cells (F. J. Rodrigues, Cedran, Bicas, & Sato, 2020).

Encapsulating material	Probiotic	Technique	Effect	Reference
Microcrystalline cellulose–trehalose–maltodextrin–vegetable wax	*Lactobacillus casei* subsp. *paracasei* LMG P-21380	Fluidized bed	o High rates of encapsulation efficiency; o Protection enhanced during the encapsulation process and greater stability during storage	Semyonov, Ramon, Kovacs, Friedlander, and Shimoni (2012)
Chitosan–alginate–inulin	*Lactobacillus rhamnosus* GG	Extrusion	o No adverse organoleptic effect on apple juice; o Improvement in the cell survival in apple juice in 90 days of storage	Gandomi, Abbaszadeh, Misaghi, Bokaie, and Noori (2016)
Alginate–shellac	*Lactobacillus paracasei* BGP-1	Fluidized bed	o Improved the microcapsule structure by reducing the porosity; o Provided protection during storage at room temperature and in in vitro gastrointestinal simulation	Silva et al. (2016)
Alginate–flaxseed or okra mucilage alginate–botryosphaeran	*Lactobacillus casei* LC 01 *Lactobacillus casei* BGP93	Extrusion	o High encapsulation efficiency; o Improvement in the stability of encapsulated cells in refrigerated storage	Rodrigues et al. (2017)
Celulose–alginate	*Lactobacillus plantarum* IS-10506	Fluidized bed	o Increased the probiotic survival rate during gastric simulation	Surono, Verhoeven, Verbruggen, and Venema (2018)
Alginate–arabinoxylan	*Lactobacillus plantarum*	Extrusion	o High encapsulation efficiency and resistance to gastrointestinal condition that than alginate beads	Wu and Zhang (2018)
Alginate–goats' milk–inulin	*Bifidobacterium animalis* spp. *lactis* BB12	Extrusion	o Inulin addition resulted in compact structure capsules; o Protection of the probiotic in simulated gastrointestinal condition	Prasanna and Charalampopoulos (2019)
Alginate	*Lactobacillus casei* ATCC 393	Extrusion	o Protection of the probiotic in simulated gastrointestinal condition	Dimitrellou et al. (2019)
Alginate–chitosan	*Bifidobacterium longum* DD98	Emulsification; internal gelation	o Improvement of heat tolerance of the encapsulated cells; o Significantly protection under gastric acid and bile salt	Ji et al. (2019)
Maltodextrin–sucrose maltodextrin–sorbitol	*Saccharomyces cerevisiae* KTP *Issatchenkia occidentalis* ApC *Saccharomyces cerevisiae* var. *boulardii*	Spray drying	o Components did not alter the characteristics of maltodextrin encapsulation; o Sucrose and sorbitol enhanced the yeast survival in simulated gastric and bile juices	Suryabhan, Lohith, and An Appaiah (2019)
Alginate–calcium carbonate	*Bifidobacterium pseudocatenulatum* G7	Extrusion	o Probiotic survived throughout gastrointestinal tract when co-encapsulated with calcium carbonate	Gu et al. (2019)
Amidated low–methoxyl pectin	*Faecalibacterium prausnitzii*	Extrusion; freeze-drying	o Stabilization of encapsulated bacteria for 14 days; o Cell protection to stomach and distal jejunum simulated conditions	Raise et al. (2020)
Alginate–persian gum–prebiotics	*Lactococcus lactis* ABRIINW-N19	Extrusion	o High encapsulation efficiency; o Probiotic cell stability during refrigerated storage in orange juice	Nami, Lorneshad, Kiani, Abdullah, and Haghshenas (2020)
Alginate–flaxseed mucilage	*Lactobacillus paracasei* spp. *Paracasei*	Emulsion	o High encapsulation efficiency; o Resistance against the harmful effects of the simulated digestive system	Shafizadeh et al. (2020)
Whey protein–chitosan	*Kluyveromyces marxianus* VM004	Spray-drying	o Increase the viability during storage for 90 days at room temperature; o Improvement of the tolerance to simulated conditions of	Vanden Braber et al. (2020

Temperature and moisture levels can affect cell viability during probiotic particle storage, mainly lipid oxidation in the cell membrane. As a result, using materials that can retain humidity improves the survivability of encapsulated cells. Furthermore, materials that entirely release encapsulated cells when suspended in gastric secretions may not be suited for cell protection during passage through the gastrointestinal tract (Rajam & Anandharamakrishnan, 2015).

Several polysaccharides, proteins, and lipids have been employed to encapsulate probiotic microorganisms, with natural water-soluble polymers and their mixtures being particularly popular. Their uses allow for the employment of gentler procedures, such as extrusion, which improves the cellular integrity of encapsulated microorganisms (Rathore, Desai, Liew, Chan, & Heng, 2013).

Alginate is still the most commonly employed wall material to entrap probiotics. Its relatively moderate characteristics and application conditions promote the encapsulation of thermosensitive agents, such as microbial cells. Nevertheless, there is growing interest in the total or partial substitution of this anionic polysaccharide by polysaccharides derived from natural sources, such as plants and microorganisms. Which can change particle properties and improve the protection and survival of encapsulated cells during storage and passage through simulated gastric and intestinal tracts (Rathore et al., 2013; F. J. Rodrigues et al., 2020).

2 PROBLEM STATEMENT

2.1 Current Shrimp Treatment

Infectious disease is still a significant issue in aquaculture around the world, and there are a variety of ways to reduce the impact of infections on farmed aquatic animals *(Table 3).* Many countries have licensed antimicrobial medications, including antibiotics, to treat bacterial infections in aquaculture animals (Kumar et al., 2016). They were using a combination of biosecurity and the practice of culturing domesticated specific pathogen-free (SPF) stocks (Lightner, 2011). Furthermore, improved nutrition and feed (Tacon, Jory, & Nunes, 2013) and the use of probiotics to avoid disease outbreaks.

Table 3: Methods of controlling diseases in aquaculture (Newaj-Fyzul, Al-Harbi, & Austin, 2014).

Method	Comment
Husbandry/ management	Includes improved hygiene including sanitary disposal of dead animals; do not overstock and over feed
Movement restrictions	Effective at preventing the spread of diseases; essential to have governmental support
Genetically resistant stock	Emotive if it involves genetic modification; useful if selecting naturally disease-resistant strains
Dietary supplements	Effective with compounds such as vitamin C
Non-specific immunostimulants	Success with some products, such as β-1,3-glucans
Vaccine	Available commercially for a minority of diseases
Probiotics	A wide range of probiotics has been considered for use in aquaculture
Prebiotics	Compounds that support the growth of probiotics; of increasing interest to aquaculture
Medicinal plant products	A wide range of plants considered particularly in China and India; may be immunostimulatory
Water disinfection	Involves chemicals which may be effective at reducing or eliminating populations of pathogens
Biological control	The application of inhibitory micro-organisms often to water; may be effective but some concerns over the fate of the inhibitors
Antimicrobial compounds	There are emotive issues in many countries about the non-medical use of medicinal compounds

Antibiotics are being used more extensively and severely in shrimp farms as a preventative treatment for bacterial infections. Antibiotic use has been linked to the development of antibiotic resistance and eliminate beneficial bacteria necessary for aquatic animals' proper development (Cabello, 2006). Antibiotic use limitations in aquaculture are being enforced, and antibiotic residues in aquaculture products are being eliminated. As a response, probiotics have emerged as a possible antibiotic alternative as well as a method for treating and preventing illnesses in aquaculture. (Defoirdt, Sorgeloos, & Bossier, 2011; Okocha, Olatoye, & Adedeji, 2018).

Probiotics are organisms that are known to have therapeutic effects on their hosts, such as avoiding harmful bacteria colonization through antagonism or increasing animal health through immune system stimulation (Domínguez-Borbor et al., 2019). Moreover, probiotics are well documented for their capacity to change the gut microbiota of shrimp. Probiotics compete against infections by secreting antibacterial chemicals, preventing their adherence to the gut epithelium, fighting for nutrition, and creating antitoxin effects (Hai, 2015; Vargas-Albores et al., 2017).

Several commercial probiotics are available, most of which are based on the *Lactobacillus* and *Bacillus* bacterial strains (Le & Yang, 2018; Talukder Shefat, 2018; Thammasorn et al., 2017). Furthermore, novel marine probiotic bacteria capable of controlling pathogenic *Vibrio* spp. to shrimp have been discovered. Such as the probiotics *Vibrio diabolicus* (Ili), *Vibrio hepatarius* (P62), and *Bacillus cereus sensu stricto* (P64) colonize internal and external surfaces of *Penaeus vannamei* shrimp larvae and protect them against *Vibrio parahaemolyticus* (Ramirez et al., 2021).

Recent aquaculture research seeks to create suitable probiotics administration requirements (viability and stability) and understanding the impact of probiotics on the structure and health effects of the host-associated microbiota (Restrepo et al., 2021). Moreover, probiotic encapsulation has been showing a promising delivery methodology for enhance probiotic bacteria' resistance to harsh environments, as well as reduce cell losses in hydrogels matrices.

3 HYPOTHESIS AND OBJECTIVES

3.1 Hypothesis

Cellulose-based beads could be produced from extracted organic/plant waste to encapsulate marine probiotics.

3.2 General Objective

To evaluate encapsulation efficiency of cellulose-based beads elaborated from organic/plant waste with the purpose of extending marine probiotics bioavailability.

3.3 Specific Objectives

- To extract cellulose from the selected organic/plant waste.
- To synthesize cellulose-based beads made from organic waste.
- To characterize the beads using X-ray diffraction (XRD), Fourier transforms infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) in order to analyze their structure, morphology, size, and composition.
- To measure the capacity of encapsulation of the different cellulose-based beads.

4 METHODOLOGY

4.1 Materials

Table 4: Materials and Reagents

Raw material	A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12
Equipment	Freezer, Pipets, Sonicator, Centrifuge, Analytic Balance, Vortex, Autoclave, Lyophilizer, Volumetric Flask, Beakers, Falcon Tube, Syringes, Syringes Filters, Cell Culture Dishes.
Probiotics Strains	*Vibrio diabolicus* (Ili), *Vibrio hepatarius* (P62), and *Bacillus cereus sensu stricto* (P64).

* To all procedures, all reagents and materials must be sterilized.

* All raw materials are derived from organic waste collected on plantations and marketplaces.

The cellulose extraction process was applied to various plant parts and fruits (Raw Material), and the extracted celluloses were lyophilized and analyzed using SEM (Scanning Electron Microscopy), XRD (X-ray Diffraction), and FTIR (Fourier-Transform Infrared Spectroscopy). After characterizing each of them, the next step was to standardize the beads protocol fabrication, test their encapsulation capabilities, and finally describe the beads using SEM. The characterizations revealed some essential descriptive properties such as crystallinity, the presence of functional groups, morphology, and encapsulation capability.

4.2 Cellulose Extraction

Raw material *(Table 4)* was collected from plants and fruits that had been cleaned, the areas of interest were extracted, oven-dried for at least one day, and ground. Cellulose extraction was performed using a chemical extraction procedure developing in the lab that remains confidential. The impurities were washed with chemicals to remove non-cellulosic components such as lignin, bioactive compounds, and pigments, in addition, followed by multiple water-washings to remove undesired components. The exact same process was applied to each sample to prevent any effect on the properties of the materials due to the extraction protocol. Finally, the cellulose samples were dried using the freeze-drying technique (Chang, Zhang, Zhou, Zhang, & Kennedy, 2010; Sescousse et al., 2011).

4.3 Beads preparation standardization

A standardization procedure was applied to improve formation, morphology, and integrity properties and reduce the capsule size of cellulose-based beads. The correlation between the shape of the beads made from pure cellulose (without particles) and the processing parameters was established. For the beads, synthesis was carried out using a syringe via dropping technique. The effect of the temperature of the solvent was studied through the bead's solution preparation. For each experiment, the temperature was repeated two times to verify any difference in the effect. The effect of the cellulose extracted *(Table 4)* was tested. Only one variable was studied at a time, and the others were kept constant. The results obtained were then used for making spherical beads with encapsulated particles.

4.4 Preparation of encapsulating beads

For the standardization of cellulose with encapsulated Probiotic Marine Bacteria P64, P62 & ILI were encapsulated within cellulose solution using a dropping. Briefly, 4% (w/v) cellulose solution was prepared. One method was applied the sterile coagulation solution (18 mL) was mixed with 2 mL of $\sim 2.6^{7}$CFU/mL probiotic organisms suspended in coagulation solution. Cellulose solutions were directly taken from the Eppendorf and extruded through a 1 ml syringe tip into the coagulation solutions. The height of the tip was manually adjusted in situ 1–1.5 cm above the surface to gain as spherical beads as possible, and stirring was applied only occasionally to even concentration gradients. Beads were prepared in beakers of 50 ml filled with 20 ml of coagulation solution in order to exclude possible effects due to changes of the surface tension of the coagulation media, sinking height, etc.

Coagulated beads were washed with solution saline and left in saline water at room temperature for further use.

4.5 Characterization

4.5.1 Cellulose Fibers

The extracted cellulose was examined using microscopic and spectroscopic approaches to study their structural features. SEM was employed as the microscopy approach. FTIR and XRD were employed as spectroscopy techniques.

The Fourier-transform infrared (FTIR) is an analytical technique that may generate an Infrared spectrum of absorption, emission, and solid-liquid or gas photoconductivity. FTIR spectrum is utilized as a chemical fingerprint to characterize new materials or to identify and validate known and unknown samples (Mandal & Chakrabarty, 2011). Then, it was used to analyze and determine the functional groups of interest from the cellulose isolated as well as the content of other compounds such as lignin. It was also utilized to compare the extracted cellulose to the spectra of a commercial control sample. The natural cellulose fibers were isolated from several plant sources using the same extraction method.

A small quantity of each sample was deposited on the equipment's disk, and scanning was

performed on the PC that generates the IR spectrum, and measurements were taken with an Agilent Cary 630 FTIR Spectrum spectrometer. In this scenario, the FTIR was used to determine the functional groups of the cellulose fibers and to ensure that no additional molecules, such as hemicellulose or lignin, were present.

The crystal lattice was seen, and the structural arrangement of atoms and molecules in the cellulose was determined using the X-ray diffraction technique. The peak intensities exhibited by X-ray diffraction patterns indicating properties like crystallinity, grain size, crystal orientation, and other structural parameters are defined by the atomic location and the lattice planes. To obtain total and uniform X-ray exposure, each cellulose sample in the form of milled powder was placed on the sample holder and leveled. In a sealed tube CuK radiation source, the X-ray generator was operated at 40 kV and 15 mA. The samples were analyzed using an of powder diffractometer Rigaku Miniflex-600, equipped with a D/tex Ultra2 detector. Configuration in the scan-axis, 0.02 ° step, and 20.0 °/min scan velocity in a range of 5-90° in 2 were used to collect data, as well as the D/tex Ultra2 detector in 1D scan mode.

The crystallinity index can be determined using the XRD patterns to evaluate the mechanical properties of the fibers based on this parameter. *Equation 1* was used to determine the crystallinity index (Mehanny et al., 2020)**.**

$$I_c = \frac{I_{(200)} - I_{(am)}}{I_{(200)}} \times 100 \qquad (1)$$

Where Ic is the crystallinity index (%), I (200) is the maximum diffraction peak intensity that represents the material, and I (am) is the amorphous material's minimum diffraction peak intensity. The material is almost amorphous at a 2θ close to ~18° (Costa et al., 2015; Mandal & Chakrabarty, 2011)**.**

4.5.2 Cellulose Beads

Scanning electron microscopy (SEM) was employed in this study to analyze the morphology and surface structure of several cellulose fibers. Besides, SEM is the most commonly used technique to characterize cellulose beads' size, shape, and morphology. It allows for a qualitative assessment of the bead surface pore structure. SEM photographs of cellulose bead cross-sections can provide

data on lateral shape (Gericke et al., 2013). A variable pressure scanning electron microscope Phenom ProX desktop was used to scan the surface topography of extracted fibers prior to the beads production and after. A layer of carbon (graphite) was put to the samples, making them conductive enough to be investigated under the scanning electron microscope with enhanced image quality.

4.5.3 Probiotic Encapsulation Efficiency (EE)

The EE was calculated using a straightforward method based on viable cell counts in solutions produced before and after the bacterial cells were microencapsulated. *Equation 2* was used to calculate the percentage efficiency of the encapsulation process using the data obtained.

$$EE\ (\%) = \frac{X_t}{X_i} \times 100 \qquad (2)$$

Where Xt is the number of cells in the microspheres and Xi is the number of cells injected into the polymer solutions at the start. (Rajam & Anandharamakrishnan, 2015; Fábio J. Rodrigues et al., 2017).

5 RESULTS

5.1 Characterization

5.1.1 Fourier-transform infrared spectroscopy (FTIR)

As illustrated in *Figures 5 & 6*, each cellulose isolate exhibited distinct physicochemical features. The fibers have characteristic cellulose peaks such as C-C, C-OH, C-H ring, and side group vibration bands that appear. The FTIR spectrum of the celluloses shows a band at 3334 cm^{-1} is assigned to hydroxyl groups stretching (–O–H stretching) and a band at 1643 cm^{-1}corresponding to the bending modes of the surface hydroxyls (Chen et al., 2018). Bands at 2894 cm^{-1} (–C–H stretching) and 1369 $cm^{-1}$are assigned to stretching and bending vibrations of C-H group in glucose unit. The peak observed in the spectra of all samples at 1054 cm^{-1} is due to the C–O–C pyranose ring (antisymmetric in phase ring) stretching vibration (Abderrahim et al., 2015; Jia, Li, Ma, Zhu, & Sun, 2011).

The C–C ring breathing band at $\sim 1155 cm^{-1}$ and the C–O–C glycosidic ether band at 1105 cm^{-1}both of which arise from the polysaccharide component is getting gradually lost in the cellulose extraction process because of hydrolysis and reduction in molecular weight. The absorption band at 898 $cm^{-1}$is characteristic of β-glycosidic linkage between glucose units (Mandal & Chakrabarty, 2011). The peaks from $900 cm^{-1}$to $1200 cm^{-1}$are associated with: –OH absorption, 900 cm^{-1}; –CH absorption $1029 cm^{-1}$; –C–OH absorption 1112 cm^{-1}; –C=O absorption $1165 cm^{-1}$; =CH2 absorption $1200 cm^{-1}$; that are all groups in the glycosyl units of cellulose (Doncea et al., 2010). Moreover, the classic fingerprint of cellulose is an absorption peak positioned between 1650 and 900 cm^{-1}. The absorption between 1430 and 896 cm-1 is employed to investigate the crystalline structure and glassy portion of cellulose, correspondingly (Auta, Adamus, Kwiecien, Radecka, & Hooley, 2017).

Spectra were beneficial in identifying cellulose content as well as remaining molecules of cell wall components like hemicellulose or lignin. The spectra of the samples match those of commercial cellulose, demonstrating that organic waste extracts are primarily constituted of cellulose (Mehanny et al., 2020). *Figure 7* depicts the typical spectra of commercial cellulose. As can be seen, both commercial cellulose and cellulose manufactured for cellulose beads exhibit the same peaks *(Figure 8).* The cellulose that did not generate beads is shown by the spectrums in *Figure 5*. There were two cellulose fibers that formed beads and ten cellulose fibers that did not form beads.

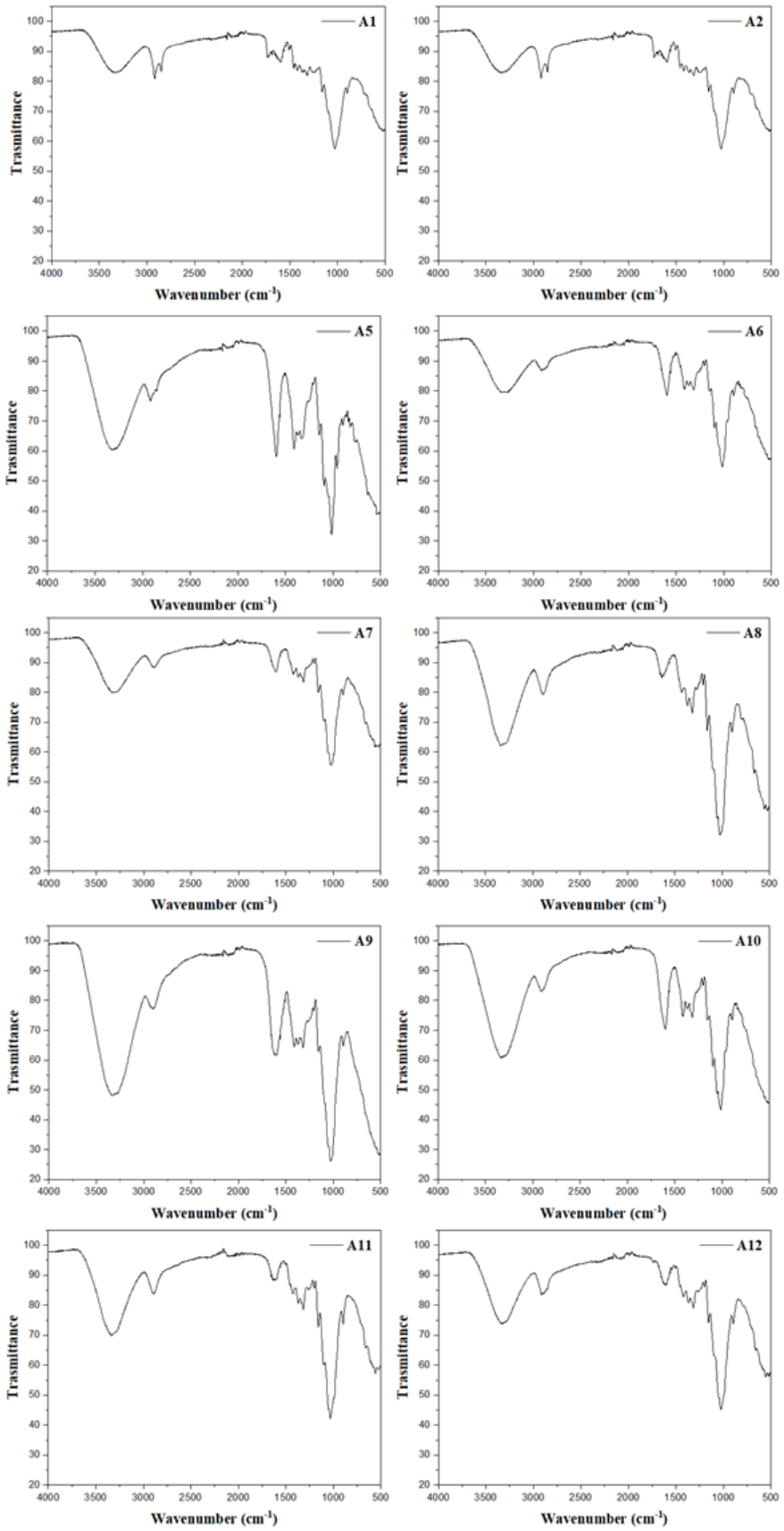

Figure 5: FT-IR comparison of the eight cellulose fiber samples that did not form cellulose beads.

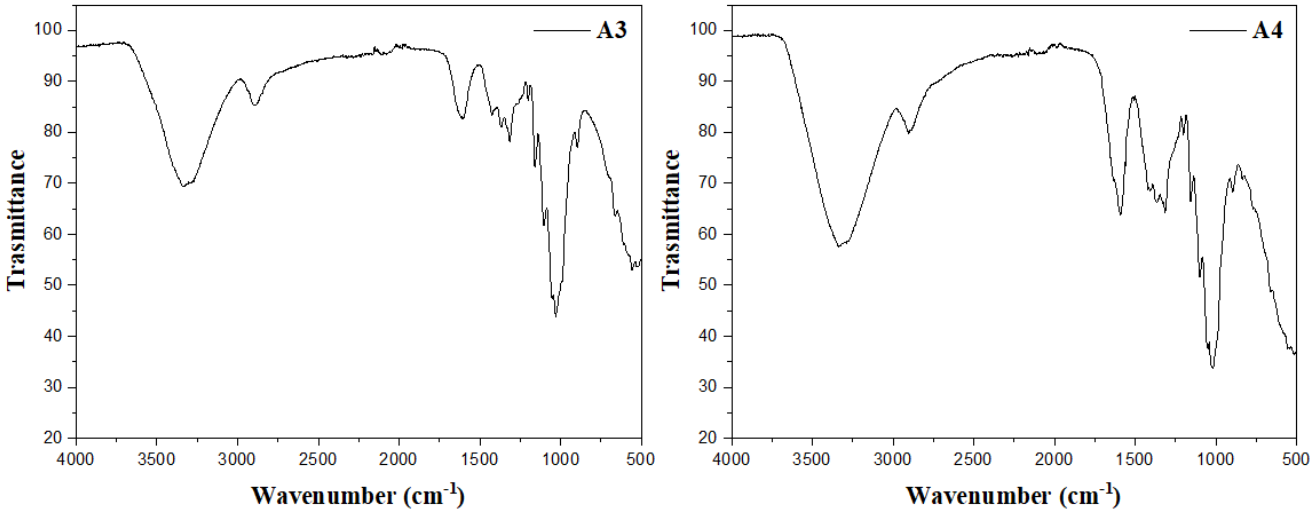

Figure 6: FT-IR comparison of the eight cellulose fiber samples that did form spherical cellulose beads.

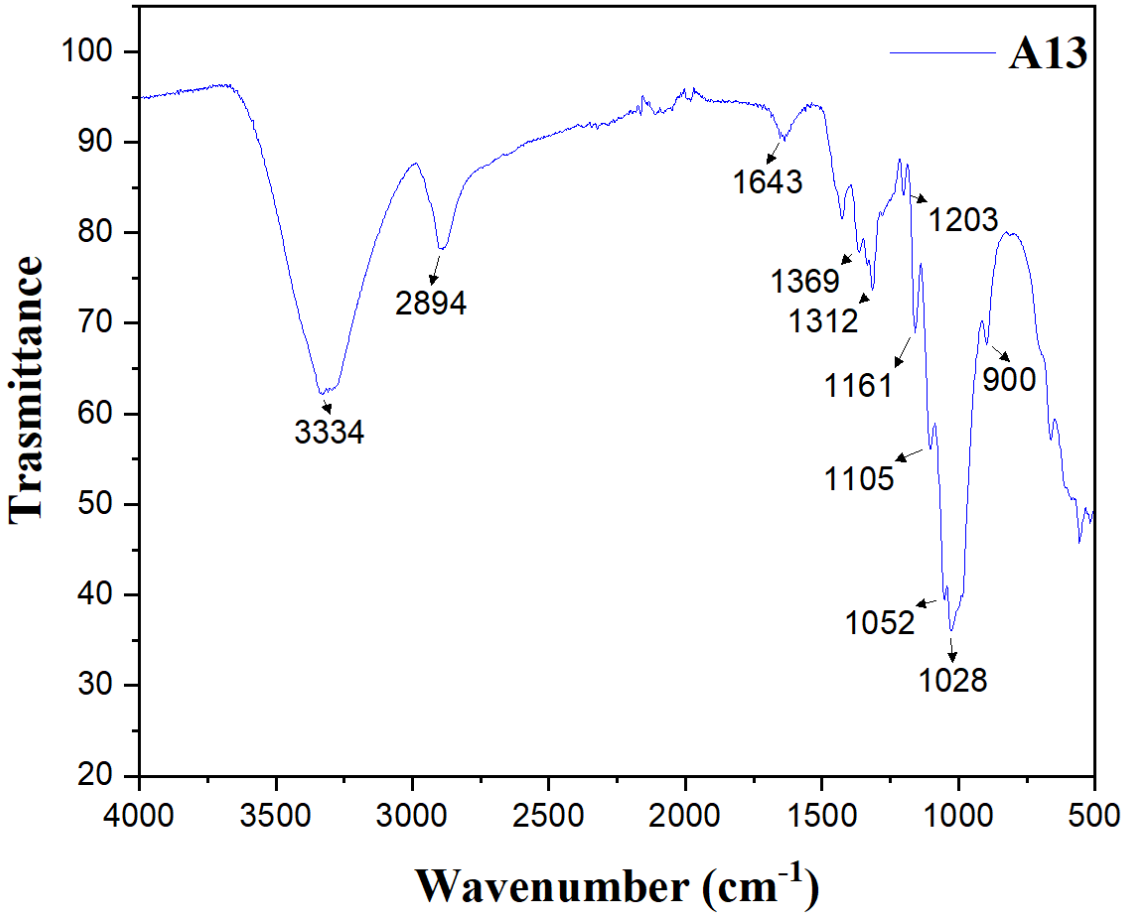

Figure 7: FT-IR spectrum of commercial cellulose.

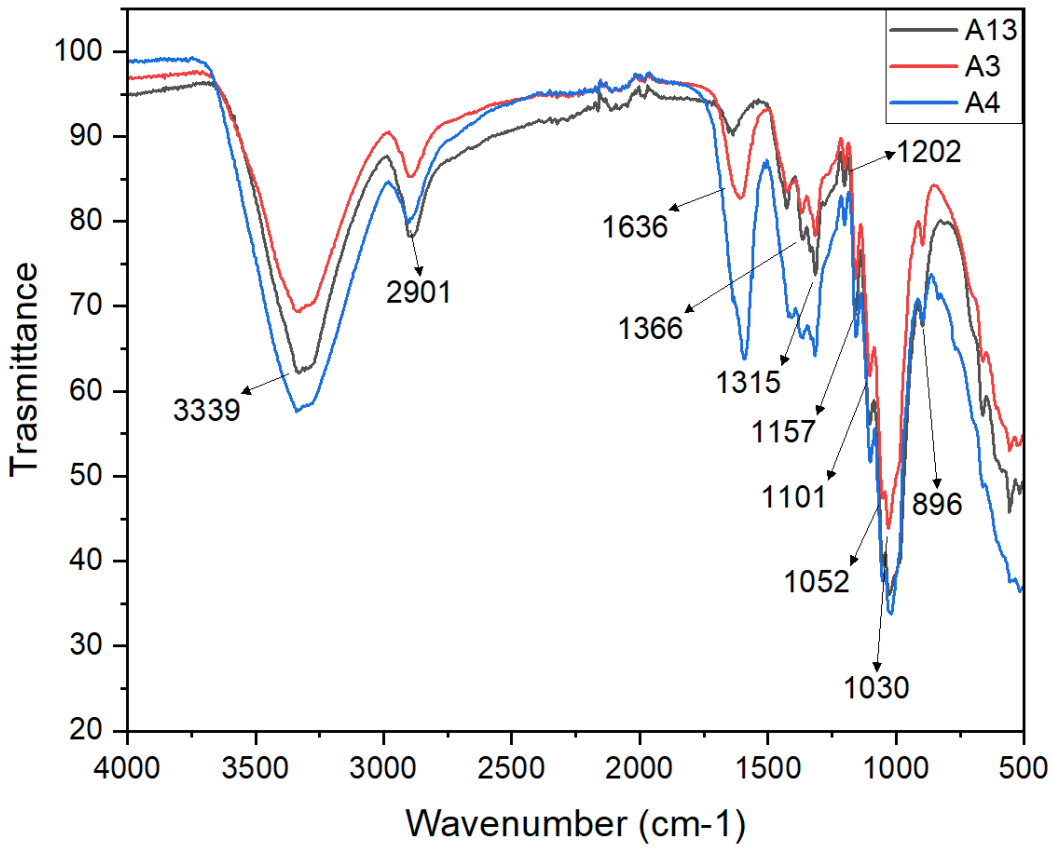

Figure 8: Comparison of FT-IR spectrum of commercial cellulose (A13) VS extracted cellulose (A3 &A4).

5.1.2 X-Ray Diffraction (XRD)

The crystallinity of the cellulose fibers was analyzed through X-Ray Diffraction. The X-ray generator was operated at 40 kV and 15 mA, using a sealed tube CuKα radiation source. XRD analyses of the twelve extracted and one commercial cellulose (A13) samples depicted distinct graphs and thus different degrees of crystallinity for each, as shown in *Figures 9 and 10*. Most of the peaks around 2θ = ~ 21.8° are cellulose crystallinity structure while the ones around 2θ = ~17.8° represent samples amorphous region (Kim, Lee, & Kafle, 2013; Mandal & Chakrabarty, 2011).

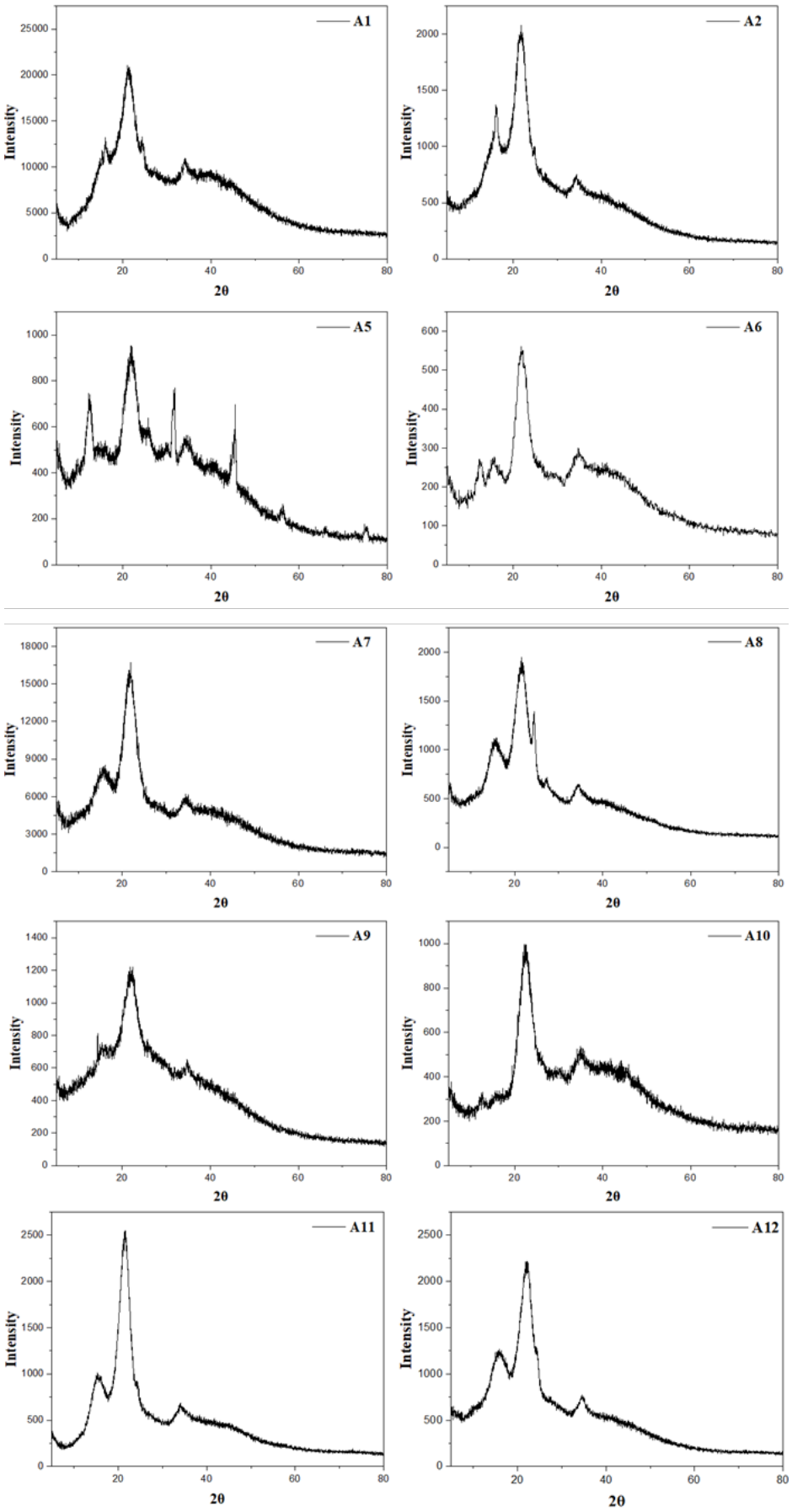

Figure 9: XRD graphs of the ten cellulose samples that did not form beads.

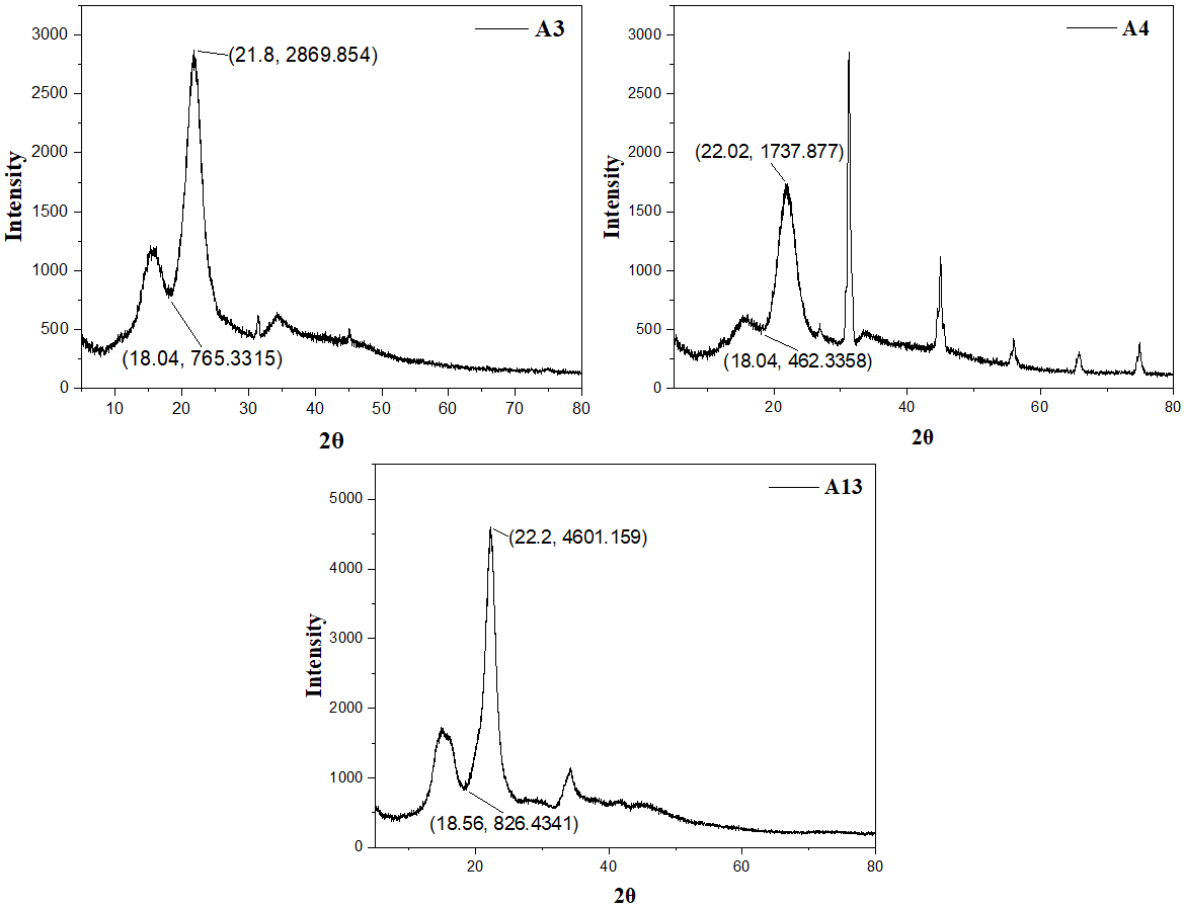

Figure 10: XRD graphs of the three cellulose samples that formed beads.

The calculation of the crystallinity index for each of the fibers *(Table 5)* shows that the cellulose fibers with a crystallinity index of more than 73.3% could form beads and had outstanding mechanical capabilities. Because the twelve cellulose samples were extracted using the same chemical process, the discrepancies in XRD patterns and crystallinity index could be related to the organic waste samples' origin.

Table 5: Crystallinity index of twelve extracted and one commercial cellulose samples, whereby the highest degrees of crystallinity belong to the ones that showed a better beads formation (Extracted=A3, A4 and Commercial =A13).

Fibers	Crystallinity Index
A1	51.12%
A2	55.05%
A3	73.33%
A4	73.40%
A5	54.62%
A6	62.91%
A7	62.70%
A8	58.86%
A9	46.53%
A10	72.31%
A11	72.60%
A12	57.56%
A13	82.04%

5.1.3 Scanning Electron Microscope (SEM)

The cellulose fibers and beads' porosity, morphology, and size were determined and analyzed using SEM. Six different cellulose samples were used for this characterization. The samples were sorted into two groups: three from those that generated cellulose beads (A3, A4 & A13) and three from those that did not (A1, A8 & A12). Even though the same extraction processes were employed for each sample, SEM studies of the cellulose fibers reveal that each sample has a distinct porosity, morphology, and size.

Figure 11 of the SEM study demonstrates the morphology of the compact cellulose fibers. However, A3 & A4 are irregular, and A4 & A13 have low levels of porosity. In addition, the structure of these fibers includes internal gaps. The cellulose fibers seen in *Figure 12* are porous, uneven, and rough. Furthermore, these fibers do not create a compact morphology.

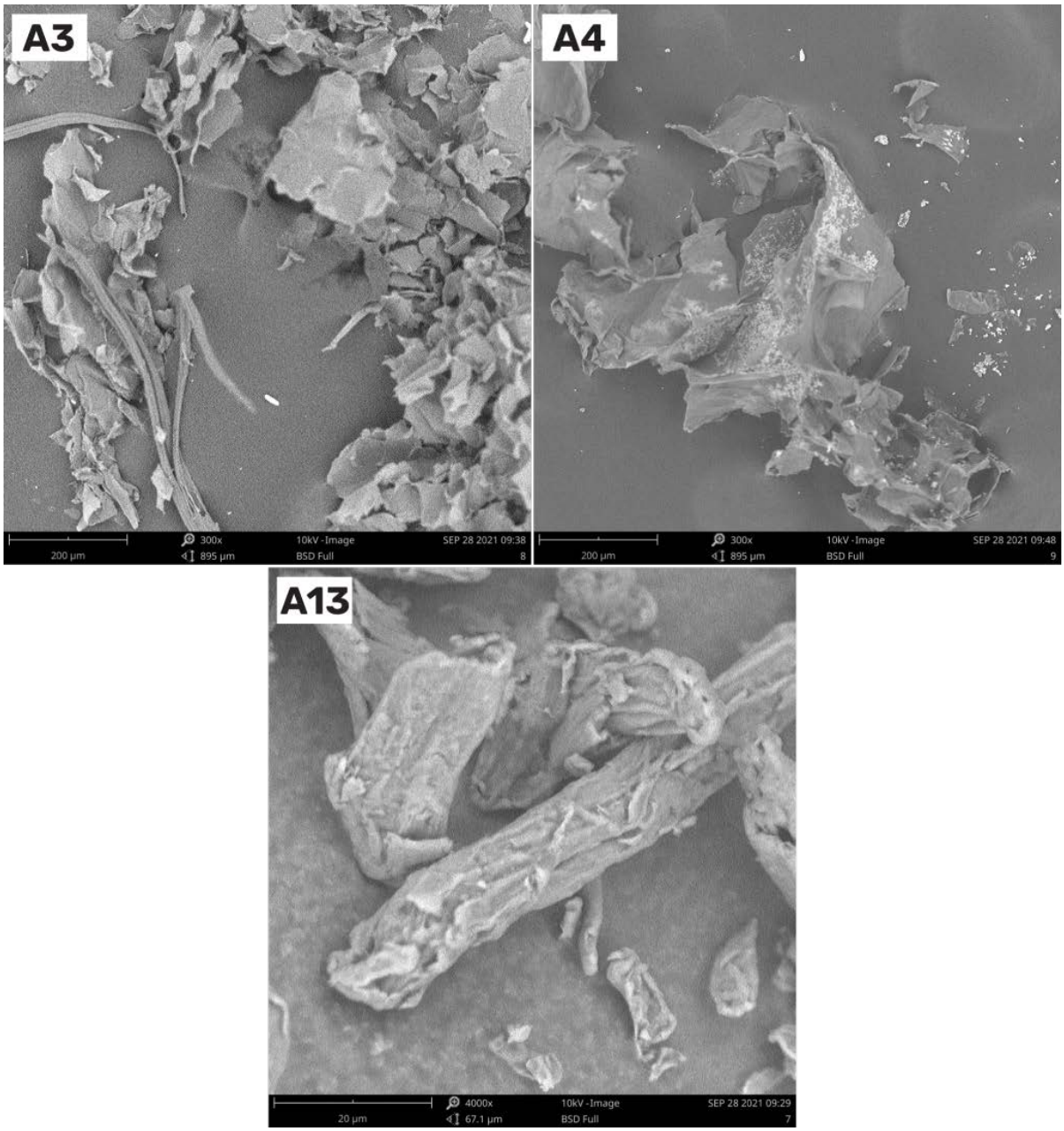

Figure 11: SEM micrographs of cellulose particles that form beads.

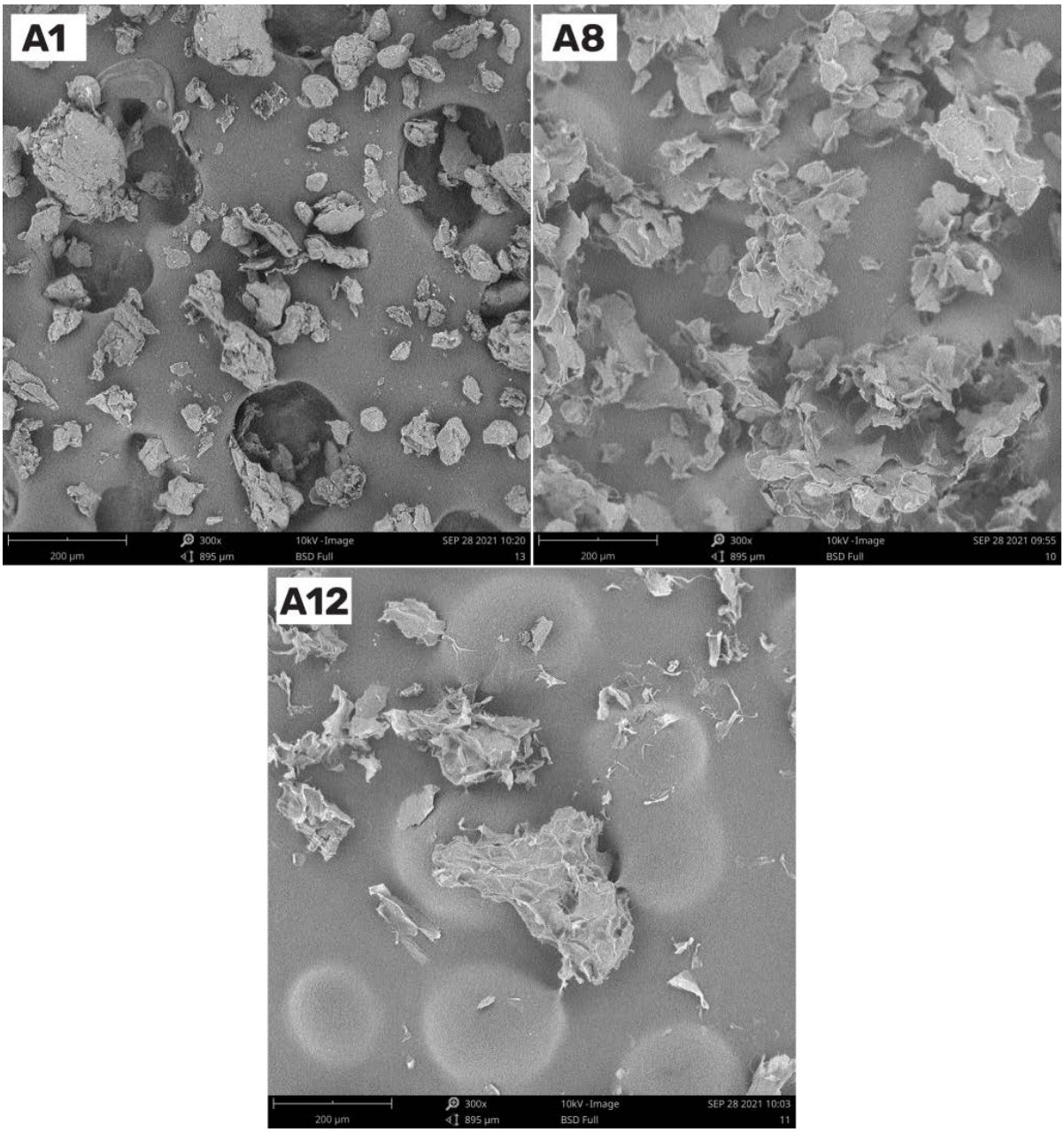

Figure 12: SEM micrographs of cellulose particles that did not form beads.

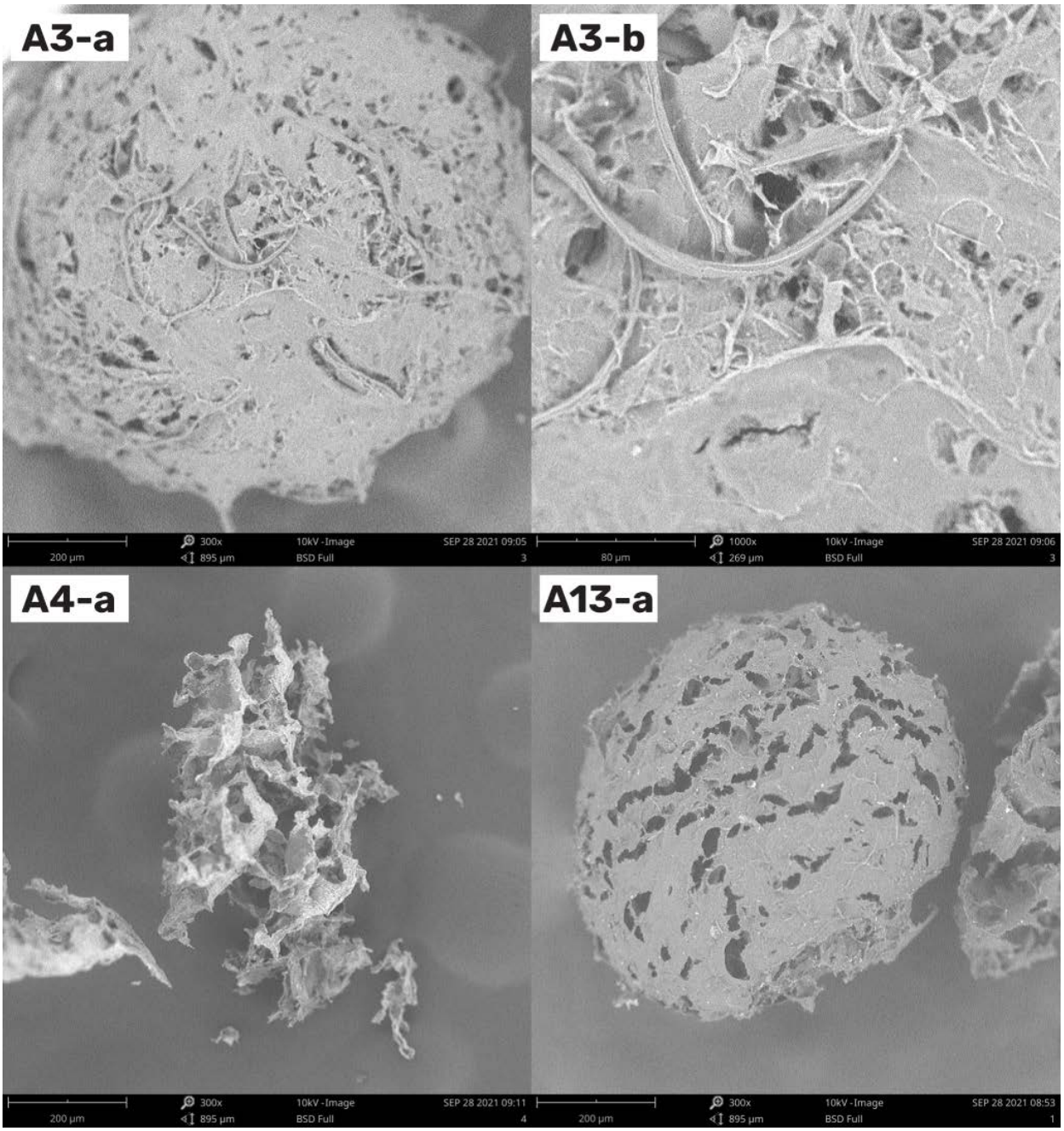

Figure 13: SEM micrographs of cellulose beads.

5.2 Beads Formation

There have been no noticed difficulties throughout the beads synthesis procedure. Thus, I am convinced that some cannot produce cellulose beads (A1, A2, A5, A6, A7, A8, A9, A10, A11, A12). *Figure 14* shows the macroscopic appearance of the cellulose fibers that did not form beads. Moreover, cellulose beads with excellent mechanical strength, spherical and micro-sized as possible by dropping technique and excellent formation were obtained in a coagulation medium by the chemical crosslinking with the extracted cellulose and commercial. *Figure 14* shows the

macroscopic morphology of the cellulose fibers, which did not form beads and confirm the characterization made previously (XRD, FTIR).

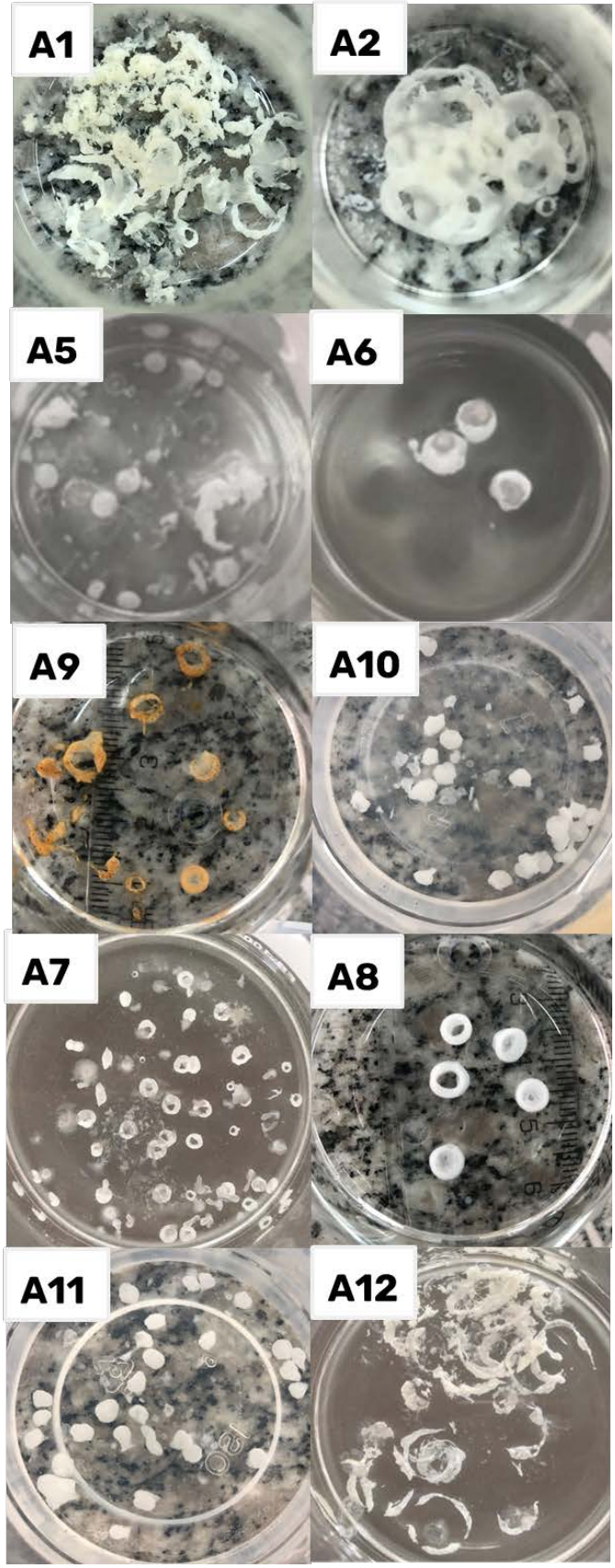

Figure 14: Macroscopic morphology of cellulose solution that did not form beads.

The other extracted cellulose: A3 & A4, did produce a spherical as possible; therefore, they resulted in beads formation. The macroscopic morphology from beads coagulation is shown in *Figure 15*. Some beads are not entirely spherical nor present in large quantity due to the mechanical properties of the beads vary depending on the material; thus, each one was made using the same procedure. The resulting beads presented a solid and hard consistency. The pH of the beads was ~10.8 as measured with pH strips. This pH can be neutralized by baths with distilled water or acidic solutions.

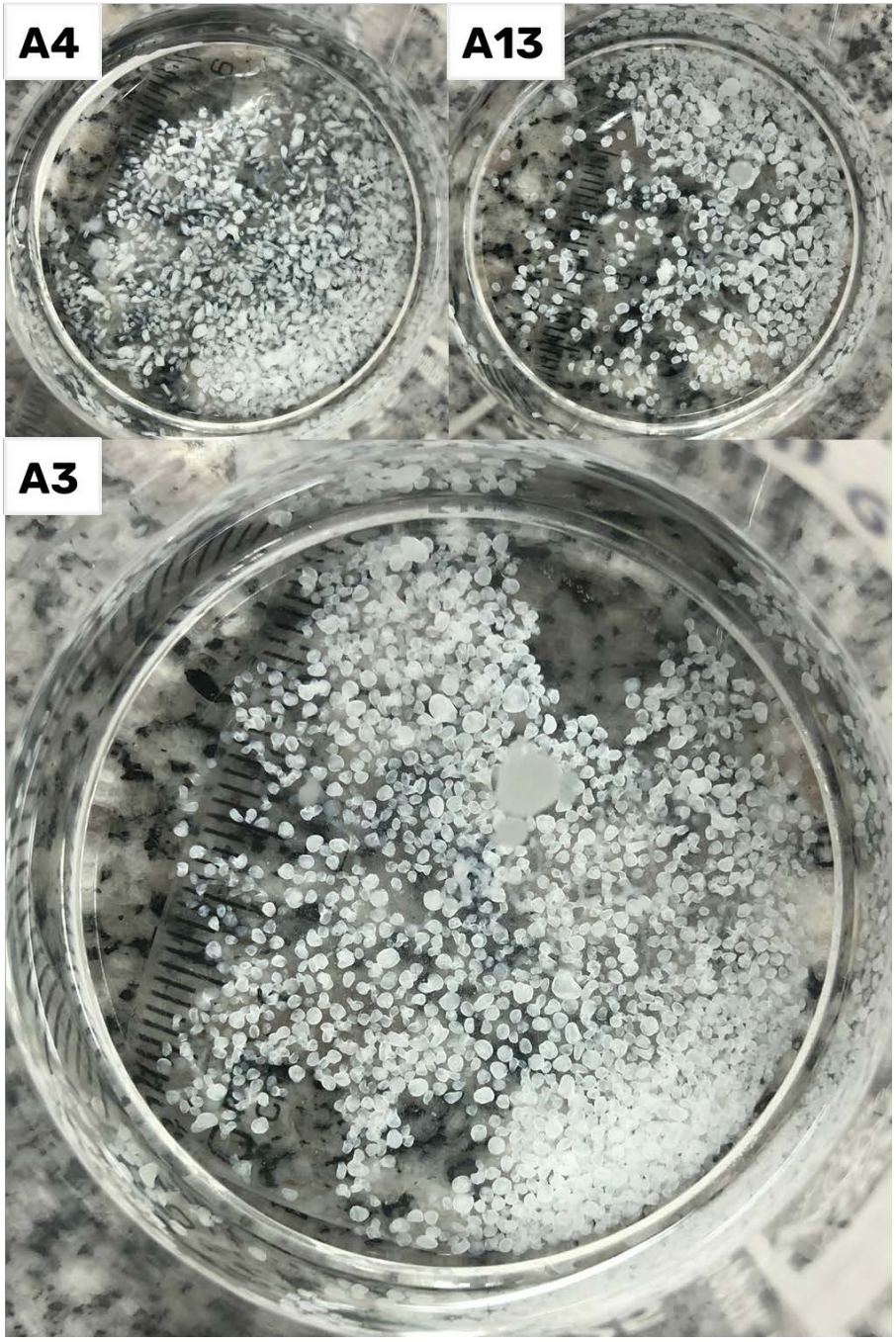

Figure 15: Macroscopic morphology of cellulose beads formed.

5.3 Encapsulation Efficiency

The encapsulation efficiency (EE) of the samples was measured by using a spread plate method and probiotic marine bacteria P64, P62 & ILI. Before the test, the initial CFU was measured by eight-time dilutions taking into account dilution -6, -7, and -8 for each bacteria. Using the hand-dropping technique with the cellulose solution into a syringe (0,5mL) was added to the coagulation bath that contained the different strain bacteria for each experiment. At this point, the beads keep a spherical aspect and capture 1.33 x 10^3, 1.08 x 10^3 and 4.25 x 10^3 CFU/mL depending on the extracted cellulose used for the encapsulation. In other words, the encapsulation efficiency was tested by capturing the bacteria with 0.5mL of the bead's solution.

Table 6 indicates the initial CFU, the maximum encapsulation, and the percentage of encapsulation efficiency of the samples that survive the encapsulation procedure. The low portion of EE (1.56% - 0.30%) represents those beads were not able to encapsulate many bacterial cells. Figure 16 shows the initial colonies concentration of P64 (Xi) and the concentration of the encapsulated ones into the beads (A3, A4 & a13).

Table 6: Probiotic Encapsulation Efficiency of P64 into different beads.

Sample	CFU/mL		Probiotic Encapsulation Efficiency (EE) %
	Initial (Xi)	Final (Xt)	
A3	2.63 x 10^7	1.08 x 10^3	0.41%
A4	2.63 x 10^7	1.33 x 10^3	0.50%
A13	2.63 x 10^7	4.25 x 10^2	0.16%

As can be seen on the TSA agar plates (Figure 16) in the presence of a few P64 colonies in the first dilutions illustrates the poor efficacy of capsules to encapsulate probiotics. However, it is essential to note that the capsules managed to encapsulate the probiotic strains. They could function as a useful tool to increase the storage time of the probiotics and facilitate their entry into the farmed shrimp by controlling the bacteria delivery and protecting probiotic bacteria from adverse environments reducing cell losses. As it is observed, some of the bacteria encapsulate are present in a dilution range between -1 to -2, and this is due to the better performance of the cellulose

extracted used to encapsulate. Also, one control was tested to use as a reference and compare how much encapsulated each of them. The cellulose commercial (A13) was used as a reference control.

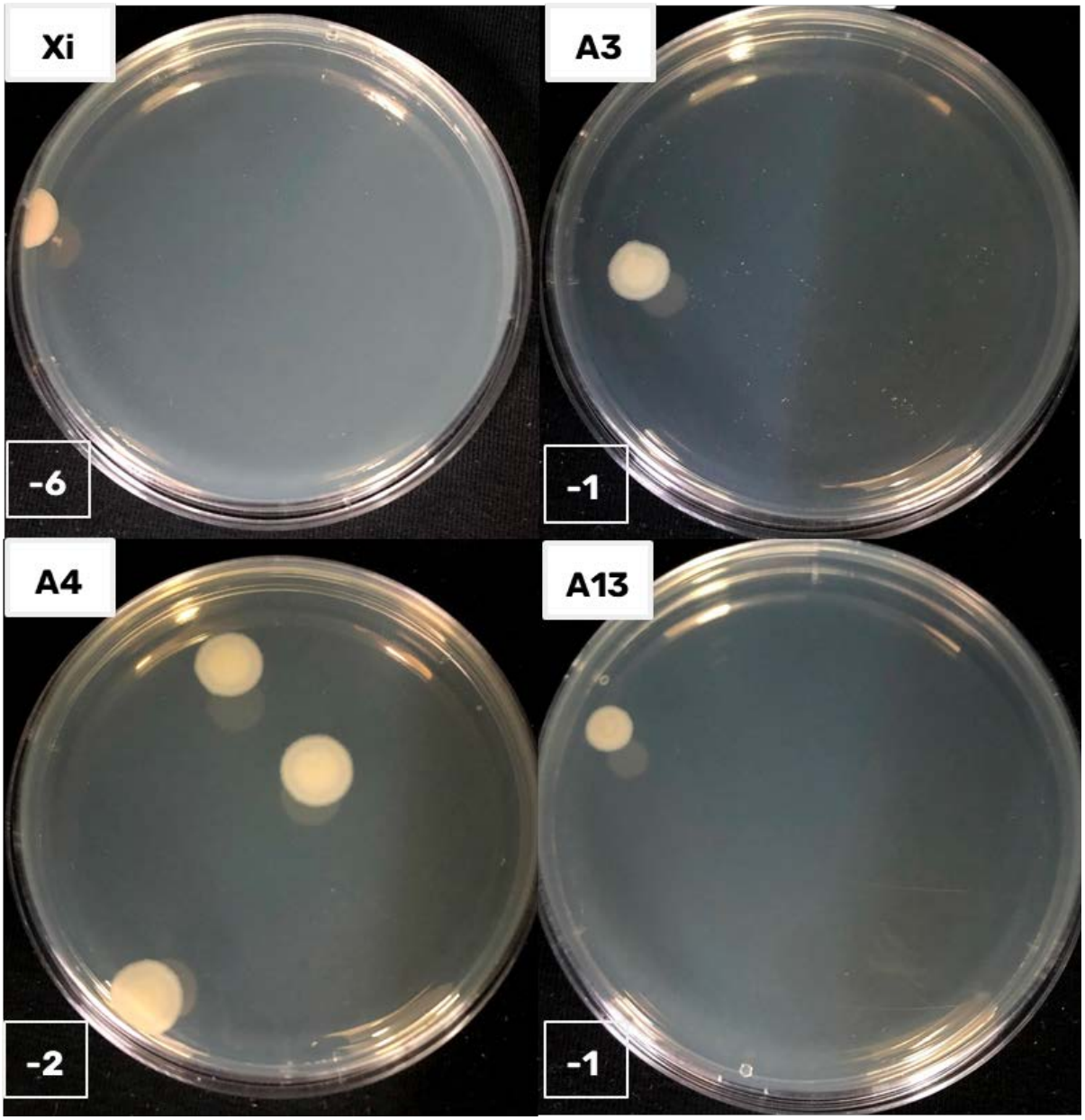

Figure 16: P64 Agar plates of the initial concentration (Xi) and final concentration for each material (A3, A4 & A13).

Figure 17 shows a notable similarity in the morphology of the final encapsulating cellulose beads between A3, A4 & A13. A3 and A4 are produced from the same extraction cellulose method, while A13 is commercial cellulose.

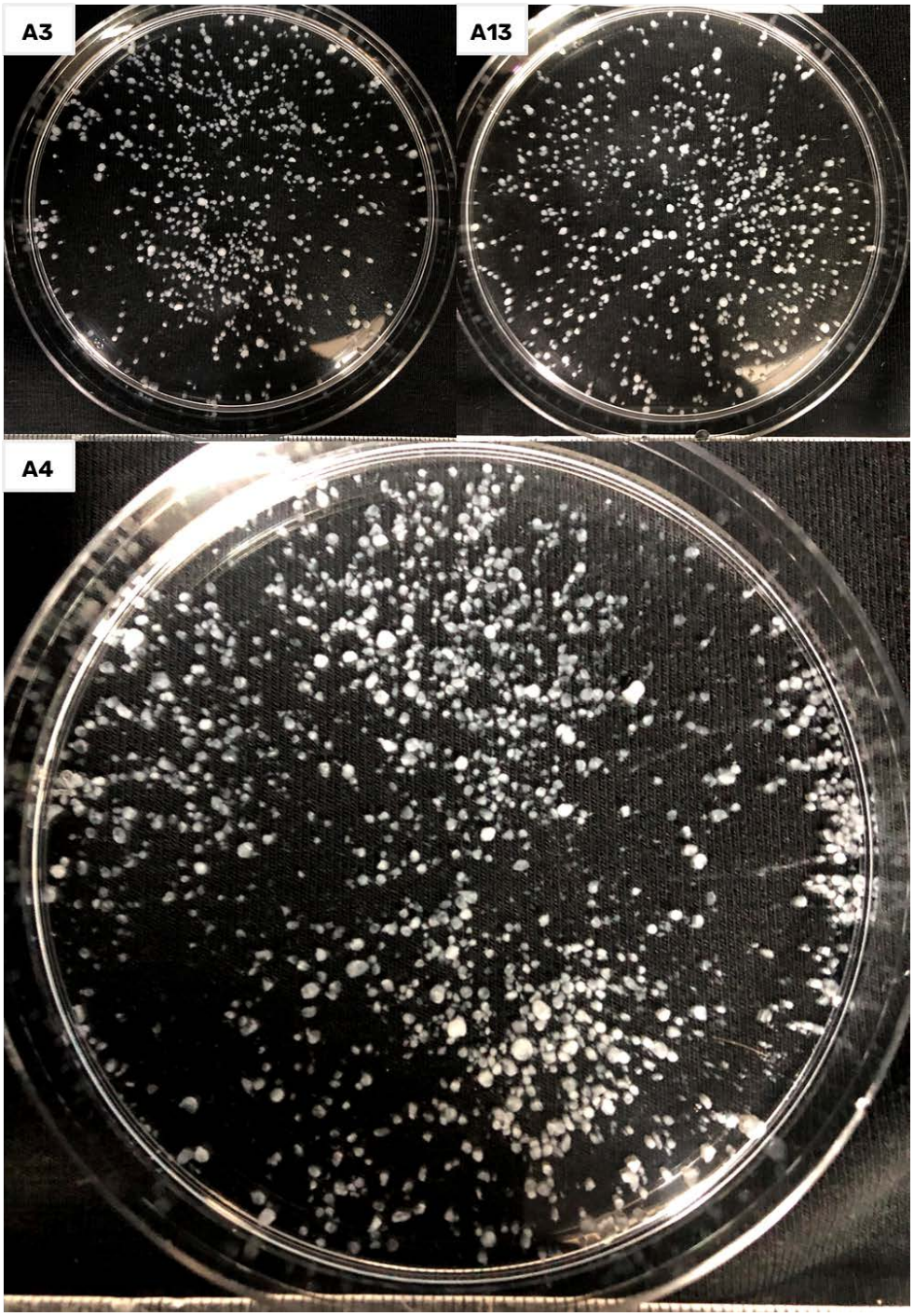

Figure 17: Shows the morphology of the various cellulose beads (A3, A4 & A13) used for probiotic bacteria P64 encapsulation.

6 DISCUSSION

This study reported the development of tailored cellulose-based beads produced from various resources for the encapsulation of marine probiotic bacteria. Then, demonstrates that the morphology, purity, and crystallinity of each cellulose sample directly impacts the efficiency of bead production. From different organic waste residues of common fruits and vegetables, twelve

cellulose fibers were effectively recovered. Cellulose fibers were removed using the same method, and they were all used to make beads. Cellulose fibers were studied using a combination of FT-IR spectroscopy, X-ray diffraction, and SEM to characterize them. Only two of the fibers were able to create cellulose beads, despite having unique physicochemical features.

According to the FT-IR results, cellulose fiber extraction from the twelve natural sources was successful. The samples' main component, conforming to FT-IR measurements, is cellulose. The typical cellulose fingerprint is an absorption peak located between 1650 and 900 cm^{-1} (Auta et al., 2017). In addition, the peaks from 900$cm^{-1}$to 1200$cm^{-1}$are associated with: –OH absorption, 900 cm^{-1}; –CH absorption 1029cm^{-1}; –C–OH absorption 1112 cm^{-1}; –C=O absorption 1165cm^{-1}; =CH2 absorption 1200cm^{-1}; all of these groups may be found in cellulose's glycosyl units. (Doncea et al., 2010). Spectra helped detect cellulose content and residual molecules of cell wall components such as hemicellulose or lignin between 1730 and 1700 cm^{-1}, indicating lignin aromatic compounds. The typical spectrum of commercial cellulose is seen in *Figure 7*. Both commercial cellulose and cellulose made for cellulose beads have the same peaks, as can be observed *(Figure 8)* (Mehanny et al., 2020).

The XRD data show standard cellulose diffraction patterns. Fibers with more than 73.3 percent crystalline indexes formed beads and have outstanding mechanical characteristics analyses of the twelve extracted. One commercial cellulose (A13) samples depicted distinct graphs and thus different degrees of crystallinity for each, as shown in *Figures 9 and 10*. Two prominent diffraction peaks can be seen in the cellulose that forms beads (A3, A4, A13) *(Figure 10).* The first occurs at 2θ = ~15.1 ° and the second 2θ = ~21.8 ° at which correspond to crystallographic planes (110) and (200) of type polymorph of cellulose (Mehanny et al., 2020). This crystal structure corresponds to the parallel arrangement of two glycosidic chains and has been referred to as a polymorph of cellulose with more excellent mechanical resistance. In the XRD patterns of A3, A4, and A13, cellulose can observe a low-intensity peak at around 2θ = ~34 °, which corresponds to cellulose's plane (040) (Costa et al., 2015). Based on the results shown in *Table 5*, the crystallinity index plays a fundamental role in forming cellulose beads.

SEM images exhibit unique morphologies and structures due to differences in porosity, shape, and size. As mentioned before, from twelve cellulose fibers extracted, only two formed beads due to their different properties are shown using characterization methods. The maximum size of droplets that may be formed restricts the size of cellulose beads produced by dropping processes to 1 to 1.5

mm. The mechanical strain that the droplets experience as they come into contact with the coagulation solution's surface has an impact on the shape of the beads (Gericke et al., 2013). The beads may flatten when the droplet stability is poor in comparison to the applied force, giving them a disk-like appearance. As a result, adjusting ejection speed, falling height, and solution viscosity are essential when using a dropping approach to make cellulose beads (Sescousse et al., 2011). Instead of droplets, cellulose solutions are forced at high speeds through a narrow hole, forming a continuous stream.

Cellulose beads are not suitable for encapsulating probiotic marine bacteria due to the high pH levels throughout the whole process. The initial cellulose solution starts with a pH of ~12.5 and ends with ~10.5. The high basic pH is due to the elevated concentration of base solvent, then this type of pH is not adequate for bacterial cells that live in intermediate pHs (5-7). The bacterial cells suffer a primary shock through the encapsulation process. The central part of the strains dies in the process, such as the probiotics *Vibrio diabolicus* (Ili) &*Vibrio hepatarius* (P62). On the other hand, some *Bacillus cereus sensu stricto* (P64) bacterial cells survive the procedure. However, the beads A3, A4, and A13's encapsulation efficiency was too low 0.41, 0.50, and 0.16 %.

XRD and SEM analyses suggest that the crystalline index and the degree of porosity of the cellulose fibers play an essential role in beads formation, thus in encapsulation efficiency. The maximum encapsulation capacity percentage was 0.50 %, and it was from sample A4 due to the high levels of pH (12.5 to 10.5) throughout the whole process. Moreover, the encapsulation efficiency of the microcapsule depends upon different factors like concentration of the polymer, the solubility of the polymer in a solvent, rate of solvent removal, the solubility of organic solvent in water, etc. The demand for cellulose-based products is growing due to several advantages: renewable, inexpensive, and biodegradable. Herein, cellulose beads have been prepared by first solubilizing cellulose, then crosslinking the cellulose chains. Cellulose-based beads as an alternative to encapsulate marine probiotics, evaluating mechanical characteristics to form tailored beads of twelve types of cellulose fibers and encapsulation efficiency properties of two of them plus the commercial one.

7 CONCLUSIONS

Successfully extracted twelve samples from organic plant waste were characterized with FT-IR, XRD, and SEM showing that cellulose is the main component with a high level of purity. Then, the application of the bead's synthesizing procedure from organic waste assures that only some of the organic materials can produce beads. The beads characterization findings indicate that the two fibers exhibit unique chemical, physical and morphological characteristics that may allow them to develop beads. One of the beads (A3) presented a solid and hard consistency, and those with a crystallinity index higher than 73.3% uniform surface, compactness, and low porosity could form beads. Based on the characterization of the beads, encapsulation process of the probiotics inside the beads was applied showing that the samples with the most outstanding encapsulation efficiency are A4 with at least 0.50% encapsulation and the others with 0.41 (A4) and 0.16 (A13). Therefore, it is concluded that it has low efficiency to encapsulate probiotics due to the high pH (12.5 to 10.5) throughout the whole process. Using a novel coagulation bath with a neutral pH (7) cannot neutralize the high level of the pH process. Overall, cellulose beads prepared using a three-step method without toxic solvents stand out as a simple green process. It is a viable protocol to design and develop large-scale cellulose-based beads with potential applications in biomedical and pharmaceutical fields as well as delivery carriers of drugs and nutraceuticals. However, it cannot be used to encapsulate living cells due to their sensibility to pH change.

8 FUTURE WORK AND RECOMMENDATIONS

It is critical to enhance bead design in terms of operability and to look for new applications based on past findings.

Research on cellulose-based beads utilizing novel organic components is required.

It is the first time in Ecuador that using organic waste to make cellulose-based beads to encapsulate marine probiotics has been recommended, highlighting the need for additional study into other cellulose encapsulation materials.

Cellulose solvents that do not significantly modify the pH of the beads should be evaluated not to affect the bacteria's viability.

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